Return to Regulated Status of Alfalfa Genetically Engineered for Tolerance to the Herbicide Glyphosate

The United States District Court for the Northern District of California has vacated the Animal and Plant Health Inspection Service's (APHIS') June 2005 determination of nonregulated status with respect to the Monsanto Company and Forage Genetics International alfalfa lines designated as events J101 and J163, which have been genetically engineered for tolerance to the herbicide glyphosate.

In a notice published in the Federal Register on June 27, 2005 (70 FR 36917-36919, Docket No. 04-085-3), APHIS advised the public of its determination, effective June 14, 2005, that the Monsanto/FGI alfalfa events J101 and J163 were no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms.

On February 13, 2007, the United States District Court for the Northern District of California issued a ruling in a lawsuit filed by the Center for Food Safety – along with several other nonprofit organizations and alfalfa growers – challenging APHIS' decision to deregulate alfalfa events J101 and J163 (referred to in the lawsuit as Roundup Ready alfalfa). The court ruled that the deregulation may have significant environmental impacts that require the preparation of an environmental impact statement (EIS), and that APHIS violated NEPA by preparing an environmental assessment (EA) instead of an EIS.

Subsequently, on March 12, 2007, the court issued a preliminary injunction order in the case. As of March 12, 2007, all sales of Roundup Ready alfalfa seed are prohibited pending the Court's issuance of permanent injunctive relief. The court decided that growers who have already planted Roundup Ready alfalfa will not be required to remove the plants. Additionally, they will be permitted to harvest, use, and sell such Roundup Ready alfalfa. The court also prohibited all future planting of Roundup Ready alfalfa beginning March 30, 2007. Growers who intended to plant Roundup Ready alfalfa before that date and who had already purchased the seed prior to the March 12, 2007, preliminary injunction order may plant the seed. Growers who intended to plant alfalfa after March 30, 2007, or who did not purchase Roundup Ready alfalfa seed prior to March 12, 2007, must plant non-genetically engineered alfalfa.

In addition, the court vacated APHIS' June 2005 decision deregulating Roundup Ready alfalfa, and the plant line is once again a regulated article. In accordance with the court’s order, APHIS is issuing the appropriate notices informing Roundup Ready alfalfa sellers and growers of the terms of the court’s preliminary injunction order. The court has scheduled a hearing on a permanent injunction for April 27, 2007.

Monsanto Company and Forage Genetics International Petition 04-110-01p for Determination of Nonregulated Status for Roundup Ready® Alfalfa Events J101 and J163

USDA/APHIS Environmental Assessment and

Finding of No Significant Impact

May 2005

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment in response to a petition (APHIS Number 04-110-01p) received from Monsanto Company and Forage Genetics International seeking a determination of nonregulated status for their genetically engineered alfalfa designated as events J101 (OECD unique identifier MON-00101-8) and J163 (OECD unique identifier MON-00163-7) under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that confers tolerance to the herbicide glyphosate. Based on the analysis documented in its environmental assessment, APHIS has reached a finding of no significant impact (FONSI) on the environment from the unconfined cultivation and agricultural use of events J101 and J163 and their progeny.

John T. Turner for Cindy Smith

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date: 6/14/05

USDA/APHIS Environmental Assessment

Monsanto Company and Forage Genetics International Petition 04-110-01p for Determination of Non-regulated Status for Roundup Ready® Alfalfa Events J101 and J163

October 2004

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment in response to a petition (APHIS Number 04-110-01p) received from Monsanto Company and Forage Genetics International seeking a determination of non-regulated status for their genetically engineered alfalfa designated as events J101 (OECD unique identifier MON-ØØ1Ø1-8) and J163 (OECD unique identifier MON-ØØ163-7) under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that confers tolerance to the herbicide glyphosate.

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

Date:

JUN 14 2005

Attachment
Finding of No Significant Impact
Response to Comments
APHIS No. 04-110-01p

APHIS received 663 comments by the close of the comment period. Comments came from alfalfa growers and seed producers, organic growers, animal producers, growers associations, consumer groups, agriculture support industries, academic professionals and individuals. Five hundred twenty respondents did not support granting non-regulated status to the petition. One hundred twenty-seven support the petition. The majority of alfalfa growers and seed producers support granting non-regulated status. They state that their markets demand a weed free product and glyphosate tolerant alfalfa offers a tool to achieve these results. Many of those that do not support the petition are concerned that certain domestic and foreign markets may be closed to growers who cannot guarantee a non-genetically engineered product. The majority of academic professionals, agricultural support industries, and grower's associations also supported the petition. Organic growers opposed the petition because of concerns that pollination of their crops by the glyphosate tolerant variety will result in the inadvertent generation of unwanted GE products, resulting in market loss. The comments raised several issues and each is addressed below.

Several comments suggested that glyphosate tolerant alfalfa may impact exports of conventional or organic alfalfa seed and hay if they contain traces of glyphosate tolerant alfalfa. Several of these comments also suggest that animal products from animals fed glyphosate tolerant alfalfa could have less market appeal. Many of the comments focused on potential impacts to export markets, especially Japan, which imports approximately $500 million of US alfalfa annually. Of the 5% of alfalfa hay exported, 75% of these exports go to Japan. Japan has not yet agreed to import glyphosate tolerant alfalfa.
USDA believes that all methods of agricultural production (conventional, organic, or the use of genetically engineered varieties) can provide benefits to the environment, consumers, and farm income. The role of Biotechnology Regulatory Services within APHIS is to provide regulatory oversight that allows for the safe development and use of genetically engineered organisms. Once a new biotech variety has been granted non-regulated status by APHIS, any decisions to produce or market that product are made by the technology providers and producers and are driven by market demand. USDA encourages the developers of new biotech varieties to seek regulatory approvals for these new products in our major export markets at the same time non-regulated status is sought within the US, to help prevent loss of markets that could result from unapproved genetically engineered products entering the export channels. The USDA Grain Inspectors, Packers, and Stockyard Administration (GIPSA) announced in August 2002 that they will be developing voluntary testing and process verification programs to facilitate the marketing of agricultural products such as non- genetically engineered varieties.

Japan is a major market for U.S. exports of alfalfa hay. The petitioner has applied for approval for glyphosate tolerant alfalfa for use in food and feed in Japan. Additionally, Japan allows up to 1% of a genetically engineered product that has not been approved in Japan in feed if that product has completed a safety assessment by a foreign government with an assessment system equivalent to the Japanese (Ministry of Agriculture, Forestry, and Fisheries Announcement 1781, 2002.) This tolerance provides an allowance for those hay producers that export their hay to Japan and are concerned about trace levels of biotech alfalfa in non-biotech shipments. By employing reasonable quality control, it is highly unlikely that the level of glyphosate tolerant alfalfa will exceed 1% in conventional alfalfa hay. This can be accomplished by appropriate oversight of planting, harvesting and transportation equipment, as well as managing storage facilities and checking seed sources.

APHIS received several comments from growers of organic and conventional alfalfa who express concern that gene flow may occur between glyphosate tolerant alfalfa and conventional alfalfa. Alfalfa is an insect pollinated crop that is mainly pollinated by bees. Commenters related that bees can travel relatively long distances and so the potential exists to move pollen from the glyphosate tolerant crop to hay fields, seed fields, and wild or feral populations of alfalfa. The commenters suggest that there will eventually be wild or feral glyphosate tolerant alfalfa plants and that these plants may serve as an additional source for glyphosate tolerant alfalfa gene flow into conventional and organic alfalfa fields.
APHIS acknowledged in the Environmental Assessment that alfalfa is insect pollinated. Insect pollination for alfalfa has been documented up to 2 miles from the pollen source. However isolation distances are not required for genetically engineered products that have been approved by EPA, FDA, and USDA for general release into the environment because the safety of these products has been thoroughly evaluated by the involved agencies. Nevertheless, the National Organic Program, which is administered by USDA's Agricultural Marketing Service, requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances, such as modified genes, from adjoining land that is not under organic management. However, the determination of the size of the buffer zones is left up to the organic producer and the certifying agent on a case-by-case basis. Furthermore, organic production operations must develop and maintain an organic production system plan that outlines the steps it will take to avoid cross pollination from neighboring operations.

Some comments expressed concern that the glyphosate tolerant trait, if established in feral alfalfa, may move from feral alfalfa to organic seed fields. Other comments provided by alfalfa and weed scientists indicated that most of the existing wild or feral populations appear to be plants from older alfalfa varieties. They speculate that these populations started during earlier times when hay was grown to a much more mature development stage resulting in the production of viable seeds. During its harvest and transportation, this hay along with the viable seed was scattered and the seeds germinated and established to form these feral populations. For the present alfalfa hay production programs, it is highly recommended that the alfalfa be harvested by 10% bloom to

maximize nutritional quality. If the alfalfa is harvested at the recommended growth stage, no mature seeds should have developed. If no mature seeds are produced, viable seeds will not be scattered during the hay harvest or transportation. If no viable seeds are scattered, the establishment of new populations of feral glyphosate tolerant-alfalfa plants should be greatly minimized.

Comments from alfalfa and weed scientists also discussed why feral alfalfa should be a very minor issue. If feral plants are pollinated from a nearby glyphosate tolerant alfalfa seed or hay field and viable seed are produced on the feral plants, seeds that drop from the plant would not germinate and establish because of autotoxicity. If the seeds drop away from the mother plants or other feral plants, alfalfa needs a fairly precise environment for germination and establishment –firm seed bed (alfalfa seed does not germinate well on a hard soil surface), neutral pH, no competition from other plants, adequate water, appropriate N-fixing bacteria, etc. All of these appropriate conditions are very difficult to have in place at the same time in unmanaged locations. APHIS agrees with this comment that establishment of feral glyphosate tolerant alfalfa should be a minor issue and subsequent pollination from those plants would also be a minor issue. The alfalfa seed producer would have the greatest influence on the number of feral plants, glyphosate tolerant or not, near the seed field. The removal of feral plants is strongly recommended near any seed field in which maintenance of varietal purity is a high priority, such as for foundation and certified seed fields of glyphosate tolerant-alfalfa, conventional alfalfa, or organic alfalfa. Good management, combined with the low likelihood of glyphosate tolerant feral alfalfa, can make this scenario very unlikely to occur.

Some commenters expressed concern that feral alfalfa is considered an invasive plant in some environments according to the USDA/ National Resource Conversation Service and the addition of glyphosate tolerance will cause control problems of glyphosate tolerant alfalfa plants in the future.

USDA/ National Resource Conservation Service Plants Database Fact Sheet for alfalfa (http://plants.usda.gov/cgi_bin/topics.cgi?earl=characteristics.html) stated that alfalfa may become invasive in some regions or habitats. However, the author of the original document from which the "invasive" terminology was derived commented: "Under NO circumstances was my intention to directly state or imply that alfalfa was invasive, or a noxious and potentially invasive plant species. Instead, I simply intended through its inclusion in the guide to note that alfalfa can, and sometimes does colonize disturbed areas and seemingly occupies a role in those areas as a ruderal." (Brett Serviss, Docket No. 04-085-1 #480).

The information provided in the petition showed that the glyphosate tolerant alfalfa has no more invasive characteristics than conventional alfalfa. Several alfalfa or weed scientists commented that alfalfa is not invasive or a serious weed that would cause major problems. APHIS concurs with this assessment.

A few comments were expressed by those opposed to granting non-regulated status to glyphosate tolerant alfalfa because total forage yields, especially during the seed establishment year, would be lower when weeds are controlled in the glyphosate tolerant

alfalfa with glyphosate as compared with the conventional alfalfa where weeds are not controlled (Doll, 2003).
Total vegetative yield is not the major consideration for alfalfa, but rather forage quality, drying time, palatability, etc. of the harvested product (Doll, 2003). It is acknowledged that vegetative yield from weeds may increase overall yield. However, weeds may be detrimental for livestock feed and decrease premiums for pure alfalfa hay because weeds in general do not have the high nutritional characteristics of alfalfa, some weeds have awns, spines and thorns that that can get stuck in the animals mouth and throat causing injury, some weeds such as wild garlic and onions result in off flavors in meat and milk, and some weeds are poisonous causing death or injury. In fact, the paper that was cited (Doll, 2003) to indicate that controlling weeds may lower overall yield also noted that glyphosate tolerant alfalfa has many advantages over conventional alfalfa. The author of this paper also commented in favor of deregulating glyphosate tolerant alfalfa (Doll, 04-085-01, Comment # 505).

Several comments suggested that glyphosate tolerant alfalfa may have an adverse effect on insectivores protected by the International Migratory Bird Treaty and state protected species that feed on species associated with alfalfa.
With specific reference to the Migratory Bird Treaty Act (MBTA)(16 U.S.C. §701 et seq.), which the commenter cites, APHIS has found no direct effects of glyphosate tolerant alfalfa or the management practices associated with glyphosate tolerant alfalfa on any non-target organism, including migratory birds. The commenter suggests that potential indirect effects on the broad environment of migratory birds by agricultural practices would violate the MBTA. The Migratory Bird Treaty Act is violated by taking, killing or possessing a listed bird (16 U.S.C. §703). According to 50 C.F.R. 10.12, a "take" within the meaning of the MBTA includes, "to pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to pursue, hunt, shoot, wound, kill, trap, capture, or collect." Since glyphosate and glyphosate tolerant alfalfa management practices associated with those crops do not constitute a taking, this act would not apply.
With respect to indirect effects on agricultural ecosystems that may result from glyphosate use irrespective of the MBTA, APHIS believes these are negligible. Agricultural areas by their very nature are subject to alternating disturbance schedules from season to season. Alfalfa, like many crops, is grown in rotation with other agricultural products. What may be a suitable site for a migratory bird one year may be inhospitable in the next. There exists no requirement that growers manage their land to maximize or optimize habitat for migratory birds.
The FDA has primary regulatory authority over food and feed safety. FDA has completed their consultation and had no further questions about the food or feed safety of alfalfa derived from events J101 or J163 submitted by Monsanto and Forage Genetics, Inc. http://www.cfsan.fda.gov/~rdb/bnfl084.html Information can be found for alfalfa along with other deregulated crops at http://www.cfsan.fda.gov/~lrd/biocon.html. Sections V.C, V.D, and V.F of the petition address the characterization and biochemical properties of the CP4 EPSPS protein produced by Events J101 and J163. Each of these sections showed that the CP4 EPSPS protein is similar to the EPSPS protein of control plants and has the same properties as the *E. coli* produced CP4 EPSPS protein. Section VI.H addresses the crop compositional assessment. This section showed the composition of the

forage derived from the plants with Events J101 and J163 in comparison with control plants and reference varieties to being very similar with any differences noted to be unlikely to have any biological significance. In addition, two feeding studies on broilers using canola meal and corn both of which were tested for their nutritional value using materials with and without CP4 EPSPS showed similar performance between the transgenic product and nontransgenic control(Taylor et al., 2003 and Taylor et al., 2004). The above information provides evidence that the glyphosate tolerant alfalfa should perform similar to nontransgenic alfalfa for birds and other animals. Similar information can be found in the 1999 OECD Consensus Document.
In relation to wildlife habitat, the National Wild Turkey Federation that supports scientific wildlife management on public, private and corporate lands commented that alfalfa is an excellent wildlife plant material providing food and shelter and voiced their support for the petition since they felt the glyphosate tolerant alfalfa should benefit wild turkeys, other birds, such as migrating and over-wintering water birds and waterfowl, and other wildlife species that utilize alfalfa fields.

Several groups and individuals involved with organic food production expressed concerns that liability or economic issues may be created by finding small amounts of glyphosate tolerant alfalfa seed in seed for sprouting used in human food, by finding transgenic genes or proteins in organic animal products (such as milk, meat and honey) or finding hay with some small level of glyphosate tolerant alfalfa. The economic loss may result from not meeting contract specifications, failing to meet certification standards, or losing market share for not meeting consumer expectations and preferences. These concerns about economic issues also raised concerns on liability issues such as who would be liable for any unexpected losses caused by identifying low levels of glyphosate tolerant alfalfa in products expected to be free of glyphosate tolerant alfalfa.
It is up to the individual organic seed or hay grower to institute those procedures that will assure meeting any specified claims or contract specifications that are beyond the normally accepted specifications or standards for the crop, in this case alfalfa seed and hay. One commenter pointed out that the official position of the International Federation of Organic Agriculture Movements (IFOAM) is that "*Organic certification shall not imply it is a "GE-free" certification. Rather it shall be presented as guaranteeing "production without GE/GMOs". As there is no guarantee that organic products are 100% free from any GMO pollution, organic products shall not be marketed as "GE-free", unless there are specific safeguards and certification procedures for that specific product. Organic producers and associations shall actively inform the consumers of this fact to ensure fair marketing claims and to avoid future debates about consumer deception.*" This statement is part of the official position Adopted by the IFOAM World Board, Canada May 2002.

A group of commenters oppose the petition because they believe that each release of another glyphosate tolerant crop, in this case glyphosate tolerant alfalfa, leads to increased use of glyphosate and other herbicides resulting in increased environmental harm to groundwater, watersheds, drinking water, fisheries, irrigation, soils, and wildlife refuges. They also suggest that increased glyphosate use leads to more glyphosate resistant weeds, which require the use of more toxic herbicides. They also suggest that glyphosate tolerance in perennial crops encourages excessive herbicide application over longer periods during the growing season and over multiple years in the same area. Some

believe that stewardship of glyphosate use should be considered to conserve this environmentally friendly herbicide. Many of these comments also suggest that the release of glyphosate tolerant alfalfa will result in the use of more toxic herbicides to control glyphosate tolerant alfalfa, because in many cases fields of alfalfa are now removed using glyphosate, so with the use of glyphosate tolerant alfalfa, herbicides with higher toxicity ratings, such as 2,4-D and dicamba, would be used to remove the glyphosate tolerant alfalfa; and glyphosate is often used to control weeds, including feral alfalfa plants, in nonagricultural environments such as roadsides, so herbicides with higher toxicity ratings would be required to control feral glyphosate tolerant alfalfa plants.

Many of the comments about herbicides incorrectly assume that herbicides are not used on alfalfa seed fields or hay fields presently. In reality, several different herbicides may be used multiple times throughout the multiyear lifespan of the typical alfalfa seed or hay field and may be applied multiple times within the same year depending on the weeds that need to be controlled. Most, if not all, of these herbicides, have a less desirable environmental profile (toxicity, decay rate, movement into groundwater, etc.) than glyphosate, most have restrictions that limit their flexibility of use, most do not control as many of the weeds, and most cause some damage to the alfalfa lowering its yield compared to glyphosate.

APHIS disagrees with the comment that glyphosate tolerance in perennial crops encourages excessive use of glyphosate because it implies that glyphosate would be used incorrectly, used more often than necessary, or used at higher than recommended rates. Applying glyphosate or any pesticide contrary to the label is illegal. Using it more often than necessary or using it at higher than recommended rates are characteristics of uninformed users that care little about the environment or about economics. Based on their comments, alfalfa seed and hay growers are informed users and they care about the environment. They also want to grow and market their products by the most economical means. Extra applications of glyphosate would take additional time and effort. It would require more glyphosate, result in greater equipment use, and may result in greater damage to alfalfa stands. All of these factors would result in greater expense to the producer. Applying glyphosate at higher than recommended rates would also result in greater expense to the producer. APHIS received comments from 34 alfalfa and weed scientists from 21 different states supporting the petition. Most of these scientists have many years (often more than 15 years) of experience working with alfalfa and/or herbicides. They are very familiar with the requirements of growing quality alfalfa while balancing the use of weed control measures to protect the environment, animal safety and the economic returns of the farmers.

Some commenters are concerned that glyphosate use on glyphosate tolerant alfalfa may result in additional glyphosate resistant weeds. APHIS agrees that this may occur. Weed species have developed resistance to every widely used herbicide. The scientists and growers in the agricultural community are very aware of this potential. A vast body of scientific literature has developed to address this issue. Alternative herbicides and strategies are available that may minimize the problem. Based on the comments, the alfalfa growers and weed scientists understand that good stewardship may be the only defense against this potential problem.

APHIS concurs that if a herbicide is used to remove glyphosate tolerant alfalfa from fields, a herbicide or combination of herbicides other than glyphosate will be used. APHIS realizes that the herbicides used may have an environmental profile that is less favorable than glyphosate. The choice that is made is based on the herbicide(s) registered for such use by EPA, and the grower's needs and preferences.
APHIS also acknowledges that if herbicides are used to remove feral glyphosate tolerant alfalfa plants along roadsides, or in other managed areas, that herbicides other than glyphosate would be used. Based on the comments received from alfalfa and weed scientists, other herbicides are registered and available for such use. In fact, based on the comments from most of these scientists, the need to control these feral plants is very small and glyphosate would not be the herbicide of choice for this control because glyphosate would destroy the companion grasses as well resulting in possible environmental damage.

Some comments suggested that increased use of glyphosate may result in shifts in weed species which in turn can ripple through the ecosystem as food sources for birds and insects change or disappear. In addition, they suggest that spray drift to field borders and nearby native vegetation causes damage to wild plants and flowers. These changes in plant populations can lead to harmful effects on birds, insects and other animals that depend on this vegetation for food and shelter.
With any wide use of a major herbicide, weed shifts are expected since plants may have more or less tolerance to any specific herbicide. This is no different for glyphosate. Maximizing yields of the highest quality crop is one of the most important goals of agriculture. Weeds are a major cause of yield and quality reduction. Therefore, it is not surprising that "diversity" of different plant species tends to be very low in agricultural fields, even in organic production. Maximizing yield in agricultural fields may lessen the need to place more area into agricultural production. APHIS believes that indirect effects on agricultural ecosystems that may result from glyphosate use are negligible. Agricultural areas by their very nature are subject to alternating disturbance schedules from season to season. Alfalfa, like many crops, is grown in rotation with other agricultural products. What may be a suitable site for a bird or insect in one year may be inhospitable in the next. There exists no requirement that growers manage their land to maximize or optimize habitat for birds or insects.

A few comments suggested that the increase in the number of glyphosate tolerant crops leads to over-reliance on one herbicide (glyphosate) with impacts on soil biota. Two examples suggested in the comments were negative effects on nitrogen fixing bacteria living in association with glyphosate tolerant soybeans (commenters were referring to King, et al., 2001),and higher incidence of fungal disease (Fusarium) on glyphosate tolerant soybeans.
Nitrogen fixing bacteria in soybeans (*Bradyrhizobium japonicum*) are different from the nitrogen fixing bacteria in alfalfa (*Sinorhizobium meliloti*). *S. meliloti* can metabolize glyphosate (Liu et al., 1991). Additionally, while the results of King et al. (2001) demonstrated a negative impact on *B. japonicum*, further investigation into the impact of using glyphosate on glyphosate tolerant-soybeans concluded that there is no significant reduction in yield. (Zablotowicz and Reddy, 2004).

Njiti et al. (2003) concluded that the development of Sudden Death Syndrome(SDS) was influenced by the genotype of the cultivar and not by the presence of the gene for glyphosate tolerance. They showed that there was no higher incidence of Fusarium on glyphosate tolerant soybeans; there was no greater root colonization by the soil-borne fungus *Fusarium solani* in the glyphosate tolerant variety and no more of the expected SDS leaf symptoms following the application of glyphosate. The petition for nonregulated status for glyphosate tolerant alfalfa also indicated that there were no significant differences between the glyphosate tolerant alfalfa and the conventional alfalfa with respect to disease susceptibility and no differences were noted for root nodulation between glyphosate tolerant alfalfa and conventional alfalfa.

A few comments noted that the petition reported an apparent increase in hard seed (seed dormancy) that may increase weediness of glyphosate tolerant alfalfa and feral glyphosate tolerant alfalfa plants. As noted in the petition, hard seed levels vary considerably in alfalfa and it appears to vary considerably with the environment. APHIS was quite aware of the significantly higher level of hard seed in the glyphosate tolerant alfalfa compared with the conventional variety in the first year that the data were collected. The data provided in the petition in Table VI-6 was the most conclusive information to indicate that glyphosate tolerance does not have an effect on hard seed level. Six scientists from four different states each with more than 20 years experience working with alfalfa and /or seed physiology commented that there was no evidence linking the increased levels of hard seed with glyphosate tolerance. They also generally noted that hard seed in the case of alfalfa was not linked to dormancy since their observations and studies in the past showed that hard seed almost always germinated within a few weeks of seeding and the seedlings that developed were generally too weak and noncompetitive to survive. From the data submitted in the petition and from the comments from these scientists, APHIS concludes that glyphosate tolerance does not cause increased seed dormancy, which may lead to increased weediness.

Some commenters expressed concern that the glyphosate tolerant alfalfa varieties will dominate the market to such an extent that conventional varieties will no longer be available and organic farmers will have no options to buy conventional varieties. This same trend toward popularity of genetically engineered varieties will further decrease the availability of non-genetically engineered varieties since forage breeders of conventional varieties will have to go to added expense and effort to assure that no transgenes are in the new variety. The commenters suggest that this same trend in variety development will further decrease diversity with a negative impact on the environment.
The varieties of alfalfa that are commercially available are driven by market forces. APHIS does not regulate the available varieties of any crop unless it is a plant pest. To do so would extend well beyond the authority of APHIS.
The concern that conventional varieties will no longer be available after the deregulation of glyphosate tolerant alfalfa assumes that this product will be so commercially successful that all future variety improvement programs would concentrate solely on genetically engineered varieties, and that the demand for new improved conventional varieties from farmers is so small that there is very little economic justification for conventional alfalfa variety improvement programs.

There are many conventional alfalfa varieties available now and much of the alfalfa presently grown east of the Mississippi River is grown in combination with forage grasses to be used for grazing or for hay. A glyphosate tolerant alfalfa variety would presumably be more expensive to purchase. To grow it with mixed forage grasses does not appear practical since its major advantage would be the elimination of all other plant species. Therefore the demand for glyphosate tolerant alfalfa would appear to be less east of the Mississippi River than west of the Mississippi River. This may result in demand for conventional varieties.

Some alfalfa seed farmers expressed concerns of seed crop contamination and felt that their respective states should be given authority to establish and monitor production zones to minimize chances for cross contamination and aid in detecting contamination when it occurs.

If APHIS grants non-regulated status to a transgenic event, APHIS does not have any further regulatory authority over this particular transgenic event. Individual states, on the other hand, often have authority to impose some type of regulation over various aspects of the agriculture enterprises within their state, such as establishing some type of production zone, to facilitate production or marketing of specific crops. APHIS would have no regulatory authority over the state to require or to forbid such a production zone.

Some comments expressed concerns of the possibility of transgenes passing from the glyphosate tolerant alfalfa to the gut micro-flora in livestock since alfalfa can be a major portion of a livestock diet.

Transgenic DNA is no different from other DNA consumed as part of the normal diet. Genetically engineered organisms have been used in drug production and microbial fermentation (cheese and yogurt) since the late 1970's. More than 500 million cumulative acres of engineered food and feed crops have been grown and consumed world wide in the past seven years (International Service for the Acquisition of Agri-biotech Applications at: http://www.isaaa.org/kc/CBTNews/press_release/briefs30/es_b30.pdf.) The FDA has not reported any significant concerns with bioengineered food and feed currently on the market. Based on lack of toxicity, the EPA has exempted from a pesticide tolerance DNA that are parts of plant-incorporated protectants FR 66 37817-37830).

There have been several studies in humans and animals following the fate of DNA once consumed (Beever and Kemp, 2000; Mercer *et al* 1999, 2001; Duggan *et al*, 2000; Chambers *et al.*, 2002; Netherwood *et al*. 2002; Einspanier *et al*., 2001; Duggan *et al*., 2003). The majority of DNA consumed is degraded in the gastro-intestinal tract although this is not 100% efficient. There is evidence that both transgenic and plant DNA can move from the gastro-intestinal tract lumen to other areas of the body and that this is a normal occurrence, but no risk has been identified.

Transfer and subsequent expression of DNA from the plant to bacteria is unlikely to occur due to impediments. First, transgene DNA promoters and coding sequences are optimized for plant expression not prokaryotic bacterial expression and the bacteria must be competent to accept DNA. Gebhard and Smalla (1998) and Schluter *et al.* (1995) have studied transgene DNA movement to bacteria, and although possible, DNA transfer would occur at extremely low rates (approximately 1 in 10^{14}). However, many genomes

(or part thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and rhizobia (Kaneko *et al.,* 2000; Galibert *et al.,* 2001, Wood *et al.* 2001, Kanekko *et al.* 2002). There is no evidence that these organisms contain genes derived from plants. Syvanen (1994), Kumar and Rzhetsky (1996), Koonin *et al*. (2001), and Brown (2003) reviews of the literature using sequencing data reveals that horizontal gene transfer from plants to bacteria occurs occasionally on an evolutionary time scale of millions of years. One of the factors limiting the frequency of horizontal gene transfer appears to be the need for homologous recombination and there are few homologous sequences between plants and bacteria. As noted in the petition, the gene for glyphosate tolerance was isolated from a soil bacterium, *Agrobacterium.*

One commenter made a reference to a no harm decision from the Fish & Wildlife Service (FWS) and indicated that the analysis is incomplete in that the Environmental Assessment fails to identify what if any species or issues it requested the FWS to address. APHIS and the Fish and Wildlife Service have a long standing agreement about these issues developed from a meeting in July 1999. The agencies agreed to use a decision tree approved by FWS to determine whether consultation with FWS would be required for a transgenic crop variety. APHIS continues to use this decision tree and policy for all petition requests. APHIS considered all threatened and endangered species, but none were identified for consultation with FWS.

Several commenters indicated that commercializing glyphosate tolerant alfalfa will benefit only Monsanto's corporate profits at the expense of consumers, farmers and the environment.
APHIS disagrees with this statement. Approximately 70 comments were received from hay growers in 20 different states (15 of which are west of the Mississippi River) as well as many of the alfalfa and weed scientists that indicated they were looking forward to using the glyphosate tolerant alfalfa to help lower costs, gain increased yields of weed free hay especially in the seeding year, use less water, gain timing flexibility in herbicide application, and decrease usage of other herbicides that are inconsistent or less effective in controlling weeds, that may injure the alfalfa crop, and that are less environmentally friendly. Of the growers that indicated the number of acres of alfalfa hay on their farms, the acreage ranged from a low of 50 acres to a high of 3000 acres. This large range implies that the size of farm is independent of the expected usefulness of this new technology. In addition, many alfalfa seed growers (10 growers from 6 states) felt that glyphosate tolerant alfalfa would help them be more efficient in controlling weeds at less cost resulting in more profit and at the same time helping to protect the environment by not having to use herbicides that may not be as environmentally friendly as glyphosate. Another group of comments suggested that the use of glyphosate tolerant alfalfa may benefit some areas because hay is commonly contaminated with invasive weeds, some of which are noxious weeds. One hay grower commented that some of their customers are public entities and private individuals who require Certified Weed Free Forage for use in Yellowstone National Park and the surrounding National Forests in the area to avoid bringing in noxious and prohibited weeds. It is obvious from this comment that if glyphosate tolerant alfalfa makes it easier and more practical to have weed free hay,

glyphosate tolerant alfalfa would help to maintain the nation's natural areas and therefore could be an environmental benefit.

Several commenters expressed concerns that glyphosate tolerant alfalfa may have adverse impacts on human or animal health. Some of these comments were concerned that the chemical composition of glyphosate tolerant alfalfa may not be the same as its conventional counterpart. The commenters were concerned that there would be downstream adverse effects in the human food supply or that since alfalfa is used primarily for animal feed, there may be impacts of glyphosate tolerant alfalfa on livestock
The FDA has primary regulatory authority over food and feed safety. FDA has completed their consultation and had no further questions about the food or feed safety of alfalfa derived from events J101 or J163 submitted by Monsanto and Forage Genetics, Inc. http://www.cfsan.fda.gov/~rdb/bnfl084.html Information can be found for alfalfa along with other deregulated crops at http://www.cfsan.fda.gov/~lrd/biocon.html
Some commenters suggested that the continued introduction of glyphosate tolerant crops, in this case glyphosate tolerant alfalfa, would lead to more glyphosate use. This would result in more human exposure to glyphosate. Reports of various symptoms have been attributed to glyphosate exposure (EPA 19080, EPA 1993). The EPA has regulatory authority over pesticide use. EPA has determined the tolerance for glyphosate residue on the alfalfa forage http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2002_register&docid=02-24488-filed and on the alfalfa seed http://a257.g.akamaitech.net/7/257/2422/01jan20051800/edocket.access.gpo.gov/2005/05-2983.htm
One commenter suggested that genetic modification could alter the nutritional value of the crop. The commenter refers to the alleged changes in phytoestrogen levels in glyphosate tolerant soybeans. However further investigations have shown that levels of soyasaponin and isoflavone are similar between genetically modified and non-genetically modified soybean lines (Goda et al., 2002) and application of glyphosate had little or no effect on estrogenic isoflavones in glyphosate tolerant soybeans (Duke et al., 2002).

Some comments suggested that this petition to deregulate glyphosate tolerant alfalfa warranted an Environmental Impact Statement under NEPA, since this is the first perennial crop to request deregulation, and since APHIS has decided to conduct an EIS on glyphosate tolerant creeping bentgrass (another perennial plant).
APHIS disagrees with these comments. APHIS has thoroughly examined the potential environmental impacts related to granting nonregulated status to glyphosate tolerant alfalfa while preparing an environmental assessment. APHIS has reached a finding of no significant impact after carefully considering the potential impacts of glyphosate tolerant alfalfa. Therefore an EIS is not needed. Some comments erroneously state that this is the first perennial crop to be granted nonregulated status. This however is not the case. The first perennial crop to be deregulated by APHIS was a virus resistant papaya (APHIS No. 96-051-01p, http://www.aphis.usda.gov/brs/aphisdocs2/96_05101p_com.pdf)
APHIS decided to conduct an EIS on glyphosate tolerant creeping bentgrass and not for glyphosate tolerant alfalfa because it was the first petition for a long-lived perennial species, that also spreads easily both vegetatively and via seed and pollen, and that has many wild, and somewhat more weedy relatives with which it can potentially hybridize

over long distances, and both creeping bentgrass and its relatives persist in non-agricultural ecologically sensitive environments in which vegetation management options may be more limited. By comparison (1) creeping bentgrass can persist for many years in non-agricultural environments including riparian areas that are sensitive and critical habitat for wildlife, whereas alfalfa tends to be a short-lived perennial that tends to die out after several years generally because of susceptibility to disease and traffic from animals and farm equipment. Alfalfa is primarily found in well-drained areas in or around agricultural fields or roadsides; (2) creeping bentgrass is wind pollinated and viable pollen can potentially spread many miles depending on wind velocity (Watrud et al.,2004 indicated finding successful hybridization 13 miles from the pollen source), whereas alfalfa is insect pollinated which generally limits potential viable pollen movement to within approximately 2 miles, (3) creeping bentgrass can easily spread vegetatively by means of stolons, whereas alfalfa has great difficulty in vegetative spread since it regenerates itself exclusively from the crown of the plants (it has no rhizomes or stolons), (4) creeping bentgrass, once seeded on the golf course is likely to serve as a potential reservoir for dispersal for 5-10 years before it is potentially replaced with another turf species or variety, whereas alfalfa whether for seed or hay will be part of a normal rotation with other crops, and (5) creeping bentgrass has at least 13 related Agrostis or Polypogon species, (both native and naturalized) within the USA with which it is has been documented to form hybrids (and the transgene could be further passed on to other species through further hybridization and introgression), whereas alfalfa has only one known relative within the *Medicago* genus in the United States, *Medicago lupulina* (black medic), and no successful hybrids have ever been documented between *Medicago sativa* and *Medicago lupulina* (black medic). APHIS evaluates each petition individually. APHIS considers many aspects of the biology as well as the intended and typical uses of the crop. APHIS diligently complies with NEPA to develop the appropriate documents to aid with the ultimate decision process.

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 04-110-01p) from Monsanto Company (St. Louis, MO) and Forage Genetics International (West Salem, WI) (hereafter FGI) seeking a determination of non-regulated status for their genetically engineered Roundup Ready® alfalfa designated as events J101 (OECD unique identifier MON-ØØ1Ø1-8) and J163 (OECD unique identifier MON-ØØ163-7). Monsanto Company and FGI seek a determination that events J101 and J163 and their progeny do not present a plant pest risk and, therefore, become no longer regulated articles under regulations at 7 CFR Part 340.

Events J101 and J163 were engineered to be glyphosate tolerant by inserting a gene that codes for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) into the alfalfa genome. The gene is from the common soil bacterium *Agrobacterium* sp. strain CP4 and was introduced into alfalfa via an *Agrobacterium*- mediated transformation protocol.

This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of events J101 and J163. It does not address the separate issue of the potential use of the herbicide glyphosate in conjunction with these plants. The United States Environmental Protection Agency (EPA) has authority over the use in the environment of all pesticidal substances, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Food and Drug Administration (FDA) has authority over food and feed issues of all plants used as food or feed.

Field trials of J101 and J163 alfalfa have been conducted under APHIS notification procedures (7 CFR 340.3). In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR 372), this EA has been prepared prior to issuing a determination of nonregulated status for J101 and J163 alfalfa in order to specifically address the potential for impact to the human environment through unconfined cultivation and use of the regulated articles in agriculture.

II. INTRODUCTION

A. Development of Events J101 and J163 Alfalfa

Monsanto and FGI have submitted a “Petition for Determination of Non-regulated Status” to the USDA, APHIS (APHIS number 04-110-01p) for genetically engineered alfalfa that is tolerant to the broad spectrum herbicide glyphosate. Glyphosate tolerant alfalfa would offer farmers a new option for weed control.

The management of weeds in alfalfa fields can be an expensive, labor intensive, and sometimes complicated operation. Often farmers use pre-emergent herbicides that will stop weed seeds from germinating. However, this assumes that weeds will always be a problem in all parts of the field. With J101 and J163 and progeny, farmers will have the option of applying herbicide after weeds have germinated and only in the areas of the field where there are weeds. Glyphosate is one of the most environmentally friendly herbicides commercially available.

These alfalfa plants were genetically engineered to be glyphosate tolerant by inserting a gene (from *Agrobacterium* sp. strain CP4) that codes for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) into the alfalfa genome. This gene, along with its regulatory sequences, was introduced into these alfalfa plants via an *Agrobacterium*-mediated transformation protocol. This is a well-characterized procedure, which has been widely used for over a decade for introducing various genes of interest directly into plant genomes.

APHIS authorized the first field testing of these alfalfa plants starting in 1998 and they have been field tested in the United States under the APHIS authorization numbers noted in Appendix B. Events J101 and J163 alfalfa have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. The field tests have been conducted in agricultural settings under physical and reproductive confinement conditions.

B. APHIS Regulatory Authority

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. These alfalfa plants have been considered regulated articles because they contain non-coding DNA regulatory sequences derived from plant pathogens and the vector agent used to deliver the transforming DNA is a plant pathogen.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

C. U.S. Environmental Protections Agency and Food and Drug Administration Regulatory Authorities

The genetically engineered alfalfa is also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces the tolerances set by the EPA. A final EPA decision is pending.

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. Monsanto/FGI submitted a food and feed safety and nutritional assessment summary for events J101 and J163 in October 2003. A final FDA decision is pending.

III. PURPOSE and NEED

APHIS has prepared this EA before making a determination on the status of J101 and J163 alfalfa as regulated articles under APHIS regulations. The developer of these alfalfa plants, Monsanto and FGI, submitted a petition to USDA-APHIS requesting that APHIS make a determination that these alfalfa plants shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 USC 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the Federal "no action" alternative, APHIS would not come to a determination that these alfalfa plants are not regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of J101 and J163 lines of glyphosate tolerant alfalfa. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of glyphosate tolerant alfalfa.

B. Determination that J101 and J163 Alfalfa Plants are No Longer Regulated Articles, in Whole

Under this alternative, these glyphosate tolerant alfalfa plants would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of glyphosate tolerant alfalfa derived from these events. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et seq*.; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 342).

C. Determination that J101 and J163 Alfalfa Plants are No Longer Regulated Articles, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (I) state that APHIS may "approve the petition in whole or in part." There are two ways in which a petition might be approved in part:

1. Approval of some but not all lines requested in the petition. In some petitions, applicants request deregulation of lines derived from more that one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others. This request is for the two events J101 and J163 and their progeny.

2. Approval of the petition with geographic restrictions. APHIS could determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In such a case, APHIS might choose to approve the petition with a geographic limitation stipulating that the approved line could only be grown without APHIS authorization in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of events J101 and J163 and its progeny in the absence of confinement.

A. Alternative A: No Action

If APHIS takes no action, commercial scale production of events J101 and J163 and their progeny is effectively precluded. These plants could still be grown in field trials for variety development as they have been for the past several years under APHIS authorizations (notifications). APHIS has evaluated field trial data reports submitted on events J101 and J163 and their progeny, and has noted no significant adverse effects on non-target organisms, no increase in fitness or weediness characteristics, and no effect on the health of other plants. The Agency expects that future field tests would perform similarly.

With respect to commercial production, if APHIS were to take no action, alfalfa growers would still have the same options available to them for weed control in their fields as they currently have. Control measures can be complicated by type of weeds (over 90 weeds were reported as being significant in alfalfa), growth stage of specific weeds, growth stage of the alfalfa, carry over effects on the following crops, and field environmental conditions. Statistics presented (Hower et al., 1999, Gianessi and Marcelli, 2000) on the usage of pesticides on the nation's alfalfa crop (seed and forage) document significant use of 19 herbicides, other than glyphosate, in the U.S. between 1988 and 1997. Planted area of alfalfa during this time period was reported to be 21,000,000 to 23,000,000 acres.

The data on the estimated use of the herbicides in alfalfa in 1997 in the following table are taken from Gianessi and Marcelli (2000) (http://www.ncfap.org/database/default.htm).

Herbicide	Application Rate (Lbs A.I./A)	Total Lbs. A.I. (1000s)
Benefin	1.2-1.35	119
Bromoxynil	0.26-0.45	37
Clethodim	0.1-0.2	4
Diruron	1.2-1.6	271
EPTC	2.6-3.5	695
Glyphosate	0.35-1.45	175
Hexazinone	0.25-1.0	316
Imazethapyr	0.03-0.11	28
Metribuzin	0.25-0.75	319
Norflurazon	1.0-1.5	43
Paraquat	0.25-0.69	355
Pronamide	1.0-1.3	24
Sethoxydim	0.11-0.5	132
Terbacil	0.50-0.64	47
Trifluralin	0.75-2.16	950
2,4-DB	0.3-1.5	389
Total		3,904

A range of application rates were reported, since recommended rates vary based on the type of weeds, environmental conditions and type of herbicide mixture.

In addition to chemical control measures, growers would also likely continue the use of mechanical and cultural practices such as mowing, tillage, burning, flash grazing and companion crops.

B. Alternative B: Approval of the Petition, in Whole

If APHIS were to grant the petition for non-regulated status in whole, alfalfa events J101 and J163 and their progeny would no longer be considered regulated articles. APHIS' assessments of the environmental impacts are discussed in the following sections.

1. Plant pathogenic properties

APHIS considered the potential for the transformation process, the introduced DNA sequences or their expression products to cause or aggravate disease symptoms in alfalfa events J101 and J163 and their progeny or in other plants. We also considered whether data indicate that unanticipated unintended effects would arise from engineering of these plants. APHIS considered information from the scientific literature as well as data provided by the developer when conducting their field trials.

Recipient organism

The plant material used for development of events J101 and J163 was FGI proprietary alfalfa clone R2336 from a high yielding, fall dormant breeding population. The initial plants, selected for tolerance to glyphosate, were designated J101 and J163, and various populations were developed from these events to provide the data presented in the petition. The breeding history and progeny resulting from events J101 and J163 can be found in Figure VI-8, p. 113 of the petition. Alfalfa is not listed as a Federal noxious weed or on other weed lists such as:

Federal Noxious Weed List (http://www.aphis.usda.gov/ppq/weeds/noxwdsa.html),
Washington State Weed Lists (http://www.nwcb.wa.gov/weed_list/weed_listhome.html),
California Weed Species Lists (http://www.extendinc.com/weedfreefeed/list-b.htm),
Montana County Noxious Weed List (http://www.weedawareness.org/weed%20list.html),
North Dakota Noxious Weeds (http://www.ext.nodak.edu/extpubs/plantsci/weeds/w1103w.htm).

Transformation system

Events J101 and J163 were developed using a disarmed (i.e. pathogenicity genes removed) *Agrobacterium*-mediated transformation system of sterile alfalfa seedling cotyledons. Post-transformation, *Agrobacterium* were eliminated from tissues by a 7-week culture on antibiotic-containing medium. Glyphosate was used to select for transformed tissues containing the *epsps* gene construct. This technique using disarmed *Agrobacterium* followed by selection has a 20-year history of safe use and has been used for transformation of a variety of plant species and tissues (Howard *et al.*, 1990).

DNA sequences inserted into alfalfa events J101 and J163

Data supplied in the petition and reviewed by APHIS (Section V.A., pp. 38-68) support the conclusion that events J101 and J163 contain the following sequences: (1) a 35S promoter from a modified figwort mosaic virus (P-FMV), (2) coding sequence for a chloroplast transit peptide from *Arabidopsis thaliana*, (3) the 5-enolpyruvylshikamate-3-phosphate synthase gene (*epsps*) from *Agrobacterium* sp. strain CP4, and (4) DNA containing polyadenylation sequences from the 3' non-translated region of the *Pisum sativum* (pea) *rbc*S E9 gene. The non-coding 35S promoter is from the plant pathogen figwort mosaic virus. This sequence, however, cannot cause plant disease and serves a purely regulatory function for the *epsps* gene. The *epsps* gene is from the soil-inhabiting bacterial plant pathogen, *Agrobacterium* sp. strain CP4. It encodes the EPSPS protein which functions to impart tolerance to the broad spectrum herbicide glyphosate. It does not cause disease and has a history of safe use in a number of deregulated genetically engineered plants (e.g., corn, cotton and soybean varieties).

Evaluation of intended effects

As expected, as a result of introduction of the *epsps* gene into the alfalfa genome, the resulting plants are tolerant to glyphosate, the active ingredient in the herbicide Roundup®.

Analysis of inheritance: Data were provided and reviewed by APHIS that demonstrate stable integration and inheritance of the *epsps* gene and its associated regulatory sequences over several breeding generations. Statistical analyses show that glyphosate tolerance is inherited as a dominant trait in a typical Mendelian manner (petition Table V-1, p. 71).

Analysis of gene expression: Data on EPSPS (5-enolpyruvylshikamate-3-phosphate synthase) protein concentrations were collected from field trials conducted at multiple locations. Using standard laboratory ELISA techniques, protein concentrations from alfalfa forage were determined (petition Table V-8, p. 97). EPSPS protein concentrations on a fresh weight basis averaged 257 µg/gram in plants with event J101, 270 µg/gram in plants with event J163, and 252 µg/gram in plants from the population containing both events J101 and J163. EPSPS enzymes are ubiquitous in plants and microorganisms and have not been associated with hazards from consumption or to the environment. Genetically engineered crops that contain this recombinant protein and have been granted non-regulated status include corn, soybean, cotton, rapeseed and sugar beet (http://www.aphis.usda.gov/brs/not_reg.html). In 2004, significant acreages of corn (10.5 million acres or 13% of the total), upland cotton (4.2 million acres or 30% of the total) and soybean (63.6 million acres or 85% of the total) grown in the U.S. were planted with herbicide tolerant varieties (http://usda.mannlib.cornell.edu/). Although the data include all herbicide tolerant varieties, glyphosate tolerant ones (containing EPSPS) predominate. All the genetically engineered glyphosate tolerant varieties have also undergone review by the FDA (http://www.cfsan.fda.gov/~lrd/biocon.html) and are allowed for food and feed use.

Analysis of the intended trait: Numerous field trials were conducted (Appendix B of this EA) to evaluate events J101 and J163 in different environments. Standard field trials evaluated (1) agronomic performance, (2) disease and pest resistance performance, and (3) seed multiplication. Agronomic practices used to prepare and maintain each field trial were characteristic of each representative region. Where the glyphosate herbicide Roundup® was used in trials, no negative impacts from application of the Roundup® were noted.

Analysis of possible unintended effects: Expression of EPSPS in events J101 and J163 alfalfa is not expected to cause plant disease or influence susceptibility of J101 and J163 or their progeny to diseases or other pests. Data addressing disease susceptibility and overall agronomic performance were collected in order to assess possible effects from introduction of the *epsps* gene and its associated regulatory sequences. The petitioner has described these trials, conducted over several years in a variety of locations, and presented these data in Section VI of the petition (starting on p. 99). Approximately 760 observations were presented in the petition from 18 location-years on susceptibility to disease, insects and weeds. All of the observations noted no differences between the populations with and without events J101 and J163. An additional 152 observations from 15 location-years were presented on abiotic stresses with no differences noted between populations with events J101 and J163 and control populations. Other phenotypic characterizations comparing J101 and J163 populations with conventional and control populations were also completed. Data were provided and assessed by APHIS on numerous characteristics related to the morphology of flowers, pollen and seed, seed germination and dormancy, seed yield, and various plant growth characteristics such as forage yield, seedling vigor, regrowth after cutting, survival, and fall dormancy. No qualitative or quantitative observations indicated any biologically meaningful differences from control populations or differences outside the range of conventional alfalfa norms.

Al-Kaff et al. (1998) have noted gene silencing effects when transgenic plants have been infected by a virus with DNA sequence homology to a portion of the introduced genes. None of the viral diseases of alfalfa is related to figwort mosaic virus (http://image.fs.uidaho.edu/vide/refs.htm and http://www.apsnet.org) (a *caulimovirus*, from which the promoter for the *epsps* gene originates), so silencing of the *epsps* gene should not occur.

In addition to field studies on agronomic parameters, Monsanto/FGI analyzed alfalfa for compositional changes as part of their submission to FDA in the consultation process. While FDA uses these data as indicators of possible nutritional changes, APHIS views them as general indicators of possible unintended changes. Compositional analyses evaluating carbohydrates, protein, ash, minerals, fiber, lignin, fat, and 18 amino acids (a total of 35 different components) identified three statistically different values compared with the control population for J101, seven statistically different values for J163, and 11 statistically different values for the paired J101 X J163 population. However, all analyses fell within the 99% tolerance interval developed from the conventional varieties grown in the same locations, providing additional evidence that J101, J163 and the paired J101 x J163 populations do not exhibit unexpected or unintended effects.

Potential Impacts on Relative Weediness of Events J101 and J163 Compared to Conventionally Bred Alfalfa

APHIS assessed whether J101 or J163 alfalfa populations are any more likely to become a weed than the non-transgenic control populations or other currently cultivated alfalfa. This assessment considers the basic biology of alfalfa and an evaluation of unique characteristics of J101 and J163 alfalfa populations.

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980; Booth et al., 2003). The parent plant in this petition, *Medicago sativa* L., is not listed as a serious weed in A *Geographical Atlas of World Weeds* (Holms et al., 1991) or as a weed in *World Weeds: Natural Histories and Distribution* (Holms et al., 1997), *Weeds of the North Central States* (http://www.ag.uiuc.edu/~vista/html_pubs/WEEDS/list.html), *Weeds of the Northeast* (Uva et al.,1997), or *Weeds of the West* (Whitson et al., 1992) nor is it listed as a noxious weed species by the U.S. Federal Government (7 CFR Part 360). Occasionally, alfalfa volunteers in fields the year after stand termination. These volunteer plants can be controlled by mechanical means or several other registered herbicides besides glyphosate. Alfalfa possesses few of the characteristics of plants that are notable of successful weeds (Baker, 1965; Keeler, 1989; Booth et al., 2003).

As part of a bilateral agreement between the United States and Canada, USDA/APHIS and the Canadian Food Inspection Agency (CFIA) have generated documents that outline basic data requirements for developers of genetically engineered plants (http://www.aphis.usda.gov/brs/international_coord.html). One of these documents, Appendix II, outlines the environmental characterization data requirements for unconfined releases. As a part of the entire package requesting a determination of non-regulated status, these data are designed to address characteristics that influence both reproductive biology and survival biology of the transgenic plant compared to its non-transgenic counterpart.

In trials conducted in the United States, no differences were observed between J101 or J163 populations and non-transgenic control populations with respect to the plants ability to persist or compete as a weed. APHIS considered data relating to plant vigor, seedling emergence, seed germination, seed dormancy and other characteristics that might relate to increased weediness. No unusual characteristics were noted that would suggest increased weediness of J101 and J163 plants. Additionally, no characteristics relating to disease or insect resistance that might affect weediness were noted. These characteristics were consistent over all field trial locations. J101 and J163 alfalfa plants are still susceptible to the typical insect and disease pests of alfalfa.

Potential Impacts from Outcrossing of Lines J101 and J163 to Wild Relatives

APHIS evaluated the potential for hybridization and gene introgression to occur from J101 and J163 to sexually compatible wild (free-living) relatives, and considered whether such introgression would result in increased weediness. Alfalfa is sexually compatible with several subspecies within the *M. sativa* complex (Small and Jomphe, 1989). The center of origin for the genus *Medicago* is generally believed to be in the Caucasus, northwestern Iran and northeastern Turkey; the genus is not native in North America (see this EA's Appendix A). An additional 18 *Medicago* species are known to be naturalized (free-living) or possibly so within the United States, of which only *M. lupulina* (black medic) is widely naturalized throughout the United States. None of these species are native to the United States, and none are sexually compatible with *M. sativa*.

Potential Impact on Threatened or Endangered Species or Non-target Organisms Including Beneficial Organisms

APHIS evaluated the potential for deleterious effects or significant impacts on non-target organisms, including those on the Federal Threatened and Endangered Species (TES) list of the U.S. Fish and Wildlife Service (FWS) (http://endangered.fws.gov/wildlife.html#Species), from cultivation of J101 and J163 alfalfa and its progeny. The gene that codes for the enzyme EPSPS that confers glyphosate tolerance is from the bacterium *Agrobacterium* sp. strain CP4. This gene is similar to the gene that is normally present in alfalfa and is not known to have any toxic property. Field observations of events J101 and J163 revealed no negative effects on non-target organisms. The lack of known toxicity for this enzyme suggests no potential for deleterious effects on beneficial organisms such as bees and earthworms. The high specificity of the enzyme for its substrates makes it unlikely that the introduced enzyme would metabolize endogenous substrates to produce compounds toxic to beneficial organisms.

A number of researchers have conducted laboratory investigations with different types of arthropods exposed to genetically engineered crops containing the CP4 EPSPS protein (Goldstein, 2003; Boongird et al., 2003; Jamornman et al., 2003; Harvey et al., 2003). Representative pollinators, soil organisms, beneficial arthropods and pest species were exposed to tissues (pollen, seed, and foliage) from genetically engineered crops that contain the CP4 EPSPS protein. These studies, although varying in design, all reported a lack of toxicity observed in various species exposed to these crops (Nahas et al., 2001; Dunfield and Germida, 2003, Siciliano and Germida 1999).

EPSPS has received an exemption from tolerance requirement from the EPA on all raw agricultural commodities (http://www.epa.gov/fedrgstr/EPA-PEST/1996/August/Day-02/pr-840.html). APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms.

From the above analysis of both qualitative and quantitative information from the petition and published data, APHIS concludes that the unconfined release of J101 and J163 and their progeny would not harm any non-target or Federally listed (or proposed) threatened or endangered species. Consistent with APHIS' U.S. Fish and Wildlife Service TES assessment requirements, this is a "no harm" decision.

Potential Impacts on Agricultural and Cultivation Practices

Current weed control practices in alfalfa can be somewhat complicated and can vary substantially among spring seedings, fall seedings, and established stands and between hay and seed production. Current practices include mechanical tillage, companion crops, mowing, flash grazing, early harvest, burning, and both pre-and post-planting application of broadleaf and grass herbicides (Hower et al. 1999). Each of these practices has its limitations and can be significantly impacted by growing conditions, soil pH, weed species, target weed size, crop size, cost, etc. Glyphosate, a non-selective herbicide (e.g., Roundup®), would provide post-planting control of most annual grass and broadleaf weeds in glyphosate tolerant alfalfa under a wide range of growing conditions. Glyphosate would control larger broadleaf weeds than currently available herbicides and allow more application flexibility when environmental conditions prevent the timely application required by some currently used herbicides. In addition, glyphosate would provide a different herbicide mode of action in the growers' crop rotation, which is important in preventing the development of herbicide resistant weeds. Glyphosate is applied like any other post-emergent herbicide used in any other crop. Glyphosate tolerant alfalfa may alter current alfalfa cultivation practices by allowing for reduced herbicide use in comparison to current practices in order to achieve the same crop yield.

Alfalfa forms a symbiotic relationship with the bacterium *Sinorhizobium meliloti* (formerly *Rhizobium melilto*), that transforms nitrogen in the atmosphere, which cannot be utilized by the plant, into a form that is useable by plants. This process of nitrogen fixation reduces the plants dependence on soil nitrogen. *Sinorhizobium meliloti* strains were able to grow on glyphosate as the sole source of phosphorus in the presence of aromatic amino acids (Liu et al., 1991), which indicates that the application of glyphosate on glyphosate tolerant alfalfa should have no effect on the nitrogen fixation process of this symbiotic relationship.

Potential Impacts on Organic Farming

The National Organic Program (NOP) administered by USDA's Agricultural Marketing Service (AMS) requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means

that are not possible under natural conditions or processes.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods.

The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic alfalfa , will be significantly impacted by the expected commercial use of this product since: (a) non-transgenic alfalfa will likely still be sold and will be available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed and labeled as glyphosate tolerant.

No transgenic varieties of alfalfa are currently in commercial production. Varieties derived from events J101 and J163 should not present new and different issues with respect to impacts on organic farmers. With the exception of seed production fields, alfalfa does not typically set viable seed in fields used to produce forage. Although these fields may produce flowers that may release pollen, the fields are typically harvested before the seed is set and allowed to mature, because high quality forage is the desired product.

Potential Impact on Sprout Production

Although alfalfa is mainly used for animal feed, humans do consume alfalfa sprouts. APHIS considered the likelihood of events J101 and J163 entering the sprouting industry. The following are the reasons why APHIS considered this use of glyphosate tolerant alfalfa to be a very low probability: (1) Only a small amount (estimated to be about 7%) of the alfalfa seed produced and marketed in the United States is used for sprouting purposes (Bass et al., 1988). (2) Glyphosate tolerant alfalfa seed will be labeled to indicate that it is glyphosate tolerant since this claim is a valuable agronomic characteristic. (3) Glyphosate tolerant alfalfa seed will use many of the conventional practices to maximize seed yield and these recommended practices include the use of pesticides during production (Hower et al., 1999). (4) The seed to be used for planting forage fields will most likely be coated with bacterial inoculant and /or fungicide and colored (petition Addendum 1). (5) Each container of glyphosate tolerant seed to be sold will have a designation (i.e. lot number) that can trace its origin to the field in which it was produced. (6) Applicants intend to produce all glyphosate tolerant alfalfa seed under production contracts that will preclude the legal sale of the seed for food use and forage producers who purchase Roundup Ready (i.e., glyphosate tolerant) alfalfa seed will be required to sign an agreement that expressly prohibits the production of

any seed. (7) The sprouting industry endorses the use of certified sprouting seed, the criteria for which include seed production practices such as field history, pesticide use and origin of seed. Each of these criteria by itself would be adequate to disqualify glyphosate tolerant alfalfa for certified sprouting purposes (DeWaal, 1998; Oregon State University, 2004; International Specialty Supply, 2004).

Potential Damage to Raw or Processed Agricultural Commodities

APHIS review of the information provided by the applicant regarding the components and processing characteristics of these plants revealed no differences in any component that could have a direct or indirect plant pest effect on any raw or processed commodity. APHIS believes that the modifications for herbicide tolerance should not affect this commodity in any significant manner.

Other Environmental Statutes and Considerations

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects. Each alternative was analyzed in its ability to affect minority and low-income populations. None of the alternatives were found to pose disproportionately high or adverse human health or environmental effects to any specific minority or low-income group.

EO 13045, “Protection of Children from Environmental Health Risks and Safety Risks,” acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency’s mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. None of the alternatives is expected to have disproportionately high or adverse human health or environmental effects to children.

EO 13112, “Invasive Species”, states that Federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. The non-engineered plant is grown in the U.S. and based on the data submitted by the applicant and reviewed by APHIS, the engineered plants are not significantly different in any fitness characteristics from their parent that might increase their invasive potential.

Potential Impacts on Biodiversity

After careful evaluation, APHIS believes that events J101 and J163 alfalfa exhibit no traits that would cause increased weediness, that their cultivation should not lead to increased weediness of other cultivated or feral alfalfa or any sexually compatible relatives, and that they are unlikely to

harm non-target organisms common to the agricultural ecosystem or Federal threatened or endangered species as recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS believes that it is unlikely that events J101 and J163 alfalfa or their progeny will pose a significant impact on biodiversity.

Consideration of Potential Environmental Impacts Associated With the Cultivation of Events J101 and J163 outside the United States

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of non-regulated status for J101 and J163 alfalfa. *Medicago sativa* is a prized forage used worldwide; the primary center of the genus is in the Caucasus, northwestern Iran and northeastern Turkey (see the EA's Appendix A). Many of the wild *Medicago* species are annuals, with which the perennial species do not hybridize or hybridize only with great difficulty under natural conditions. None of the *Medicago* species were noted to be serious weeds.

Any international trade in alfalfa subsequent to this determination would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention. In addition, issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international forums. APHIS continues to play a role in working toward harmonization of biosafety and biotechnology guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection. APHIS also participates regularly in biotechnology policy discussions at forums sponsored by the European Union, and the Organization for Economic Cooperation and Development (OECD). APHIS periodically holds bilateral or multilateral discussions on biotechnology regulatory issues with other countries (most often Canada and Mexico), and has participated in numerous conferences intended to enhance international cooperation on safety in biotechnology. APHIS has sponsored several workshops on safeguards for planned introductions of transgenic crops, most of which have included consideration of international biosafety issues. All the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new alfalfa cultivars internationally apply equally to those covered by an APHIS determination of non-regulated status under 7 CFR Part 340.

In 1992, world leaders agreed on a strategy for "sustainable development". One feature of this agreement was the Convention on Biological Diversity (CBD). The U.S. government has not ratified the CBD. One part of the CBD is the Cartegena Protocol on Biosafety, which entered into force on September 11, 2003. The Biosafety Protocol is designed to ensure an adequate level of safety in the transfer, handling and use of "living modified organisms" addressing the potential adverse effects on conservation, sustainable use of biological diversity, taking into account risks to human health. Signatory countries are required to implement a system to address these issues. The CEQ report's biodiversity considerations for incorporation into environmental impact analysis

under the National Environmental Policy Act are similar to those addressed by the Biosafety Protocol.

C. Determination that J101 and J163 Alfalfa Plants are No Longer Regulated Articles, in Part

If APHIS were to grant the petition for non-regulated status in part, alfalfa events J101 and/or J163 and their progeny would no longer be considered regulated articles, with some restriction. If an in-part determination would be a geographic restriction, all of the environmental considerations under Part B would be applicable to such a determination.

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VII. CONSULTATIONS

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Appendix A: Alfalfa biology and the potential for introgression into related species

Medicago sativa L., alfalfa (sometimes called lucerne), is a perennial herbaceous legume (Lesins and Lesins, 1979; Hill, 1987) that is grown worldwide, and is a member of the family Fabaceae (Leguminosae), tribe Trifolieae (Mabberley, 1998; Small and Jomphe, 1989; Quiros and Bauchan, 1988). Alfalfa is insect pollinated, primarily by a few bee species; the flower is specialized and only pollinated once, after which it is tripped and no longer available for insect visitors to pollinate it again (Viands et al., 1988; Hill, 1987). The mature plant has a deep taproot, making it possible to use soil moisture from depths of about 20 feet (6 meters) or more (Teuber and Brick, 1988; Barnes and Sheaffer, 1995). In addition, alfalfa forms a symbiotic relationship with *Sinorhizobium meliloti* (formerly *Rhizobium meliloti*), thereby reducing its dependence on soil nitrogen (Vance et al., 1988; Ferguson et al., 2002). Flowers develop at the shoot apex when the plant transitions from vegetative to reproductive growth, which generally takes place between the 6^{th} and 14^{th} nodes depending on genetics and the environment. The stems are indeterminate, so vegetative and reproductive growth can occur simultaneously (Teuber and Brick, 1988; Viands et al., 1988).

The Old World genus *Medicago* L. (including some *Trigonella* species) consists of about 85 species (Small and Jomphe, 1989; Bena, 2001; Mabberley, 1998), with most being annuals and one quarter or fewer being perennial herbs (and a few shrubs). Domesticated alfalfa (*M. sativa*) originated in Asia Minor, Iran, Transcaucasia and Turkmenistan several millennia ago (Quiros and Bauchan, 1988; Muller et al., 2003). It can now be found growing wild (free-living) from Spain (Muller et al., 2001) to China and North Africa to Sweden. It has also become acclimatized as a crop in South Africa, Australia, New Zealand, South America and North America. The *M. sativa* complex, which was introduced into North America early by Europeans for forage and includes all the commercial alfalfa varieties, is a group of closely related subspecies, including the cultivated *M. sativa* ssp. *sativa* and *M. sativa* ssp. *falcata* (synonym *M. falcata*) (Small and Jomphe, 1989). Of the 11 other species in *Medicago* section *Medicago* (all of which are perennials), only *M. hybrida* is found free-living in North America (Small and Jomphe, 1989; Quiros and Bauchan, 1988; Michaud et al., 1988; Kartesz, 2004).

In addition to the *M. sativa* complex within which all of the subspecies are sexually compatible to some degree, an additional 17 and possibly 18 *Medicago* species have been recognized as being naturalized (free-living) or possibly so in the United States (USDA-NRCS, 2004; Kartesz, 2004). All of these 18 species are annual species, except for the species *M. hybrida* (in *Medicago* section *Medicago*) hybrids of which have only been produced experimentally by embryo culture. No annual species are known to hybridize with *M. sativa* (Quiros and Bauchan, 1988; McCoy and Bingham, 1988; and the petition's Appendix 4).

Medicago lupulina (black medic) is the species that might be of most concern within this list of 18 species. It is considered a weed in lawns and waste places and in forages since its seeds frequently contaminate forage legume seed crops. Black medic is an annual (possibly sometimes short-lived perennial) self-pollinating species and is known to occur throughout the United States. Successful hybridizations between *M. sativa* and *M. lupulina* have been reported (Southworth, 1928; Fryer, 1930; Shrock, 1943). However, because of many subsequent experiments, there is general agreement that these putative "hybrids" were most likely not hybrids but due to self-fertilization

(Lesins and Gillies, 1972; Fridriksson and Bolton, 1963; Valizadeh et al., 1996). For more details on this topic, see Section E.1 (p. 284) and Appendix 4 of the petition. Based on all the recent data available on this subject, APHIS' opinion is that hybridization between *Medicago sativa* and *M. lupulina* has an extremely low to non-existent probability of occurring in a non-experimental or even in an experimental setting.

Alfalfa is not considered a serious weed, a noxious weed or an invasive species in the United States, even though feral (free-living) populations are fairly common and volunteers may occur in succeeding crops. Generally feral populations, many of which are along roadsides, are not a problem, and generally no attempts are made to control these populations. In some instances, these feral populations are considered advantageous and are encouraged (petition Appendix 3, p. 375, 12/31/02 Letter from South Dakota State University). More detailed information concerning feral populations of alfalfa and control of feral populations is in the petition's Section E.4 (p. 287), F.3 (p. 293) and Appendix 3 (p. 369). Alfalfa is frequently used in different crop rotations, varying with the region. The use of glyphosate tolerant alfalfa is not expected to change current crop rotation options or patterns. More detailed information on crop rotations is addressed in Section F.1 (p. 291) and Section F.4 (p. 302) of the petition. Less than 100% stand termination can result in volunteer alfalfa plants in the following crop. Therefore good stand termination procedures would still be a good method of eliminating volunteer glyphosate tolerant alfalfa plants. More detailed information on stand termination is addressed in the petition's Section B.6 (p. 259) and specifically for glyphosate tolerant alfalfa in Section E.2 (p. 292). If volunteers of glyphosate tolerant alfalfa are in a crop, management practices and recommendations to control these volunteers can be found in its Sections F.3 (p. 293) and F.5 (p. 303). Based on the available information on this subject, APHIS' opinion is that alfalfa is not an important weed in the United States, but care should be taken with other glyphosate tolerant crops that may be chosen to follow glyphosate tolerant alfalfa.

Possible movement of the transgene via pollen from events J101 and J163 to other members of the *Medicago* genus would be species and geographically specific. Movement of the transgene to plants within the *M. sativa* complex can be expected if the plants are located relatively near each other. Based on a search for *Medicago* populations in the United States (http://www.natureserve.org/explorer) 14 matches were found. All of the matches were considered to be non-native species.

APHIS believes that if the glyphosate tolerance trait moves from J101 and J163 to other sexually compatible *Medicago* species in the United States, this will not have a significant impact. Since all *Medicago* species are not native to the Western Hemisphere, there will be no impact on the natural genetic resources of these species from release in the United States. If glyphosate tolerant individuals did arise through interspecific hybridization, the tolerance would not confer any competitive advantage to these plants unless challenged by glyphosate. This would only occur in managed ecosystems where glyphosate is applied for broad spectrum weed control, or in plant varieties developed to exhibit glyphosate tolerance and in which glyphosate is used to control weeds. As with glyphosate tolerant alfalfa volunteers, these individuals, should they arise, could be controlled using other available chemical and/or mechanical means. Undesired crosses, if they developed, could potentially result in the loss of glyphosate as a tool to control them. However, this can be avoided by the use of sound crop management practices.

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Appendix B: APHIS authorizations for field tests of events J101 and J163 alfalfa

1998 Field Trials
98-093-08n

1999 Field Trials
99-047-03n

2000 Field Trials
00-040-10n
00-053-07n
00-053-14n
00-053-17n
00-055-03n
00-063-18n
00-069-04n
00-139-01n
00-139-02n
00-171-02n
00-182-04n
00-207-01n
00-243-06n
00-272-04n

2001 Field Trials
01-009-04n
01-009-05n
01-009-08n
01-010-09n
01-011-03n
01-016-33n
01-017-08n
01-017-09n
01-029-12n
01-053-08n
01-058-09n
01-058-10n
01-080-05n
01-092-07n
01-092-08n
01-107-01n
01-108-09n
01-156-01n
01-159-01n
01-163-01n
01-163-02n
01-164-01n
01-164-02n
01-164-03n
01-164-04n
01-164-05n
01-164-06n
01-164-07n
01-205-04n
01-205-05n
01-205-06n
01-211-06n
01-211-08n
01-211-09n
01-219-02n
01-236-03n
01-243-10n
01-275-02n

2002 Field Trials
02-004-12n
02-004-13n
02-004-14n
02-004-15n
02-004-17n
02-007-08n
02-010-08n
02-010-09n
02-010-10n
02-010-11n
02-011-01n
02-011-02n
02-028-29n
02-028-30n
02-044-10n
02-044-11n
02-044-15n
02-046-16n
02-046-19n
02-046-22n
02-046-24n
02-046-25n
02-046-26n
02-051-11n
02-051-17n
02-051-20n
02-051-21n
02-051-23n
02-051-24n
02-051-26n
02-051-27n
02-052-06n
02-053-04n
02-056-08n
02-056-12n
02-060-08n
02-060-09n
02-077-14n
02-077-22n
02-078-04n
02-084-19n

2002 Field Trials

02-093-09n
02-099-01n
02-105-04n
02-170-02n
02-193-02n
02-205-02n
02-206-01n
02-212-04n
02-212-05n
02-214-09n
02-220-16n
02-247-07n
02-346-12n
02-346-14n
02-346-15n
02-346-16n
02-346-17n
02-346-18n
02-352-01n
02-352-02n

2003 Field Trials

03-021-15n
03-021-17n
03-021-18n
03-021-19n
03-021-21n
03-021-22n
03-021-23n
03-022-03n
03-022-04n
03-034-30n
03-043-09n
03-043-10n
03-052-19n
03-052-21n
03-062-03n
03-062-04n
03-098-02n
03-098-03n
03-098-04n
03-098-06n
03-121-05n
03-121-06n
03-184-03n
03-184-04n
03-184-05n
03-184-06n
03-191-01n
03-191-02n
03-191-03n
03-191-04n
03-191-05n
03-191-06n
03-202-10n
03-218-01n
03-247-01n
03-247-02n
03-247-04n
03-304-03n
03-304-04n
03-304-05n
03-310-02n
03-314-03n
03-318-04n
03-318-05n
03-324-01n
03-324-02n

2003 Field Trials

03-325-01n
03-328-02n
03-345-01n
03-345-03n
03-350-01n

2004 Field Trials

04-005-01n
04-007-01n
04-013-02n
04-030-10n
04-030-14n
04-036-02n

Appendix C: Summary table of critical data submitted with petition 04-110-01p for alfalfa events J101 and J163

Molecular genetic characterization data	**Figure/ table number and page in petition**
Plasmid map of PV-MSHT4	Fig. III-1 p. 31
DNA insert diagram with restriction sites and predicted fragment sizes	Fig. V-1A p. 49, Fig. V-1B p. 50
Southern blots verifying intactness of insert, promoter, coding region, polyadenylation signal and gene copy number	Fig. V-2 p.51, Fig. V-3 p.52, Fig. V-4 p.53, Fig. V-5 p.54, Fig. V-6 p.55, Fig. V-7 p.56, Fig. V-8 p.57, Fig. V-10 p.59, Fig. V-11 p.60, Fig. V-12 p.61, Fig. V-13 p.62, Fig. V-14 p.63, Fig. V-15 p.64, Fig. V-16 p.65
Southern blots verifying stability of inheritance of the *epsps* gene over multiple generations	Fig. V-19 p.68
Western blot characterization of EPSPS protein in events J101 and J163	Fig. V-21 p.78, Fig. V-22 p.80, Fig. V-25 p.89, Fig. V-26 p.91
Statistical analysis of genetic segregation pattern of multiple generations of events J101 and J163	Table V-1, p.71
Agronomic characterization data	
Seed germination and dormancy	Table VI-2 pp.119-120, Table VI-3 p. 121, Table VI-4 pp.124-125, Table VI-5 pp.128-129, Table VI-6 p. 132, Table VI-7 p. 135
Seedling emergence and vigor	Table VI-10 pp.148-151, Table VI-11 p.152, Table VI-12 pp.153-156, Table VI-13 p.157
Vegetative growth	Table VI-10 pp.148-151, Table VI-11 p.152, Table VI-12 pp.153-156, Table VI-13 p.157, Table VI-14 p.158, Table VI-15 p.158, Table VI-17 pp.169-170, Table VI-20 p.175, Table VI-21 p.179, Table VI-23 p.181
Diseases, Insects, and Abiotic stresses	Table VI-16 pp.159-166, Table VI-18 pp.171-172, Table VI-19 pp.173-174, Table VI-20 p.175
Survival and fall dormancy	Table VI-22 p.180, Table VI-24 p.182
Flowering properties	Table VI-26 p.188, Table VI-27 p.189, Fig. VI-10 p.191, Fig. VI-11 p.192, Fig. VI-1 Fig. VI-10 p.1912 p.193, Fig. VI-13 p.194, Fig. VI-15 p.196, Fig. VI-16 p.197, Fig. VI-17 p.198, Fig. VI-18 p.199, Table VI-28 p.203, Table VI-29 p.203, Fig. VI-19 pp.204-205, Fig. VI-20 pp.206-207, Fig. VI-215 pp.208-209

Seed yield	Table VI-30 p.212, Table VI-31 p.214, Table VI-32 p.216
Plant tissue compositional analyses	Table VI-34 pp.226-243, Table VI-35 p.244, Table VI-36 p.245, Table VI-37, p.246, Table VI-38, p.247, Table VI-17, p. 82, Table VI-18, p. 83
Symbiotic organisms	Table VI-39 p.251, Fig. VI-22 p.252, Fig. VI-23 p.253
Comparisons of Roundup® with other herbicides used in alfalfa production	
Relative efficacy on a variety of weed species	Table VII-3 pp.271-272, Table VII-4 p.273, Table VII-5 pp.274-276
Herbicides used in production	Table VII-6 p. 278
Miscellaneous information	
Stand-out and volunteer control of alfalfa	Table VII-7 p.295, Table VII-8 p.296, Table VII-9 pp.297-298, Table VII-10 p.299, Table VII-11 p.300, Table VII-12 p.301
Annual Roundup Ready alfalfa use estimates	Table VII-13 pp.305-307
Glyphosate resistant weeds	Appendix 2 pp.350-368
Gene Flow in Alfalfa	Appendix 5: Table 1 p.404, Table 2 p.405, Fig. 1 p.406

Appendix D. Determination of Non-regulated Status for Roundup Ready® Alfalfa Events J101 and J163

In response to a petition (designated 04-110-01p) from Monsanto Company and Forage Genetics International, APHIS has determined that genetically engineered alfalfa transformation events J101 and J163 and progeny derived from them will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for J101 and J163 alfalfa and its progeny. Importation of seeds and other propagative material would still be subject to APHIS foreign quarantine notices at 7 CFR Part 319 and the Federal Seed Act regulations at 7 CFR Part 201. This determination is based on APHIS' analysis of field, greenhouse, and laboratory data and references provided in the petition and other relevant information as described in this environmental assessment that indicate that J101 and J163 will not pose a plant pest risk for the following reasons. (1) It exhibits no plant pathogenic properties - although a plant pathogen was used in the development of this alfalfa, these plants are not infected by this organism, nor do they contain genetic material from this pathogen that can cause plant disease. (2) It exhibits no characteristics that would cause it to be more weedy than the non-transgenic parent alfalfa line or other cultivated alfalfa. (3) Gene introgression from J101 and J163 to native, introduced, or naturalized species of *Medicago* in the United States is extremely unlikely, and it is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity any more than would introgression from other cultivated alfalfa. (4) Disease and insect susceptibility and compositional profiles of the plants of J101 and J163 are similar to those of its parent variety and other alfalfa cultivars grown in the United States, therefore, no direct or indirect plant pest effect on raw or processed plant commodities is expected. (5) Field observations, compositional analyses, and data on the safety of the engineered EPSPS protein all indicate that J101 and J163 should not have a greater potential than other cultivated alfalfa to damage or harm organisms beneficial to agriculture. (6) Compared to current alfalfa pest and weed management practices, cultivation of J101 and J163 should not reduce the ability to control pests and weeds in alfalfa or other crops. In addition to our finding of no plant pest risk, there will be no affect on threatened or endangered species resulting from a determination of non-regulated status for J101 and J163 and its progeny.

APHIS also has concluded that there may be new varieties bred from J101 and J163 alfalfa; however, they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for alfalfa descended from J101 and J163, or those observed for other alfalfa varieties not considered regulated articles under 7 CFR Part 340.

John T. Turner for Cindy Smith

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Date: 6/14/05