Approval of USDA-ARS Request (04-264-01P) Seeking a Determination of Non-regulated Status for C5 Plum Resistant to Plum Pox Virus

Finding of No Significant Impact and Decision Notice

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared a final environmental assessment (EA) prior to approving a petition (APHIS Number 04-264-01p) for a determination of nonregulated status received from USDA-ARS under APHIS regulations at 7 CFR part 340. The subject of this petition, plum line C5, is genetically engineered to express the coat protein gene of plum pox virus, which confers resistance to the virus. On May 16, 2006, APHIS published a notice in the ***Federal Register*** (71 *FR* 28296-28298, Docket no. 2006–0084) announcing the availability of the draft EA for public review and comment for the designated 60-day comment period ending on July 17, 2006. One thousand, seven hundred twenty-five comments were received and addressed, where appropriate, in the preparation of the final EA, which is attached to this document. This includes additional discussion analysis in the final EA that addresses concerns raised in public comments.

In the draft EA, APHIS considered three alternatives: Alternative A – No Action Alternative; Alternative B – Determination that C5 'HoneySweet' plum trees are No Longer Regulated Articles, in Whole; Alternative C - Determination that C5 plums are No Longer Regulated Articles, in Part. APHIS proposed Alternative B as its preferred alternative because of the lack of plant pest characteristics displayed by the C5 plum. Based upon analysis described in the final EA, APHIS has determined that the preferred alternative, to grant the petition in whole, will not have a significant impact on the quality of the human environment and no Environmental Impact Statement will be prepared regarding this decision.

Pursuant to its regulations (7 CFR part 340) promulgated under the Plant Protection Act of 2000, APHIS has determined that the C5 'HoneySweet' plum lacks plant pest characteristics and therefore this determination of non-regulated status will not pose a risk of the introduction or dissemination of a plant pest for the following reasons:

1. In assessing potential risks associated with gene introgression from the C5 'HoneySweet' plum into its sexually compatible relatives, APHIS considered two primary issues: a) the potential for gene flow and introgression; and b) the potential impact of introgression. There are few reports of the successful production of interspecific hybrids between *P. domestica* and other *Prunus* species. Additionally, the 'HoneySweet' variety of plum that is the subject of this petition is a self-incompatible variety and would be expected to have a very low chance of out-crossing with other *Prunus* species. Since the potential for gene flow and introgression are insignificant, the potential for any plant pest risk posed by gene flow and introgression from C5 'HoneySweet' plum is also insignificant.

2. The C5 ‘HoneySweet’ plum is a highly domesticated variety, and like other domesticated plums, is unlikely to thrive in unmanaged ecosystems. Therefore, it is unlikely that there would be any weed impact, nor any plant pest risk posed by a determination of nonregulated status for the C5 ‘HoneySweet’ plum variety.

3. ‘HoneySweet’ plum relies upon a RNA gene silencing mechanism for resistance to plum pox virus and does not produce the plum pox virus coat protein or any other novel proteins. Nucleic acids (*i.e.*, RNA and DNA) are present in all living organisms and are not known to have any toxic properties. Nucleic acids are considered to be “generally recognized as safe” (GRAS) by the U.S. Food and Drug Administration (FDA) (FDA 1992) and exempt from the requirement of a tolerance under the Federal Food Drug and Cosmetic Act by the U.S. Environmental Protection Agency (EPA) (EPA 2001). Because of these characteristics, there are unlikely to be any adverse impacts on non-target organisms, including beneficial organisms and threatened or endangered species.

4. The C5 ‘HoneySweet’ plum does not exhibit any traits that would be expected to cause increased weediness or invasiveness. Unconfined use of the C5 plum should not lead to increased weediness of other plum species. The C5 plum has been engineered to resist infection by plum pox virus. Other than its resistance to plum pox virus, the C5 plum does not exhibit any change in disease or pest susceptibility. Based on this analysis, there is no apparent potential for significant impact on biodiversity and no apparent plant pest risk.

5. In assessing risks posed by viral interactions, APHIS considered the potential for recombination, heterologous encapsidation and synergy. Extensive scientific knowledge is available about plum pox virus, and other members of the potyvirus group, based upon research performed around the world. Analysis of all available scientific information suggests that the likelihood of development of new viruses, or viruses with novel/altered properties is very low to non-existent. The low likelihood of risk posed by viral interactions suggests the lack of a plant pest risk in C5 ‘HoneySweet’ plum.

6. While plum pox virus distribution is currently limited in the United States, if C5 ‘HoneySweet’ plum were to be grown commercially, the impact on the environment would likely be no different than from cultivation of other domesticated plums. If, in the future, plum pox virus becomes more widespread or invasive, the C5 plum could provide a measure of resistance against the virus disease and provide growers with an alternative to other domesticated plums which are susceptible to the virus. Further, given the plum pox virus is considered an invasive species in the United States, wide-scale deployment of C5 plum could help reduce potential virus inoculum in the environment, similar to what has occurred with papaya ringspot resistant papaya in Hawaii (Gonsalves et al. 2004).

Based upon the above information, APHIS has concluded that C5 'HoneySweet' plum is not a plant pest and hereby approves APHIS petition no. 04-264-01p. Therefore, C5 'HoneySweet' plums will no longer be considered regulated articles under the regulations at 7 CFR part 340.

for Rebecca Bech
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: JUN 27 2007

Attachment to
Finding of No Significant Impact and Decision Notice
Response to Comments
APHIS No. 04-264-01p

APHIS received 1,725 comments by the close of the comment period. These comments came from state farm bureaus, organic growers, growers associations, consumer groups, agriculture support industries, academic professionals and individuals. There were 1,708 respondents that did not support granting the petition for non-regulated status to the C5 plum. The majority of the comments in opposition of deregulation were similar in content. There were 17 comments that supported deregulation.

The majority of academic researchers, as well as the state farm bureaus that submitted comments, support granting non-regulated status to the C5 plum. They state that there is a need for a plum tree that is resistant to plum pox virus and cite the lack of alternative methods of control for the disease. The majority of those who submitted comments opposing granting non-regulated status were submitted by organic grower or consumer groups, organic growers, those who favor organic agriculture or those who are opposed to genetic engineering technology in general. Many of those commentors that do not support the petition expressed concern about cross-pollination of their organic or conventional plum crops. In addition, many individuals expressed concerns that certain domestic and foreign markets may be closed to growers who cannot guarantee a non-genetically engineered product. The comments raised several issues and each is addressed below.

Several comments suggested that gene flow from the C5 plum to conventional or organic plum may impact exports of conventional or organic plum. There were a number of issues that were considered by APHIS when addressing these comments during review of the petition for non-regulated status, including: APHIS' authority; plum biology; and plum compatibility groups.

APHIS authority under the Plant Protection Act requires APHIS to assess the potential for being a plant pest by the organism under consideration for a determination of non-regulated status. In the assessment of C5 plum, APHIS used data submitted by the developer, as well as all other available scientific literature to assess whether the C5 plum is a plant pest and whether widespread deployment of C5 plum could cause an adverse plant pest effect. APHIS analysis has determined that pollen derived from C5 plums is not a plant pest.

Plum biology plays a significant role as well in the discussion of any suggested impacts on conventional or organic plum growers/exporters. Plum trees are normally propagated by grafting, not by seed. This is significant because when plum trees are pollinated and produce fruit, the flesh of the resulting fruit is solely derived from the maternal tree. Therefore, in the event that pollen flow was to occur from a transgenic plum tree to a non-transgenic plum tree, the resulting flesh of the fruit of the pollinated non-transgenic plum tree would still be non-transgenic. While the seed of the plum would be derived

from both parents, these seeds are not used for food or feed purposes because of the high levels of cyanide; nor are these seeds normally used for producing new trees, as plum trees are normally propagated by grafting, not by seed.

The final issue considered for this group of comments is the ability of plum species to hybridize. The C5 'HoneySweet' plum, is of the genus and species *Prunus domestica* L. *Prunus domestica* is not native to the United States (USDA/NRCS 2006). It will hybridize with other *Prunus domestica* L. trees if they are of a compatible group (Nyeki 1997) including the Bullace or Damson plum (not native to the United States) which is variously classified as *Prunus domestica* L. ssp. *insititia* (L.) Schneid., *Prunus* ×*domestica* L. var. *insititia* (L.) Boivin (pro nm.), or *Prunus insititia* L. (USDA/NRCS 2006).

Prunus domestica, the European or prune plum, which is the species that is the subject of this petition for non-regulated status, does not normally interbreed with other *Prunus* species such as those that are native to the United States and colloquially termed "native plum varieties." This does not exclude man-made hybrids that may be produced through artificial methods including direct application of large amounts of pollen from one species onto stigmas of another species, in vitro embryo culture, and special germination techniques for the rescue of interspecific seedlings. In a list of over 750 of "The varieties of plums derived from native American species" (Wright 1915) there were no varieties classified as hybrids of *P. domestica* and only one that was specifically bred that had *P. domestica* listed in its pedigree as a parent in a cross three generations separated from the final variety ('Alhambra').

There are few reports of the successful production of interspecific hybrids between *P. domestica* and other *Prunus* species. According to the developer of the C5 'Honeysweet' plum, one of the most extensive reports involving *P. domestica* hybrids was published by Olden (Olden 1965). In this report *P. domestica* was used as a female and also as a male parent with each of the following *Prunus* species or hybrids: *americana, besseyi, hortulana, nigra, salicina* x *americana, salicina* x *munsoniana, salicina* x *simonii, salicina, simonii* x *americana,* and *simonii* x *salicina* x *munsoniana*. In total, 35,751 flowers were cross-pollinated. From these, 210 plants were produced (0.5%) and of this total 181 were the product of hybridization of *P. domestica* with *P. nigra* (Canada plum) (96 seedlings, 0.26%) and *P. salicina* (Japanese plum) (85 seedlings, 0.23%). When *P. domestica* was used as a pollen parent, only 17 hybrid seedlings were produced. This suggests that what little gene flow may occur between *P. domestica* and the species tested would be in the direction of species pollen entering the *P. domestica* plum orchard. Transgenic *P. domestica* pollen that would leave the orchard would be much less likely to hybridize with other *Prunus* species. As part of this same study (Olden 1965), both self-compatible and self-incompatible *P. domestica* varieties were tested for their ability to hybridize with diploid plums. Of 14,857 flowers pollinated, a total of 192 seedlings were produced (1.3%). With self-incompatible *P. domestica* varieties (10 were tested, 4833 flowers pollinated), only 2 hybrid seedlings were produced (0.04%). These hybrids were from *P. nigra* while hybridizations of *P. domestica* x *P. salicina*, *P. simonii*, *P. americana*, and *P. munsoniana* failed.

'HoneySweet' is a self-incompatible variety and, therefore, from the data presented in this study it is expected to have a very low chance of out-crossing with diploid *Prunus* species. The weakness of the relatively few hybrids of *P. domestica* that were produced was also noted by Olden: "The weakness of many hybrids as traced already in the germinating seeds and stunted embryos were found not being able to break the stones and grow out. Several seedlings died soon due to weak growth and poor development and from 225 germinating seeds only 129 plants survived. A great deal of the weak hybrids derived from the cross *P. domestica* x *P. nigra* which, however, is of special interest for further breeding for hardiness." Finally, the few hybrids of *P. domestica* x *P. nigra* that were produced generally showed a low degree of fertility (Olden 1965), essentially acting as a genetic dead end.

These data, taken together, suggest that there is little chance for gene flow from genetically engineered plums to native species in the United States based on 1) very low percentages of fruit set from artificial or "forced" hybridization; 2) the tendency for hybrids to be produced using *P. domestica* as a female parent rather than as a male or pollen parent; 3) the extremely low hybridization rate with self-incompatible *P. domestica* ('HoneySweet' being self-incompatible); 4) the weakness of hybrid seedlings; and 5) the low fertility of hybrids that do survive.

One commenter in support of deregulation suggested that organic growers would not be significantly impacted by deregulation of C5 plum. Similar to the assessment provided in Section VI.7 of the final EA, this commenter discussed the issue of propagation by grafting, rather than seed, which reduces concerns about organic growers using non-organic trees. In addition, the commenter discussed that the flesh of the fruit is derived from the mother tree and because of this, even if gene flow was to occur between transgenic and organic trees, the flesh of the fruit from the organic tree would be non-transgenic. Finally, this commenter discussed the issue of wild plums versus cultivated plums. The commenter indicated that the plums that are consumed are a different species than wild plum and because of the very low level of potential cross-pollination, there would likely be no difference compared to conventional plums. APHIS agrees with these comments.

APHIS received several comments inferring that the approval of C5 plums would be a precedent-setting step by USDA, allowing for approval of other tree varieties in the future. The C5 plum has undergone an assessment of its potential for increased plant pest risk based upon the information provided by the developer, as well as all other available information. A determination of non-regulated status for C5 plum would not result in predetermination of non-regulated status for other engineered trees in the future by APHIS. Each year, developers apply for permits to conduct field trials on a wide range of plant species (including trees) with a wide range of phenotypes (ISB 2006). However, for a variety of reasons, only selected products advance to the point where a petition for non-regulated status is submitted by developers. APHIS has no influence on which particular species a developer might choose to petition for non-regulated status.

Between 1992 and 2006, APHIS has deregulated a number of genetically engineered plants, including one tree – papaya ringspot resistant papaya (USDA/APHIS 2006). Each submission/crop has been reviewed individually and a safety determination for each was based upon the characteristics of that crop/trait combination, and data provided by the developer. All future submissions will be handled in the same manner. If applications for non-regulated status for other trees are received in the future, APHIS will assess them based upon all available data that pertains to that specific crop/trait combination.

Some comments expressed concern about the genetic stability of the inserted genes in the C5 plum trees. These comments questioned the stability of these genes over the lifetime of the tree. In addition, these comments discussed the possibility of viral recombination and creation of new viruses.

As discussed in Appendix B.5 of the final EA, data provided by the developer indicates stable inheritance of the of the PPV CP gene. This conclusion is supported by laboratory data showing that inheritance of the PPV CP gene followed the expected Mendelian segregation. In addition, 10 years of field tests of C5 trees, in multiple locations and environments, have shown the virus resistance to be stable.

Section VI.5.2 of the final EA contains a discussion about the likelihood of virus recombination occurring and the resulting potential to present a plant pest risk. As discussed in the final EA, it is common in nature to find plants that are infected with multiple viruses. The presence of more than one virus in an infected plant provides the opportunity for viral interactions, including recombination. However, based upon published literature and unpublished data collected by researchers, natural development of functional recombinant viruses producing new and/or different diseases are not common.

Theoretically, virus sequences incorporated into a plant genome could generate a new virus, if a series of unlikely events were to occur. Whether such recombination could occur and whether the recombinant could pose a significant risk are the important questions. In considering this issue, two primary questions must be considered about potential recombination in transgenic viral coat protein (VCP)-expressing plants: 1) Are any recombinants formed likely to be successful in competition with parental viruses? 2) Are these recombinants likely to be different from those that naturally occur? According to Bruening (2000), it is highly unlikely given the high background of recombination known to occur naturally in mixed infections of both crop and wild plants that the risks would be any different (Bruening 2000). The 2005 EPA SAP Panel (EPA 2006) agreed and stated that, "It was agreed that recombination rarely results in an incrementally higher probability of a virus arising with new, and possibly undesirable properties."

In addition to considering the likelihood of recombination occurring, important consideration should be given to the risk posed by recombination, under the unlikely scenario that it were to occur. It is widely accepted that recombination is a significant part of virus evolution. However, the frequency of recombination that results in some significant outcome is considered to be extremely low. The 2004 and 2005 EPA SAP Panels (EPA 2004; EPA 2006) agreed that the important questions are not the relative

likelihood for recombination to occur, but rather whether recombinants in transgenic plants are different from those in non-transgenic plants and whether they are viable. The 2005 SAP Panel further stated that, "While the discussion was wide-ranging, the consequences of any viable recombination event were considered to be minimal. This conclusion was based on the fact that nearly every plant on the planet is harboring multiple virus infections with both closely related and taxonomically distinct viruses, with essentially no new viruses emerging with substantially different properties and causing wide pandemics or undesirable environmental effects. Since we have had the ability to identify viruses, essentially all new viruses that have emerged as major pathogens pre-existed and have emerged due to altered host genotypes, cultural practices, or change in climate. This conclusion is also strongly supported by the results of 10 years or more of field experience with transgenic crops. " (EPA 2006).

Therefore, based upon available scientific information, it appears very unlikely that widespread deployment of C5 plum trees would increase the likelihood for recombination to occur between the PPV coat protein and other viruses. It is even less likely that any resulting recombinant would cause new or different disease characteristics.

Somewhat related to the issue of gene stability is the issue of fitness. Several comments questioned the fitness of the C5 plum trees, relative to that of non-transformed trees. In addressing this issue, APHIS-BRS considered two primary issues: differences between the C5 plum and its non-transgenic comparator and whether plum pox virus naturally plays a role in controlling plum trees in natural settings. In terms of how the C5 plum differs from its non-transgenic comparator, the data provided by the developer shows that the only significant difference is the presence of the PPV coat protein gene in the C5 plum. Agronomic, phenotypic and composition data show that there are no significant differences that would suggest that the C5 plum would be more competitive in a natural environment than wild or non-transgenic plum trees. Currently in the United States, PPV is not considered to be widespread nor is it a virus that inhibits the growth of a significant portion of wild or cultivated plum populations. While the virus has been detected in some areas of Pennsylvania, Michigan and New York and is considered to be an invasive species (USDA/APHIS 2006), its impact to this point has been very limited geographically. Therefore, widespread use of the C5, PPV resistant trees would not be expected to alter the natural populations of plums in the United States. The only scenario in which APHIS-BRS can envision the C5 plum being more competitive than non-transgenic plum would be in a location where PPV is widely prevalent and causes significant destruction of non-transgenic trees. Given the known devastation that the virus can cause, such a result is not unexpected as the C5 trees would provide a measure of resistance against the virus disease.

Some comments questioned the need for a plum pox virus resistant tree variety. Some of these comments suggested that there was no need for a virus resistant tree when the virus was not present in the United States Other comments suggested that either traditional breeding and/or chemical control were a more appropriate means of virus control.

Recent reports have shown that PPV has again been detected in Pennsylvania, and for the first time, PPV has been detected in New York and Michigan (Agriculture 2006;

USDA/APHIS 2006; USDA/APHIS 2006). While PPV eradication efforts have been conducted in Pennsylvania for several years (USDA/APHIS 2004), eradication is not efficient and not always 100% effective. As stated in Section V.A.1 of the final EA, plum pox virus can be spread over short distances and long distances by insect vectors and infected propagative material respectively (Scorza 1994; Isac, Preda et al. 1998; Kegler, Fuchs et al. 1998; Lopez-Moya, Fernandez-Fernandez et al. 2000; Gianessi 2002; Hull 2004). Given the presence of the virus in parts of Canada that border the north-central and northeastern United States, it is likely that the virus will continually be a threat to the United States for the foreseeable future.

APHIS regulations require a determination as to whether the transgenic plant variety that is the subject of a petition for non-regulated status poses more of a plant pest risk than its non-transgenic counterpart. While the APHIS assessment does not focus solely on whether or not the transgenic variety fulfills a particular perceived or actual need, such issues can be taken into consideration when assessing the potential for increased plant pest risk. In the case of the C5 plum, PPV is considered to be an invasive species by USDA. Given the invasive status of the virus and the lack of other effective control measures, it is possible that the C5 plum, if commercialized, could play a role in control or eradication of PPV in the United States

Finally, as discussed in the final EA, greater than 50 years of traditional breeding for plum pox disease resistance has had only limited success (Fuchs, Gruntzig et al. 1998; Hartmann 1998; Minoiu, Maxim et al. 1998; Paprstein and Karesova 1998; Ravelonandro, Scorza et al. 2000; Moustafa, Badenes et al. 2001; Gianessi 2002). In addition, unlike other plant pests, plant viruses cannot be controlled by chemical or biological pesticides. While insecticides have been used to help control insect vectors of plant viruses, these insecticides can only help slow down the spread of a virus disease from plant to plant. Virus infected plants are not impacted by application of pesticides.

Other comments questioned APHIS' consideration of potential impacts on bees and animals. Some of the same comments questioned whether the potential impact on consumers of honey was considered. APHIS considered data submitted by the developer, as well as numerous other scientific resources in its assessment of potential impacts on non-target organisms, including bees and other pollinator species (see Section VI.3 of the final EA). In this assessment, consideration was given to whether wide-spread cultivation of C5 plum would pose a greater risk than its non-transgenic counterpart.

Available data shows that the C5 plum differs genetically from its non-transgenic counterpart only in the addition of the coat protein gene from PPV. Nucleic acids, RNA and DNA, are essential components of all life-forms. Nucleic acids are non-toxic and non-allergenic. There have not been reports in available scientific literature that suggest that nucleic acids can cause adverse effects on non-target organisms, including pollinators. The EPA has exempted residues of nucleic acids that are part of plant-incorporated protectants (PIPs) from the requirement of a tolerance because of their known safety - they are non-toxic and ubiquitous in all foods and feeds (EPA 2001).

Therefore, based on the knowledge that non-target organisms will only be exposed to non-toxic RNA, there is virtually no potential for adverse effects to non-target organisms.

Further, the developers of C5 plum have shown through laboratory and field studies that the C5 plum does not produce any virus coat protein or other non-native proteins. Plant viruses are ubiquitous in the environment which provides opportunities for constant exposure to non-target organisms (Hull 2004). While production of the PPV coat protein would not significantly increase the risk of adverse effects on non-target insects and animals, the lack of any coat protein production in the C5 plum further supports APHIS' conclusions that there is not an increased risk for non-target organisms.

Finally, as discussed in Section VI.8 of the final EA, APHIS' analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the plums indicate no significant differences between C5 plum and non-transgenic counterparts that would be expected to cause either a direct or indirect adverse effect on non-target organisms. Field trials conducted over a 10 year period with the C5 plum in both the United States and Europe have not shown any observable significant differences between the C5 plum and non-transgenic controls. Because of this, there is no scientific reason to expect that the transformed C5 plum trees themselves would have a negative impact on non-target organisms. In addition to the APHIS-BRS environmental assessment, the developer is in the process of completing a consultation with the FDA for a food and feed safety analysis.

Other comments questioned the safety of the small RNAs that are responsible for providing resistance in C5 plum. One comment specifically points out the use of RNA interference (RNAi) gene therapy and its potential for adverse human health effects. As discussed above, the safety of nucleic acids is widely accepted. Both RNA and DNA are part of all food products that we consume. Further, given that plant viruses infect a tremendous amount of the fruits and vegetables that we consume, it is highly likely that humans have been exposed to the same or similar viral RNA that may be expressed in a coat-protein expressing plant. In terms of the concern about RNAi used in gene therapy, such RNA's would be specifically designed and intended for targeted use in humans, and they would be significantly different than those found in the C5 plum.

A few comments expressed concern about the presence of the *nptII* kanamycin resistance marker gene in the construct inserted into the C5 plum and its potential transfer to soil bacteria and then to animal pathogens. As discussed in Appendix B.2, the *nptII* gene is a commonly used marker gene and is found in soil-inhabiting *Escherichia coli*. These bacteria are not plant or human pathogens, and do not cause disease symptoms or the production of infectious agents in plants. If such a transfer was going to occur, the presence of the *nptII* gene in normal populations of soil-inhabiting *E. coli* would provide ample opportunity for such. This conclusion is supported in opinions developed by both the U.S. FDA and the European Food Safety Authority.

In 1998, FDA developed draft guidance on the use of antibiotic resistance markers in transgenic plants (http://www.cfsan.fda.gov/~dms/opa-armg.html). In this document,

FDA concluded that the likelihood of such transfer of antibiotic resistance is remote. In this draft guidance the FDA stated, "FDA acknowledges that the likelihood of transfer of an antibiotic resistance marker from plants to microorganisms in the gut or in the environment is remote and that, such transfer, if any, would likely be insignificant when compared to transfer between microorganisms, and in most cases, would not add to existing levels of resistance in bacterial populations in any meaningful way."

Further, to evaluate safety, the European Food Safety Authority reviewed the antibiotic selection markers used in genetically engineered plants (http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html). In this 2004 document, various antibiotic resistance genes were assigned into groups based on the criteria of therapeutic use in humans and in animals and presence in the environment; Group I is composed of kanamycin and hygromycin resistance genes. The opinion states that because of the frequency of horizontal transfer plants to other organisms is very rare, previous existence in the environment and the history of use of the kanamycin resistance, that there is no rationale for restricting Group I antibiotics.

Several comments suggested that there has been no short-term or long-term safety testing or feeding trials for toxicity and other potential adverse effects of the genes inserted into the GE plum trees on human or animal health. Section II.A and II.B discuss the roles of USDA and FDA as part of the coordinated framework for regulation of genetically engineered organisms and products. The USDA is responsible for ensuring that the organism or product does not cause an increased plant pest risk. The FDA assesses the food and feed safety of products produced through genetic engineering. In addition, the EPA is responsible for assessing the safety of the pesticidal product to ensure that it will not cause unreasonable adverse effects on the environment. The EPA is also responsible for assessing the safety of the pesticidal product found in food and feed.

As discussed above, C5 plums express the coat protein gene from PPV. Based upon molecular biology, composition, and field data, the C5 plums differ from their non-engineered counterparts only by addition of this one gene. There is no scientific reason to expect that the PPV coat protein gene could have adverse effects on living organisms. APHIS' analysis of the composition of the plum fruit did not reveal any differences between the C5 plum and its non-transgenic comparators that would suggest the risk of adverse effects. Finally, a complete food safety analysis of the C5 plum will be conducted by FDA as part of their biotechnology consultation process.

Several comments in support of granting non-regulated status suggested that use of the C5 trees could act to reduce pest pressure by reducing the potential inoculum of PPV and reduce the need/use of pesticides. APHIS agrees with these conclusions. When consideration is given to the limited host range of PPV, if plums were removed as the primary source/reservoir of the virus, then the potential for disease spread would be significantly reduced. In some areas of the world where virus disease is significant enough, some growers use pesticides to control the insect vectors of plant viruses. While use of pesticides has no impact on the viruses themselves, they can in some instances, reduce the spread of plant viruses. Therefore, if C5 trees were used in areas of high pest

and insect pressure, there could be a reduction in the amount of pesticides used to control the insect vectors of PPV.

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USDA/APHIS Final Environmental Assessment

In response to USDA-ARS Petition 04-264-01P seeking a Determination of Non-regulated Status for C5 Plum Resistant to Plum Pox Virus

OECD Unique Identifier ARS-PLMC5-6

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

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I. Summary

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 04-264-01p) from USDA, Agricultural Research Service (USDA-ARS). The petition requested a determination of non-regulated status for genetically engineered (transformed) ARS-PLMC5-6 plum (*Prunus domestica* L.) derived from their transformation event C5 (referred to hereafter as C5 plum). The genetically engineered C5 'HoneySweet'[1] plum (*Prunus domestica)* was developed to resist infection by plum pox virus (PPV). This C5 plum is currently a regulated article under USDA regulations at 7 CFR part 340, and as such, interstate movements, importations, and field tests of C5 plum have been conducted under a permit issued by APHIS (Permit #95-205-02r). USDA-ARS petitioned APHIS requesting a determination that C5 plum does not present a plant pest risk and that C5 plum and its progeny derived from crosses with other non-regulated plum should no longer be regulated articles under these APHIS regulations.

II. Introduction

Plum pox (also referred to as Sharka disease) is the most devastating virus disease in plums and other *Prunus* species and considered an invasive species in the United States. Once established, plum pox virus can occur over a broad area and cause significant losses, with estimates of 100 million stone fruit trees in Europe currently infected (APSnet 2000). While disease severity can vary between plum cultivars, the impact on susceptible cultivars can result in 80-100% loss of yield (APSnet 2000). In plums and other *Prunus* species, fruit deformation is a characteristic of disease infection. In addition, other virus symptoms can appear on leaves, fruits, flowers, and seeds. Leaves and fruit can show yellow (chlorotic) and brown (necrotic) ring patterns, as well as yellow bands or blotches (APSnet 2000).

C5 plum was developed by using genetic engineering techniques to introduce the plum pox virus (PPV) coat protein (CP) gene into plum trees. Incorporation of the PPV-CP gene into the plum via *Agrobacterium*-mediated transformation does not cause plant disease, but rather enables C5 plum to resist infection by PPV. The PPV-CP gene was introduced into the plum as part of genetic construct that also included two plant-expressible genetic marker genes, *nptII and uidA (gus).* These marker genes enable researchers to easily select those plant tissues that have been successfully transformed with the genetic construct.

PPV coat protein gene expression in C5 plum is under the control of the cauliflower mosaic virus (CaMV) 35S promoter, however, expression of the PPV coat protein gene in C5 plum does not result in production of PPV coat protein. The DNA regulatory

[1] C5 plum has been patented under the name 'HoneySweet' US PP15,154 P2.

sequences derived from the plant pathogens *Agrobacterium tumefaciens* and CaMV cannot cause plant disease by themselves or in conjunction with the genes that they regulate in the C5 plum.

Analysis of the C5 plum shows that it exhibits the characteristics of resistance based upon gene silencing. Multiple years of field trials of C5 and other transgenic plums have been conducted in both the United States and Europe. These field trials have provided evidence that C5 plum resistance to plum pox disease is both effective against the major serotypes of PPV and stable under field conditions.

In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR part 372), this EA has been prepared for C5 plum in order to specifically address the potential for impact to the human environment through the unconfined cultivation and use in agriculture of the regulated article.

A. USDA 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 regulations and is also a plant pest, or if there is reason to believe that it is a plant pest. These plum trees have been considered regulated articles because they were genetically engineered with regulatory sequences and a viral coat protein gene derived from plant pathogens.

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.

B. Food and Drug Administration (FDA) Regulatory Authority

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. USDA-ARS has submitted a food and feed safety and nutritional assessment summary to FDA for the C5 plum.

III. PURPOSE and NEED

The developer of the C5 plum trees, USDA-ARS, submitted a petition to USDA-APHIS requesting that APHIS make a determination that these plum trees shall no longer be considered regulated articles under 7 CFR part 340. Under regulations in 7 CFR part 340, APHIS is required to give a determination on the petition for nonregulated status. APHIS has prepared this EA before making a determination on the status of C5 plum as regulated articles under APHIS regulations.

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 § 1b; 7 CFR part 372).

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the Federal "no action" alternative, APHIS would not alter the current regulatory status of the C5 plum. Under this alternative, C5 plum trees would continue to be regulated articles under the regulations at 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of C5 plum trees. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of plum trees engineered to express the coat protein of PPV. Under this alternative, the petition would be denied.

B. Determination that C5 plum trees are No Longer Regulated Articles, in Whole

Under this alternative, C5 plums 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 plum pox virus resistant plum derived from this transformation event. APHIS might choose this alternative if there were sufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of plum trees engineered to express the coat protein gene of PPV and marker genes (*nptII* and *gus*).

C. Determination that C5 plums are No Longer Regulated Articles, in Part

The regulations at 7 CFR § 340.6(d)(3)(i) state that APHIS may "approve the petition in whole or in part." APHIS might approve a petition in part if this partial approval would mitigate a potential plant pest risk. Appropriate conditions and/or limitations would be

placed on the importation, interstate movement, and environmental release of C5 plum trees to mitigate plant pest risks.

Preferred Alternative

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

Alternative Eliminated from further Consideration

APHIS has eliminated Alternative C from further consideration based upon the analysis included in this EA. APHIS has utilized all available scientific information, in addition to data supplied by the developer, to reach a determination that there is not likely to be a plant pest risk posed by potential widespread deployment of the C5 'HoneySweet' plum trees. Because APHIS is not able to envision a scenario where mitigation of any plant pest risk posed by these trees would be necessary, selection of Alternative C would not provide an outcome different from selection of Alternative B, the preferred alternative.

V. Affected Environment

Plum species (*Prunus domestica*) are found native throughout the Northern Hemisphere with descriptions of plum dating back 2000 years (OECD 2002). The OECD Consensus Document on *Prunus* species <http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)13> provides a thorough overview on the biology of plum.

Prunus domestica (European or common plum) is an apparent natural alloploid between *P. cerasifera* which is diploid and *P. spinosa* which is tetraploid (OECD 2002). Many *P. domestica* cultivars are self-incompatible and may be cross-incompatible or cross-compatible. Pollen spread normally occurs via insect vectors (e.g., bees). Pollen of *Prunus* species is normally not spread by wind, and self-pollination typically requires mechanical intervention of insects (OECD 2002). Most cultivated *Prunus* species (e.g., peach, nectarine, etc.) are diploid and do not naturally hybridize with *P. domestica* which is hexaploid (OECD 2002). This does not exclude man-made hybrids that may be produced through artificial methods including direct application of large amounts of pollen from one species onto stigmas of another species, in vitro embryo culture, and special germination techniques for the rescue of interspecific seedlings. In a list of over 750 of "The varieties of plums derived from native American species" (Wright 1915) there were no varieties classified as hybrids of *P. domestica* and only one that was specifically bred that had *P. domestica* listed in its pedigree as a parent in a cross three generations separated from the final variety ('Alhambra'). While the *Prunus* OECD Consensus Document reports that sterile hybrids are normally produced between peach (*P. persica*) and *P. domestica*, there are reports of successful crosses between apricot *(P. armeniaca)* and other plum groups with *P. domestica* (OECD 2002).

While it is physically possible, introgression[2] between cultivated *Prunus* sp. and wild relatives has been rarely seen (OECD 2002). Escapes of cultivated *Prunus* sp. are frequently found in woods, pastures, and abandoned orchards, but intercrosses with wild populations are very unlikely because wild plums are extremely different in morphology and adaptation. In other words, hybrids could only be expected to survive in a protected environment (OECD 2002). Gene flow[3] to naturalized *Prunus* species in the United States is limited because of ploidy differences (Table 3, page 18-19 of petition) and the limited success of interspecific hybrids produced through controlled breeding.

According to the developer of the C5 'HoneySweet' plum, one of the most extensive reports involving *P. domestica* hybrids was published by Olden (Olden 1965). In this report *P. domestica* was used as a female and also as a male parent with each of the following *Prunus* species or hybrids: *americana, besseyi, hortulana, nigra, salicina* x *americana, salicina* x *munsoniana, salicina* x *simonii, salicina, simonii* x *americana,* and *simonii* x *salicina* x *munsoniana*. In total, 35,751 flowers were cross-pollinated. From these, 210 plants were produced (0.5%) and of this total 181 were the product of hybridization of *P. domestica* with *P. nigra* (Canada plum) (96 seedlings, 0.26%) and *P. salicina* (Japanese plum) (85 seedlings, 0.23%). When *P. domestica* was used as a pollen parent, only 17 hybrid seedlings were produced. This suggests that what little gene flow may occur between *P. domestica* and the species tested would be in the direction of species pollen entering the *P. domestica* plum orchard. Transgenic *P. domestica* pollen that would leave the orchard would be much less likely to hybridize with other *Prunus* species. As part of this same study (Olden 1965), both self-compatible and self-incompatible *P. domestica* varieties were tested for their ability to hybridize with diploid plums. Of 14,857 flowers pollinated, a total of 192 seedlings were produced (1.3%). With self-incompatible *P. domestica* varieties (10 were tested, 4833 flowers pollinated), only 2 hybrid seedlings were produced (0.04%). These hybrids were from *P. nigra* while hybridizations of *P. domestica* x *P. salicina*, *P. simonii*, *P. americana*, and *P. munsoniana* failed.

'HoneySweet' is a self-incompatible variety and, therefore, from the data presented in this study it is expected to have a very low chance of out-crossing with diploid *Prunus* species. The weakness of the relatively few hybrids of *P. domestica* that were produced was also noted by Olden: "The weakness of many hybrids as traced already in the germinating seeds and stunted embryos were found not being able to break the stones and grow out. Several seedlings died soon due to weak growth and poor development and from 225 germinating seeds only 129 plants survived. A great deal of the weak hybrids derived from the cross *P. domestica* x *P. nigra* which, however, is of special interest for further breeding for hardiness." Finally, the few hybrids of *P. domestica* x *P. nigra* that

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[2] Introgression is the introduction of genes from one species into the gene pool of another via sexual crossing. The process begins with hybridization between the two species, followed by repeated backcrossing to one of the parent species.

[3] The spread of genes from one population to another by the movement of individuals, gametes, seeds, or spores.

were produced generally showed a low degree of fertility (Olden 1965), essentially acting as a genetic dead end.

These data, taken together, suggest that there is little chance for gene flow from genetically engineered plums to native species in the United States based on 1) very low percentages of fruit set from artificial or "forced" hybridization; 2) the tendency for hybrids to be produced using *P. domestica* as a female parent rather than as a male or pollen parent; 3) the extremely low hybridization rate with self-incompatible *P. domestica* ('HoneySweet' being self-incompatible); 4) the weakness of hybrid seedlings; and 5) the low fertility of hybrids that do survive.

A. Plum Pox Virus and Pathogen Derived Resistance

Plant viruses are ubiquitous in the environment and represent a significant threat to global agriculture because of their ability to reduce the quality and, more importantly, the yield of food and fiber crops (Matthews 1991; AIBS 1995; Hadidi, Khetarpal et al. 1998; Pappu 1999; Gonsalves, Gonsalves et al. 2004). Plant virus diseases cause damage to fruits, leaves, seeds, flowers, stems, and roots of many important crop species (OECD 1996). Hundreds of plant viruses have been described, affecting a wide range of plants and trees (ICTV 2005). These viruses infect virtually every plant species, and under natural conditions, certain plant viruses are nearly always present on particular crop or weed hosts (OECD 1996; Waterhouse 2001). The severity of virus infection can vary depending upon location and from one growing season to the next (OECD 1996).

Despite some diversity in size, shape and host range, plant viruses are very simple organisms that have small genomes and contain a small number of genes (Matthews 1991; OECD 1996; Goldbach, Bucher et al. 2003). Most viruses are composed of proteinaceous coatings called capsids that contain either RNA or DNA genomes. Some capsids may also contain carbohydrates and lipids (Matthews 1991; OECD 1996; Goldbach, Bucher et al. 2003). This proteinaceous coat plays an important role in protecting the genetic material, as well as in insect vector specificity and virus movement inside plants (Callaway, Giesman-Cookmeyer et al. 2001; Culver 2002).

Most plant viruses are obligate parasites that move from plant to plant via vector-mediated transmission[4] (Matthews 1991; OECD 1996). Plant viruses can also be spread in a number of other ways, depending upon the virus type, including seed transmission, pollen transmission, and/or mechanical[5] transmission (Matthews 1991; OECD 1996). In some agricultural regions, certain crop species cannot be grown effectively because of the persistent presence of infected plant populations and/or potential virus vectors (OECD 1996). In other areas around the world, chemical pesticide sprays are used to help control

[4] Vector-mediated transmission can include: insects (e.g., aphids and whiteflies), nematodes, mites, and fungi.

[5] Mechanical transmission can include: intentional transfer of infected plant sap or purified virus in solution, vegetative propagation, infected host tissue, or contaminated equipment.

insect vectors, but while these pesticide sprays provide the only means of relief, they are both expensive and not very effective in controlling virus disease spread (OECD 1996).

1. Plum Pox Virus

Plum pox virus is the causal agent of plum pox or Sharka disease, which is the most serious viral disease of plum and other *Prunus* species including: peach, apricot, nectarine, sweet cherry and sour cherry (Dunez 1988; Lopez-Moya, Fernandez-Fernandez et al. 2000p; Ravelonandro, Scorza et al. 2000; Moustafa, Badenes et al. 2001; Gianessi 2002; Manganaris, Economou et al. 2003). Two major strains, or subgroups (PPV-M and PPV-D), and two minor strains of PPV (PPV-EA and PPV-C) have been identified (Glasa 2005). The PPV-EA and PPV-C strains represent a geographically-limited isolate (Egypt) and an isolate that is naturally able to infect cherries, respectively (Glasa 2005). Glasa and Candresse also report that there may be both a third major and third minor subgroup of PPV (Glasa 2005).

Plum pox virus can be spread over short distances, such as from tree to tree or orchard to orchard, via several species of aphid vectors. Aphids transmit PPV in a non-persistent manner[6] and therefore can acquire the virus from an infected tree and transmit the virus to a healthy tree only within a few minutes (Scorza 1994; Isac, Preda et al. 1998; Kegler, Fuchs et al. 1998; Lopez-Moya, Fernandez-Fernandez et al. 2000; Gianessi 2002; Hull 2004). This is especially important when considering reports which estimate that between 50,000 and 300,000 aphids can visit a single fruit tree within a one year period (Gianessi 2002). The virus can also be transmitted over both short and long distances through infected propagative material (i.e., budwood), which represents the primary source of PPV inoculum (Scorza 1994; Isac, Preda et al. 1998; Kegler, Fuchs et al. 1998; Lopez-Moya, Fernandez-Fernandez et al. 2000; Gianessi 2002).

Infected trees exhibit leaf and fruit chlorosis, fruit deformation, premature fruit drop, and in co-infections with other *Prunus*-infecting viruses, tree decline (APSnet; Moustafa, Badenes et al. 2001; Gianessi 2002). Since the disease was originally reported in Bulgaria (Atanassov 1932; Gianessi 2002; ICTV 2005) the virus has spread throughout Europe, where it is considered to be the most serious disease affecting stone fruit production and has destroyed more than 100 million trees (Lopez-Moya, Fernandez-Fernandez et al. 2000; Ravelonandro, Scorza et al. 2000; USDA/APHIS 2000; Moustafa, Badenes et al. 2001). More recently, the virus has spread to and caused significant damage in Asia, South America and North America (Levy 2000; Thompson 2001; Boulila 2004). Other than eradication of infected trees, there are no measures available to treat a PPV infection. Once a tree becomes infected with PPV, it can serve as a reservoir

[6] In non-persistent aphid transmission, the viruses are acquired rapidly from plants (i.e., seconds), maintained in the aphid stylet, and can only be transmitted for a very short period of time (usually minutes) Hull, R. (2004). Matthew's Plant Virology. San Diego, CA, Elsevier Acadamic Press.

for virus transmission to other trees. This could be especially important in cases where a tree is tolerant of PPV infection and is not removed because of a lack of PPV symptoms (Minoiu, Maxim et al. 1998; Gianessi 2002).

In the United States, where PPV is considered an invasive species (Clinton 1999; USDA/APHIS 2006), PPV-D was first detected in 1999 in Adams County, Pennsylvania (USDA/APHIS 2000; USDA/APHIS 2004). Since that time, local (Pennsylvania Department of Agriculture) and federal (USDA-APHIS) identification, control and eradication efforts have shown the virus to be limited to about 1600 acres of trees in three counties in Pennsylvania (USDA/APHIS 2004). Despite the relatively small affected area in Pennsylvania, eradication efforts have exceeded $40 million there (USDA/APHIS 2000; USDA/APHIS 2004). In addition to Pennsylvania, PPV has recently been detected in both New York and Michigan (USDA/APHIS 2006; USDA/APHIS 2006).

In Canada, where the disease is more widespread, the Canadian government has instituted a new seven year plum pox eradication program that essentially renewed the original three year eradication program that began in 2000 (CFIA 2005). This new Canadian program began in April 2004, with an initial allocation of Can$85 million from the Canadian government for plum pox virus eradication.

Currently, plum pox disease prevention relies upon the use of certified virus-free planting material in addition to quarantine and eradication of infected materials. Greater than 50 years of traditional breeding for disease resistance has had only limited success (Fuchs, Gruntzig et al. 1998; Hartmann 1998; Minoiu, Maxim et al. 1998; Paprstein and Karesova 1998; Ravelonandro, Scorza et al. 2000; Moustafa, Badenes et al. 2001; Gianessi 2002). In cases where resistance has been identified, the resistance is controlled by multiple genes, which makes it very difficult to breed into new varieties (Gianessi 2002). If disease develops, the only control measure is tree destruction. However, eradication is not always a simple task. PPV is known to infect more than 30 *Prunus* species, as well as other plant species, all of which could potentially serve as reservoirs of the virus making eradication of the virus extremely difficult (Kegler, Fuchs et al. 1998; Ravelonandro, Scorza et al. 2000; Moustafa, Badenes et al. 2001; Damsteegt 2004; Scorza 2005).

2. Pathogen Derived Resistance

In general, the tools available for plant virus disease control are limited, as is their effectiveness in most instances. In cases where plants are susceptible to viruses, common control or management strategies have relied upon ineffective conventional measures of disease control such as use of virus-free planting material, vector control, or eradication (Gooding 1985; Superak, Scully et al. 1993; Swiezynski 1994; OECD 1996; Khetarpal, Maisonneuve et al. 1998). Unlike other agricultural pests (e.g., insects), there are no chemical control measures that can be used directly to prevent or control plant virus disease outbreaks (OECD 1996; Hadidi, Khetarpal et al. 1998; Pappu 1999).

As an alternative approach, the concept of pathogen-derived resistance (PDR) was described about two decades ago (Sanford and Johnston 1985; Grumet, Sanford et al.

1987). Pathogen-derived resistance is based upon the use of pathogen-derived genes to generate specific host resistance (Goldbach, Bucher et al. 2003). One form of PDR is cross-protection which was first identified in 1929 (McKinney 1929) and involves intentional inoculation of crop plants with a closely related mild virus strain (Gooding 1985; Fulton 1986; Sherwood 1987; Beachy 1999; Goregaoker, Eckhardt et al. 2000; Culver 2002; Abbas M. 2005). Prior infection with a protecting or mild strain of a virus can prevent or interfere with infection by a related, more severe strain of the virus (Gooding 1985; Fulton 1986; Sherwood 1987; Beachy 1999; Goregaoker, Eckhardt et al. 2000; Culver 2002; Abbas M. 2005).

The mechanisms for cross protection have been determined to be either RNA-based or protein-mediated. RNA-based cross protection likely results from a gene silencing (post transcriptional gene silencing—PTGS) mechanism that targets viral RNA for destruction (Angell and Baulcombe 1997; Jan, Pang et al. 1999; Goregaoker, Eckhardt et al. 2000; Savenkov and Valkonen 2001; Culver 2002; Lacomme, Hrubikova et al. 2003; Lu, Martin-Hernandez et al. 2003; Baulcombe 2004; Chang, Chen et al. 2005). Protein-mediated cross protection likely relies upon several different mechanisms, including interference (Sherwood 1987; Beachy 1999; Goregaoker, Eckhardt et al. 2000; Culver 2002). This interference relies upon the coat protein of the mild strain of a virus to properly associate with and block disassembly of a more virulent strain of a virus, thus preventing replication and hence infection by the more virulent strain of the virus (Culver 2002).

In recent years, much of the research and development for plant virus disease control has focused on development of transgenic virus resistant plants. Building upon the concept of PDR and mechanisms previously described for cross protection, genetic modifications of host plants and trees are made that allow for expression of viral genes or proteins. Plant expression of viral genes or proteins often acts to delay or prevent infection by the same or related viruses. This form of PDR was first accomplished in 1986 by Roger Beachy and colleagues (Abel, Nelson et al. 1986) in which tobacco plants engineered to express tobacco mosaic virus (TMV) coat protein were resistant to TMV infection.

Since the initial successful development of a virus resistant transgenic plant, numerous other virus resistant plants and trees have been developed and field tested (Tepfer 2002; ISB 2006). Over the past 15 plus years, nearly 900 virus resistant plants and trees have been authorized by USDA-APHIS for field testing in the United States. Some of these crops have been deregulated by APHIS and grown commercially in the United States, including plants that express viral coat protein genes (e.g., papaya ringspot virus resistant papaya and ZW-20 squash) or a replicase protein gene (potato leafroll luteovirus resistant potato) (EPA 1998; Gonsalves 1998; ISB 2006). Most of this virus resistance is based on the pathogen-derived resistance, and most often using VCP or VCP gene expression as the basis for resistance (Tepfer 2002; ISB 2006).

In the early 1990's, several researchers expressed PPV coat protein in transgenic plants (mostly tobacco) to determine if expression of PPV coat protein would provide an effective tool to combat plum pox disease development (Ravelonandro, Monsion et al. 1992; Ravelonandro, Monsion et al. 1993; Wypijewski, Musiao et al. 1995). Based upon

this and other previous experience with transgenic virus resistant plants, transgenic plum was developed by Scorza and colleagues (Scorza 1994). The mechanism for resistance in the C5 plum was determined to be RNA-based (PTGS) (Scorza, Callahan et al. 2001; Hily, Scorza et al. 2004; Hily, Scorza et al. 2005). C5 plum trees do not produce detectable PPV coat protein and have shown stable and effective resistance to each of the major serotypes of PPV in field tests that have been conducted in three European countries over the past eight years (Scorza, Callahan et al. 2001; Hily, Scorza et al. 2004; Hily, Scorza et al. 2005).

APHIS authorized the first field testing of these plum trees in 1995 and they have been field tested in the United States under APHIS authorization (APHIS Permit # 95-205-02r) in subsequent years. No virus inoculations were allowed for field trials because of the invasive nature of this virus. However, field testing performed in the three European countries (Spain, Poland, & Romania) under appropriate permits from each country, included virus challenge experiments. C5 plum and its progeny have been evaluated extensively to confirm stability and that they exhibit the desired agronomic characteristics and do not present a plant pest risk. Field tests have been conducted in agricultural settings under physical and reproductive confinement conditions.

VI. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of C5 plum and its progeny in the absence of confinement.

1. Potential impacts from gene introgression from C5 plum into its sexually compatible relatives.

In assessing the risk of gene introgression from C5 plum into its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression; 2) the potential impact of introgression.

Despite the low likelihood of introgression into relatives of C5 plum, consideration was given to what potential impact introgression could have on the environment if it was to occur. In the case of C5 plums, the primary concern is that transgene introgression would result in a domesticated, wild or weedy relative of plum becoming invasive because its acquired virus resistance (Tepfer 2002; Stewart, Halfhill et al. 2003; Fuchs, Chirco et al. 2004(a); Fuchs, Chirco et al. 2004(b)). To consider this potential risk, several aspects of virus and plant biology should be considered.

In general, gene flow from cultivated agricultural crops to domesticated, wild or weedy relatives has most likely occurred ever since the domestication of a particular crop, assuming sexually compatible species are present (Stewart, Halfhill et al. 2003; Fuchs, Chirco et al. 2004(a)). Gene flow also can occur between virus resistant transgenic crops and non-transgenic crops (Fuchs, Chirco et al. 2004(a)). What is not as well understood is how much gene flow from a transgenic virus resistant crop to a wild or weedy relative, results in introgression of the gene(s), and what ecological impact this introgression

would have. Stewart et al (2003) and others, discuss the basic difference between gene flow, such as through pollen, and introgression of genes, as well as the frequency of introgression (NRC 2000; Stewart, Halfhill et al. 2003; Fuchs, Chirco et al. 2004(a)). Based upon currently available data, introgression is not a frequent event. There have been a relatively low number of confirmed cases of introgression from non-transgenic crops to their wild relatives (Stewart, Halfhill et al. 2003; Fuchs, Chirco et al. 2004(a)).

Even if it was shown that gene flow and introgression could occur with C5 plum, there is no clear evidence that shows the introgression of a virus resistance transgene into a plum relative would be any different than introgression of a naturally-occurring virus resistance gene from a non-transgenic plum (Tepfer 2002; Fuchs, Chirco et al. 2004(a)). Further, there is no evidence that indicates that a weedy plant would become more competitive, if it gained virus resistance via gene flow from VCP-expressing plants (EPA 2004). This is because, as discussed earlier, plant viruses are obligate parasites, and because of this, total destruction of their plant hosts would lead to the extinction of that virus (EPA 2004). It is assumed that there is a certain level of tolerance by some hosts – probably wild and weedy hosts – that allow for persistence of the virus. In fact, many virus infections do not produce visible symptoms in weeds (Falk and Bruening 1994; EPA 2004). Because of this, there likely exists a number of wild or weedy plant species that contain resistance genes that allow these plants to survive virus infection and serve as reservoirs for the virus (Raybould, Maskell et al. 1999).

This is somewhat different than the relationship between cultivated crops and plant viruses. Most of the major crop species used in today's agriculture (e.g. soybean, rice, wheat, beans) have been subjected to intense artificial selection over centuries and only have low survival under most natural conditions. The vast majority of the crops used in agriculture are much less fit, under natural conditions, than wild or weedy plants. Because of this, the impact of virus infection is potentially more severe than with some wild or weedy plants.

Finally, as mentioned earlier, PPV is an invasive species in the United States and has been the focus of significant eradication efforts. These eradication efforts, while successful in Pennsylvania, have been very expensive and were conducted on a relatively small scale – the efforts only involved three counties in Pennsylvania (USDA/APHIS 2000; USDA/APHIS 2004). Eradication efforts in Canada have been much more complicated because of the more widespread occurrence of the disease (Canadian Food Inspection Agency 2005). Similar difficulties have also been encountered in other parts of the world where PPV is present. Therefore, even though it is very unlikely that gene flow and introgression of the PPV-CP resistance gene into plum relatives will occur, the net impact of introgression could be positive. This is because a critical part of virus disease development and spread is the availability of hosts or reservoirs for the virus. In the case of PPV, if related tree species were to become resistant to PPV, the result could be a reduction in potential virus reservoirs and hence an increase in the potential for disease control. Based on this, choosing Alternative B, granting non-regulated status may decrease the overall incidence of plum pox infection in cultivated and wild plants.

If APHIS chooses the no action alternative (Alternative A), APHIS would continue to regulate the environmental release of this resistant plum. There would be fewer plum pox resistant trees in the environment. The potential reduction in the plum pox reservoir would not occur. When plum pox re-enters the United States the resulting impact will be unchanged from its current state.

2. Potential impacts based on the relative weediness of C5 plum

P. domestica is not described as a weedy species and none of the *Prunus* species that may be sexually compatible with *P. domestica* are described as weedy species. In addition, plum 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).

Because *P. domestica* is not described as a weedy species and none of the *Prunus* species that may be sexually compatible with *P. domestica* are described as weedy species, there would be no weed impact from deregulating this variety (Alternatives B and C). If APHIS chooses the no action alternative (Alternative A) there would also be no weed impact from this variety.

3. Potential impact on non-target organisms, including beneficial organisms and threatened or endangered species

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 United States Fish and Wildlife Service (FWS) (http://endangered.fws.gov/wildlife.html#Species), and species proposed for listing from cultivation of C5 plum and its progeny.

APHIS first considered an analysis that was performed by the developer to determine if there were changes to insect fauna associated with trees expressing the PPV-CP or marker genes associated with the C5 plum. Data presented in Table 8 (page 66 of the petition) indicates that there was no correlation between insect damage and the transgenic or non-transgenic plum trees used in those field trials.

APHIS further considered the biology of the C5 'HoneySweet' plum trees with respect to their potential to affect non-target organisms such as beneficial insects (including pollinators such as bees), and biocontrol organisms. The C5 plum does not express detectable coat protein from PPV, which eliminates concern of protein exposure to non-target organisms. Even if C5 did express viral coat protein, however, this would not increase the issue of potential impacts to non-target organisms as the PPV coat protein is not known to have any toxic properties. Plant viruses are ubiquitous in the environment and cause damage to fruits, leaves, seeds, flowers, stems, and roots of many important crop species (Matthews 1991; AIBS 1995; Hadidi, Khetarpal et al. 1998; Pappu 1999; Gonsalves, Gonsalves et al. 2004). Hundreds of plant viruses have been described, affecting a wide range of plants and trees (ICTV 2005). These viruses infect virtually every plant species, and under natural conditions, certain plant viruses are nearly always present on particular crop or weed hosts (OECD 1996; Waterhouse 2001). Viral coat proteins are therefore routinely ingested by virtually all mammals when virus-infected fruits and vegetables are consumed. The small-interfering RNAs (siRNA) responsible for the PTGS resistance mechanism in C5 plum are also not of concern. Nucleic acids are a normal part of every living organism and do not have toxic or allergenic properties. Further, nucleic acids are considered to be "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (FDA) (FDA 1992) and exempt from the requirement of a tolerance under the Federal Food Drug and Cosmetic Act by the U.S. Environmental Protection Agency (EPA) (EPA 2001). Because of the ubiquitous nature of plant viruses, likelihood of previous exposure and the lack of protein production, the likelihood of impact on non-target organisms is virtually non-existent.

The *nptII* and ß-glucuronidase genes are commonly used marker genes found in soil-inhabiting *E. coli* bacteria. These bacteria are not plant or human pathogens, and do not cause disease symptoms or the production of infectious agents in plants. In addition, these marker genes are not known to cause adverse effects to non-target organisms and both have been granted exemption from the requirement of a tolerance by EPA for use in or on all raw agricultural commodities (EPA 1994; EPA 2001).

In addition to the analysis of potential impact to non-target organisms described above, APHIS also considered potential impact on TES. In this analysis, APHIS considered the biology of the C5 'HoneySweet' plum trees, as well as typical agricultural practices associated with cultivation of plum. As mentioned previously, the C5 'HoneySweet' plum tree differs from non-transgenic plum only in the expression of the RNA sequence representing the CP of PPV that is responsible for virus resistance. These C5 'HoneySweet' plum trees do not express additional proteins, natural toxicants, allelochemicals, pheromones, hormones, or other chemicals that could directly or indirectly result in killing or interfering with the normal growth, development, or behavior of a TES or endangered species or species proposed for listing. Further, data submitted on the composition of the C5 'HoneySweet' plum fruit indicate that these plums are not significantly different from non-transgenic plums and would not be expected to have any impact on TES that would be different from non-transgenic plum. The C5 'HoneySweet' plum is not sexually compatible with a federally listed TES or a species proposed for listing. Finally, cultivation of C5 'HoneySweet' plum is not

expected to differ from typical plum cultivation. As such, plum orchards are typically highly managed agricultural areas that would be expected to be dedicated to orchard production for many years. Plum trees do not typically grow in unmanaged habitat and would not be expected to invade and/or persist in the natural environment.

As required by Section 7 of the Endangered Species Act, APHIS has analyzed the best available data and has reached a determination that granting a petition to deregulate C5 plums will have "no effect" on Federally listed threatened and endangered species or designated critical habitat or habitat proposed for designation. Consequently, no written concurrence or consultation with FWS is required for this analysis.

4. Potential impacts on biodiversity

Analysis of available information indicates that C5 plum exhibits no traits that would cause increased weediness, that its unconfined cultivation should not lead to increased weediness of other cultivated plum or other sexually compatible relatives, and that it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the FWS. Based on this analysis, there is no apparent potential for significant impact to biodiversity. If APHIS chooses the no action alternative there would also be no impact on biodiversity.

5. Potential for viral interactions and development of new viruses

APHIS has considered the physical and biological properties of PPV and its interactions with both its insect vectors and its host plants. PPV is considered to be an invasive species in the United States (Clinton 1999; USDA/APHIS 2006) and has been the focus of an eradication program since it was first detected in the United States in 1999 (USDA/APHIS 2000; USDA/APHIS 2004). While PPV is not currently present in the United States, the aphid vectors for PPV are widely prevalent in the United States in areas where plums are grown.

1. Heterologous Encapsidation

Heterologous encapsidation occurs when the coat protein of one virus is able to encapsidate the nucleic acid of a second virus. Heterologous encapsidation was first described by Rochow (1970) and has been the subject of numerous reviews (Rochow 1977; Falk and Duffus 1981; Falk, Passmore et al. 1995; Miller, Koev et al. 1997; Tepfer 2002). In some cases, these two or more viruses may be related, while in other scenarios, the viruses may be completely unrelated (Falk, Passmore et al. 1995; Tepfer 2002). The majority of heterologous encapsidation interactions that have been identified involve luteoviruses (Rochow 1977; Falk, Passmore et al. 1995; Miller, Koev et al. 1997). These interactions occur naturally in both agricultural crop and weed plants, and are a natural part of virus-virus and virus-plant interactions (Rochow 1977; Falk and Duffus 1981; Falk, Passmore et al. 1995). In some cases, heterologous encapsidation is a specific interaction between two viruses that plays an important role in both virus biology and

survival (such as in the case of helper-dependent transmission[7]) (Falk, Passmore et al. 1995).

In the case of C5 plum, the potential for heterologous encapsidation is essentially non-existent. Data on the C5 plum shows that the mechanism of resistance is based upon PTGS. Therefore, because it appears that no PPV coat protein is produced in these trees, there is essentially no potential for C5 plum expressed PPV-CP encapsidating RNA from other plant viruses.

2. Recombination

It is theoretically possible for new plant viruses to arise in the C5 plum through recombination and APHIS has considered this issue in its evaluation of this petition. Recombination is defined as the exchange of nucleotide sequences between two nucleic acid molecules (USDA/APHIS 1996; USDA/APHIS 1999). Recombination between viral genomes can result in heritable, permanent change (USDA/APHIS 1996; USDA/APHIS 1999). The persistence of the recombined viral genome depends upon its fitness with respect to its ability to replicate within the original host cell, its ability to replicate in the presence of the parental viruses, its ability to spread systemically within the host, and its successful transmission to other host plants.

Recombination events in plant viruses contribute to evolution of the viral genome (Falk and Bruening 1994; Gibbs and Cooper 1995; Roossinck 1997; Aaziz and Tepfer 1999; Rubio, Borja et al. 1999; Worobey and Holmes 1999; Tepfer 2002). RNA-RNA recombination occurs between closely related RNA molecules, but also between dissimilar RNAs, possibly at sites of similar RNA structure (Falk and Bruening 1994; Roossinck 1997).

Under normal agricultural conditions, plant viruses have numerous opportunities to interact genetically (Falk and Bruening 1994). Multiple or mixed infections, where more than one virus infects a crop or weed host, are common in nature. Some reports have shown five or more different viruses infecting the same plant (Falk and Bruening 1994; Falk, Passmore et al. 1995; EPA 2004). Falk and Bruening suggest that these mixed infections probably occur more frequently than what has been reported and have likely already brought together numerous combinations of virus genes (Falk and Bruening 1994). Therefore, under natural field conditions, it is possible for viruses that cannot systemically infect a particular plant to interact with viruses that are capable of systemic infection (Falk and Bruening 1994). Although there is potential for these viruses to continuously interact under natural settings, new viral diseases are normally due to minor variants of existing viruses as opposed to new viruses resulting from recombination (Falk and Bruening 1994). The idea of new variants arising from existing viruses, and being responsible for virus diseases is strongly supported by the level of variability that occurs within individual viruses (Falk and Bruening 1994; Gibbs and Cooper 1995; Roossinck

[7] Helper-dependent transmission often involves a virus that lacks a coat protein becoming encapsidated into the coat protein of another virus allowing for subsequent insect transmission of the coat protein-lacking virus.

1997; Aaziz and Tepfer 1999; Rubio, Borja et al. 1999; Worobey and Holmes 1999; Tepfer 2002).

According to Bruening (2000), it is highly unlikely given the high background of recombination known to occur naturally in mixed infections of both crop and wild plants that the risk of recombination would be any different in transgenic plants (Bruening 2000). Most scientific literature suggests that such an event would be a rare occurrence (Falk and Bruening 1994; USDA/APHIS 1999; EPA 2004). In further considering this issue, one must also consider what risk such a recombination event would pose. Given that recombination is widely accepted as a significant part of virus evolution and that multiple viruses are commonly found in a single plant providing ample opportunity for interaction, the likelihood that transgenic viral coat protein-expressing plants present a greater risk to the environment is low.

Plum pox virus is a member of the potyviridae which is a large group of RNA plant viruses that infect a wide range of plant species (Matthews 1991; ICTV 2005). Other than PPV, there have not been other reports of potyviruses infecting *Prunus* species (Hull 2004; ICTV 2005). Therefore, while there have been reports of recombination between PPV strains (Glasa 2001; Glasa 2002), the lack of potential potyvirus interactions occurring in the C5 plum suggests that the likelihood of recombination between the PPV-CP and other potyviruses in C5 plum trees is very low. Further, most of the viruses that occur in *Prunus* species in Europe also occur in the United States, and there have not been reports of recombination events between PPV and other viruses in Europe under natural conditions and where the C5 trees have been tested. Based upon what we know about the biology of plant viruses, and data that we have gathered from Europe, the likelihood of recombination events between the C-5 plum expressed PPV-CP and other plant viruses is very low.

3. Synergy

Synergy occurs when two independent viruses infect a plant simultaneously and the resulting disease symptoms are more severe than when either virus infects the plant individually (Matthews 1991; OECD 1996; Pruss, Ge et al. 1997; Tepfer 2002). Synergistic infections typically result in agronomic problems, producing diseased, unmarketable crops, rather than environmental impacts. Their occurrence would not likely be any different in transgenic crops than in naturally mixed infections (USDA/APHIS 1996).

Several naturally-occurring synergistic virus interactions have been described, with the majority of the combinations involving at least one potyvirus (Rochow and Ross 1955; Vance 1991; Vance, Berger et al. 1995; OECD 1996; Pruss, Ge et al. 1997; Tepfer 2002). Vance and colleagues have shown that when plants are co-infected by both a potyvirus (e.g., potato virus Y virus – PVY; tobacco vein mottling virus – TVMV; pepper mottle virus - PeMV) and potato virus X virus (PVX), the disease symptoms are significantly worse than plants infected with either of the viruses alone (Vance 1991; Vance, Berger et al. 1995). In addition to the change in disease symptoms, there was a significant increase in PVX virus particles without any corresponding increase in PVY virus particles (Vance 1991).

While there is potential for synergistic interactions to occur between PPV and other viruses, there is no evidence to suggest that potyviral coat protein genes alone are involved in synergy. Therefore, it is unlikely that use of C5 plum would increase the potential for synergistic interactions.

6. Potential impacts on commercial use

If APHIS takes no action, commercial scale production of C5 plum and its progeny is effectively precluded. These trees could still be grown under APHIS permit as they have been for the past several years. However, widespread, unconfined use of the trees would not be allowed as long as the C5 plum is considered a regulated article. APHIS has evaluated field trial data reports submitted on this event and 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 if these trees were grown under permit in the future, that they would perform similarly. If APHIS were to grant the petition for non-regulated status in whole, C5 plum and its progeny would no longer be considered regulated articles. The unrestricted cultivation and distribution of C5 plum would be allowed and would not be subject to regulation by APHIS under 7 CFR part 340.

From a commercial perspective, current methods for control of this virus are both ineffective and expensive. The USDA-APHIS began an eradication program in 2000 in an effort to remove PPV infected trees in three counties in Pennsylvania. While this eradication program appears to have been successful, it was expensive, and was conducted on a relatively small scale as the virus had been detected in these three counties in Pennsylvania (in addition to the initial presence of PPV in Pennsylvania, there have been more recent reports of virus-infected trees in Pennsylvania, Michigan and New York). As mentioned earlier, eradication efforts in Canada have been much more expensive and more complicated given the widespread nature of the virus. The presence of the virus in Canada, near the Canada-United States border, presents a potential long-term challenge for plum growers in the United States. If C5 plum was no longer considered a regulated article (Alternative B), it could add a potentially more effective, cheaper and preemptive means of control of an invasive species in the United States. The C5 plum trees could be used in breeding programs throughout the United States and progeny of C5 plums could be grown on a large-scale basis without confinement restrictions that are imposed in release permits.

In addition, if PPV becomes more widespread in the United States, the C5 trees would not only provide resistance to the virus, but could potentially reduce PPV virus inoculum to levels where impacts of the virus on nontransgenic plum production (traditional and/or organic) could be greatly reduced. Such a scenario has taken place in Hawaii following widespread deployment of papaya ringspot virus- (PRSV) resistant transgenic papaya (Gonsalves, Gonsalves et al. 2004). Gonsalves noted that “it is critical that Hawaii continues to produce nontransgenic papaya to supply the market in Japan (Gonsalves, Gonsalves et al. 2004).” Because of this, in 1999, the Hawaii Department of Agriculture began a program to ensure production of nontransgenic papaya that took advantage of the reduced virus inoculum levels provided by the transgenic papaya (Gonsalves, Gonsalves

et al. 2004). Gonsalves states, "The goal of this strategy was to reduce initial infection rates and secondary virus spread, thus slowing the PRSV epidemic in the Kahuwai management area" (Gonsalves 2003). Further, based upon observational data from Hawaii, Gonsalves (2004) states, "Although definitive experiments have not been carried out, it seems that transgenic papaya can provide a buffer zone to protect nontransgenic papaya (from PRSV) that are planted within the confines of the buffer."

Given that PPV is a member of the same potyvirus group as PRSV, use of the C5 plum in the United States, particularly in areas impacted by PPV, could provide similar results as seen in Hawaii for papaya. Traditional or organic growers of not only plums, but other stone fruits (e.g., peaches, apricots, cherries, etc.) that are hosts of PPV could benefit from the reduction in inoculum levels provided by the C5 plum. Whether this is accomplished by using buffer zones of C5 plum or simple widespread deployment of the C5 plum, the C5 plum potentially could aid in reducing virus inoculum levels, allowing continued production of nontransgenic varieties of stone fruits.

Therefore, if APHIS were to take no action (Alternative A), and growers do not have PPV resistant varieties of plum trees derived from C5 plum, they would likely have to rely upon cultural practices to reduce the potential impact of PPV. USDA-APHIS-Plant Protection and Quarantine (USDA/APHIS/PPQ) conducted an environmental assessment (EA) in 2000 to assess the potential impact of a PPV eradication program in Pennsylvania (USDA/APHIS 2000). In this EA, PPQ described the limited effectiveness of using cultural practices to control PPV and reached a determination that the adverse effects of selecting the no action alternative to PPV eradication could have significant environmental impacts (USDA/APHIS 2000). Other than eradication and use of clean propagative material, there are no other effective control measures for plum pox. If the disease were to occur in the United States with wider geographical distribution than has been seen in Pennsylvania, and as has occurred in Canada and Europe, the disease could cause devastating losses to both commercial and private stone fruit trees. As stated in the USDA plum pox eradication environmental assessment document (USDA/APHIS 2000), a widespread plum pox infestation could greatly reduce the plum supply, a valuable agricultural commodity.

Plum pox virus has been shown to have a host range that includes ornamental and wild *Prunus* species, some common weeds (clover and lamb's quarters) as well as some garden plants (tomatoes, petunias and zinnias) (USDA/APHIS 2000). These infected trees and plants could serve as hosts for the virus and reservoirs for further spread of the virus. Therefore, while the occurrence of the disease in the United States has been limited to date, there is significant potential for widespread impact on a much larger scale if the virus were to be re-introduced into the U.S in the future, which could occur given the close proximity of the disease in Southern Canada.

Field tests conducted over the past eight or so years have shown the C5 plum trees to be resistant to infection by PPV, even under conditions of high disease pressure. Further, the PPV resistance has been shown to be stable and inheritable. Despite the fact that the PPV-CP gene is derived from a plant pathogen, the coat protein gene itself cannot cause plant disease. The data provided in this petition indicate that the mechanism for

resistance is based upon PTGS. Because of the lack of protein production, there would be no adverse effects from protein exposure and no potential for heterologous encapsidation. The potential for synergy and recombination would be low. While PPV is not currently widely prevalent in the United States, there is a tremendous amount of knowledge about potyviruses. In addition, most of the viruses related to PPV that occur in the United States also occur in Europe and other areas where PPV is more widespread, yet there have not been any reports of new or more pathogenic viruses/diseases developing from their interactions with PPV. Finally, as discussed previously in this EA, gene transfer from C5 plum to naturalized *Prunus* species is limited due to ploidy differences and the limited success of interspecific hybrids produced through controlled breeding.

Another issue considered by APHIS is the use of insecticides for control of insect virus vectors. As discussed previously, while there are no chemical treatments that can be used to directly control plant viruses, use of insecticides to attempt to control plant virus vectors is used in some instances. However, such insecticide use is both ineffective and expensive (OECD 1996; Hadidi, Khetarpal et al. 1998; Pappu 1999). Nevertheless, insect vector control is often the only means of providing any relief against virus infection. If PPV were to become widespread in the United States and no new method for control of the virus is developed, insecticide use could increase in and around plum and other stone fruit production areas. Therefore, APHIS expects the use of the C5 plum might also prevent increased pesticide use in stone fruit production areas.

7. Potential impacts on non-adopters[8]

It is not likely that farmers, including organic farmers, who choose not to plant transgenic plum varieties or sell transgenic plum, will be significantly impacted by the expected commercial use of this product. Nontransgenic plum will likely still be sold and will be readily available to those who wish to plant it. Plum trees are normally propagated by grafting, not grown from seed. If USDA-ARS receives regulatory approval from all appropriate agencies, it will make the C5 'HoneySweet' plum available to growers or breeders. ARS plans to license this plum variety labeled as 'Honey Sweet' plum pox virus resistant plum. As with other varieties of plums, growers or breeders obtaining budwood or grafted plants will inquire about the genetic background of this plum variety and therefore know that this product is a transgenic PPV resistant plum.

It is important to note that the flesh of plum fruit is exclusively derived from the maternal tree and the cells of the flesh are genetically identical to the cells of the maternal tree (Esau 1965). Therefore, even in the extremely rare instance that cross pollination was to occur between a transgenic C5 tree and a receptive non-transgenic tree, the resulting edible portion of the plum fruit (i.e., flesh) of the non-transgenic tree would contain no transgenic cells. Although the plum seed (not including the stone surrounding the seed because the stone is maternal), resulting from the cross pollination described above,

[8] Includes organic and other plum growers who choose not to grow genetically engineered plum.

would contain transgenic cells, plum trees are not propagated from seeds. Instead, plum trees are clonally propagated from another tree of the desired variety. A twig from such a tree is either induced to produce its own roots (not normally done commercially), or it is grafted onto an existing rootstock. The plum seed is essentially a waste product of the pollination: it is used neither as food nor to produce new plum trees. In fact, plum seeds are toxic. Most importantly, cross pollination does not alter the genetic background of the tree receiving pollen from a C5 tree.

Therefore, the biology of plum trees and the method used for their propagation ensure that in the unlikely event of a cross pollination of an organically produced tree, the edible portion of the fruit and the tree itself cannot be genetically altered (Esau 1965). Additionally, as discussed in part 6 above, the adoption of the C5 plum tree should result in a decrease of the overall amount of plum pox virus in conventional orchards that may lower the likelihood of an organic orchard becoming infected, an important benefit to organic plum producers.

This particular product should not present new and different issues than those associated with non-transgenic plum, with respect to impacts on non-adopters. APHIS has considered that gene transfer to naturalized *Prunus* species in the United States is limited because of ploidy differences (Table 3, page 18-19 of petition), a lack of documented natural outcrossing and the limited success of interspecific hybrids produced through controlled breeding.

If APHIS chooses the no action alternative there would be impacts on organic or other non-transgenic plum farmers similar to those impacts on commercial use discussed in part 6 above, since the current cultivation practices are unlikely to change. As also discussed above, in the absence of plum pox resistant plum, the opportunity for plum pox to establish in plum orchards is greater. This may provide more routes to infect organic orchards.

8. Potential impacts on raw or processed agricultural commodities

APHIS analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the plums indicate no significant differences between C5 plum and non-transgenic counterparts that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of line C5. In addition, as discussed earlier, the only addition to the C5 plum is the coat protein gene from PPV. This nucleic acid is not unlike all other nucleic acids that are considered to be "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (FDA) (FDA 1992) and exempt from the requirement of a tolerance under the Federal Food Drug and Cosmetic Act by the U.S. Environmental Protection Agency (EPA) (EPA 2001). Finally, the C5 plum is currently undergoing review by the FDA for use in food and feed (http://www.cfsan.fda.gov).

9. Cummulative Impacts

APHIS considered whether the proposed action could lead to significant cumulative impacts, when considered in light of other past, present, and reasonably foreseeable

future actions, regardless of what agency or person undertakes such actions. Typically, fruit tree production occurs on land that can be dedicated to an orchard for 20 or more years. As with most orchard tree production, continuous production of plum would normally include the use of resources to limit the growth of weeds, limit the potential impact caused by insects, animals or disease, and to maximize production. Widespread use of C5 'HoneySweet' plum is expected to have an insignificant impact on typical plum production. The virus resistance trait of these trees will help limit the impact of PPV, if the virus becomes widely-established in the United States. However, other than the CP gene (nucleic acid) of PPV, the C5 'HoneySweet' plum will not produce any other substance that is not normally produced by plum trees, nor is the composition of the fruit produced by these trees significantly different from unmodified plum. Therefore, APHIS does not expect accumulation of a novel substance in soil, nor does APHIS expect impacts on organisms living in and around these orchards because of exposure to the C5 'HoneySweet' plum.

Data supplied by the applicant, including results of 10 years of field tests in various environments, suggest that the C5 'HoneySweet' plum trees have not had observable or measurable impacts on the ecosystems in which they have been allowed to grow. Based upon available information, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create significant cumulative impacts or significantly reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the ecosystem in which C5 'HoneySweet' plum is planted.

VII. CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

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.
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. Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or 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. Non-engineered plum is widely grown in the United States. Based on historical experience with non-engineered plum and the data submitted by the applicant and reviewed by APHIS, the engineered plant is sufficiently similar in fitness characteristics to other plum varieties currently grown and it is not expected to have an increased invasive potential.

Introduction of C5 plum trees results in the introduction of a genetic portion of plum pox virus, which is considered an invasive species in the United States (USDA/APHIS 2002; USDA/APHIS 2006). However, the coat protein gene of PPV cannot itself cause disease. In addition, the PPV-CP gene expressed in C5 plum could provide a means of resistance to the PPV which supports EO 13112 to "provide for their control and to minimize the economic, ecological…..impacts that invasive species cause".

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the United States, its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should non-regulated status be determined for C5 plum or if one of the other alternatives is chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new plum cultivars internationally, apply equally to those covered by an APHIS determination of non-regulated status under 7 CFR part 340. Any international traffic of C5 plum subsequent to a determination of non-regulated status for C5 plum would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control" (https://www.ippc.int/IPP/En/default.jsp). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (137 countries as of April 2005). In April, 2004, a standard for pest risk analysis (PRA) of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). Under these standards, the C5 plum would be classified as an LMO. The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through

biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 132 countries are Parties to it as of March 6, 2006 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, US exporters will still need to comply with domestic regulations which importing countries that are Parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the US Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (http://usbiotechreg.nbii.gov). This data will be available to the Biosafety Clearinghouse. APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States and in the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the United States, Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea. Many countries, e.g. Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, the United Kingdom, and the European Union have already approved biotech varieties to be grown or imported for food or feed (http://www.agbios.com/dbase.php).

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IX. PREPARERS AND REVIEWERS

Biotechnology Regulatory Services
Cindy Smith, Deputy Administrator
Rebecca Bech, Associate Deputy Administrator for Emerging and International Programs

Permits and Risk Assessment Staff
Neil Hoffman, Ph.D., Division Director

Michael Watson, Ph.D., Supervisory Biotechnologist (Preparer of EA)
John Cordts, M.S. Biotechnologist (Reviewer)

BRS, Policy and Coordination Division
John Turner, Ph.D., Director
Rebecca Stankiewicz-Gabel, Ph.D., Regulatory Analyst (Reviewer)
Terri Dunahay, Ph.D. Lead Agriculturist (Reviewer)

X. AGENCY CONTACT

Ms. Cynthia Eck, Document Control Officer
USDA, APHIS, BRS
4700 River Road, Unit 147
Riverdale, MD 20737-1237
Phone: (301) 734-0667
Fax: (301) 734-8669
Cynthia.A.Eck@aphis.usda.gov

Appendix A: Summary table of data submitted with petition 04-264-01p for C5 Plum

Appendix B: Summary of petition data and information considered in completing environmental assessment

1. Description of Transformation System:

The *Agrobacterium* transformation system used to develop C5 plum has been previously described by Mante et al. and Scorza et al. (Mante 1991; Scorza 1994). Transformation with *Agrobacterium* should not lead to crown gall disease in C5 plum because the *Agrobacterium tumefaciens* strain was disarmed by removing the native T-DNA from C58/Z707. The native T-DNA, which contains the plant hormone genes necessary for the formation of crown gall tumors, was replaced by the PPV-CP cassette. Further, antibiotics were used to kill any remaining *Agrobacterium* after transformation.

The C5 plum was transformed using the previously described binary plasmid pGA482GG (Fitch 1990; Ling 1991). This plasmid was also used in the previously deregulated papaya ringspot virus resistant papaya (APHIS, 1996). The pGA482GG plasmid contains the *nptII* and *uidA* (gus) marker genes, as well as tetracycline and gentamicin antibiotic resistance genes. The *nptII* gene is under control of the nopaline synthase promoter (nos) and nos terminator. The *uidA* gene is under control of the 35S promoter and nos terminator. The tetracycline and gentamicin marker genes are under control of prokaryotic promoters and therefore are not expressed in plants. In addition to these intact genes, pGA482GG contains an interrupted *ß*-lactamase gene. Sequencing analysis show that this gene is interrupted by a *cos* site that renders the gene non-functional.

The PPV-CP gene cassette, containing the 35S promoter, from plasmid pBIPCP (Ravelonandro, Monsion et al. 1992) was subcloned into *Hind*III-digested pGA482GG and the resulting plasmid was designated pGA482GG/PPV-CP-33 (see Figure 1, page 20 of Petition for schematic diagram of the PPV-CP cassette). This plasmid was used to electrotransform *Agrobacterium tumefaciens* strains C58/Z707. This is the same *A. tumefaciens* that was previously used in the deregulated papaya ringspot resistant papaya (USDA/APHIS 1996). The transformed *A. tumefaciens* was grown overnight at 28°C in 10 ml Luria broth with 50 µg/ml kanamycin and 50 µg/ml gentamicin; centrifuged at 4000 x g for 10 min; resuspended in 10 ml bacterium resuspension medium (Murashige 1962) with 2% sucrose, 100 µM acetosyringone and 1 mM betaine phosphate; and shaken for 6 hr at 20°C before use.

2. Characterization of DNA inserted into C5 plum

A series of analyses were conducted to characterize the DNA inserted into C5 plum, including Southern blot analysis and DNA sequencing. Briefly, DNA was isolated from C5 plum, four other putatively transformed plums (C2-C4 & C6), and non-transformed 'Bluebyrd' plum. DNA was digested with restriction enzymes *Bam*HI and *Eco*RI. Southern blot analysis of *Bam*HI digested C5 DNA show the expected 1.2 kb fragment, in addition to a second, larger fragment (> 2kb). The developers suggest that this larger than expected fragment likely resulted from a rearrangement. DNA signal intensity analysis suggests that the C5 contains between 1 and 4 copies of the PPV-CP gene (Figure 4, page 26 of petition). Southern blot analysis of EcoRI digested C5 DNA

showed the expected 7 kb fragment, along with other larger and smaller fragments, which suggest multiple insertions of the PPV-CP gene (Figure 4, page 26 of petition).

Further analysis was performed to more fully characterize the PPV-CP insert in C5 plum. DNA from the C5 plum was digested with *Eco*RI, *Hind*III, and *Bam*HI and analyzed by Southern blot analysis using either the1 kb from the PPV-CP gene, the 1.1 kb fragment from the *nptII* gene, or the 0.8 kb fragment from the *uidA* gene as a probe. Figure 5 (page 27 of the petition) shows the results of the *Eco*RI digest. Each of the digestions showed that the full-length PPV-CP gene was incorporated into the C5 plum genome.

In addition to the Southern analysis of the PPV-CP insert, a bacterial artificial chromosome (BAC) library was developed from C5 plum and sequenced. Because of the complexity of the insert, including sequence repeats, DNA methylation and the bacterial plasmid origin of replication, sequencing results represent approximately 80% of the insert. The combination of this sequencing and the restriction analysis allowed for development of a schematic diagram of the components of the transgene inserted in C5 plum (Figure 6, page 30 of petition). In addition, the sequence analysis provided evidence that the ß-lactamase gene in C5 plum is interrupted by a fragment containing a bacterial *cos* site (Figure 1, page 20 of petition) and is therefore inactivated.

The *nptII* and *ß*-glucuronidase genes, commonly used as marker genes, are found in soil-inhabiting *E. coli*. These bacteria are not plant or human pathogens, and do not cause disease symptoms or the production of infectious agents in plants. The PPV-CP cassette contains the leader sequence from the TMV coat protein and an ATG start codon fused to the PPV coat protein gene from the PPV-D strain (Ravelonandro et al., 1992; Takamatsu et al., 1987). Both the TMV leader sequence and the PPV coat protein gene are components of naturally occurring plant viruses, but neither of these genes is capable of causing plant or human disease. The commonly used 35S promoter is derived from cauliflower mosaic virus which is a plant pathogen. Cauliflower mosaic virus (CaMV) causes disease primarily in cruciferous plants. However, the CaMV 35S promoter does not cause disease symptoms in plants, nor does it encode for an infectious agent.

3. RNA and Protein Characterization and Expression:

Northern blot analysis was performed on each of the five transformed plum lines (C2 - C6) and a non-transformed control plant (‘Bluebyrd’). Figure 2 (page 23 of the petition) shows the expected 1.4 kb transcript present in each line, as well as the relative amounts of PPV-CP RNA found in each line. These results show that the amount of transcript RNA present in C5 plum was much less than that found in C2-C4 plum. These results are consistent with those previously described by Scorza, et al (Scorza 1994). As expected, no transcript RNA was found in the non-transformed control.

Western blot analysis was used to analyze protein production in each of the five transformed lines (C2-C6). Figure 3, page 24 of the petition shows the results of the immunoblot that was performed with monoclonal antibodies raised against the PPV coat protein. Results of this testing showed protein production in transformed lines C2-C4,

but no detectable protein produced in the C5 and C6 lines. This lack of detectable protein is consistent with the lack of protein produced in C5 plum field trials, as well as the suggested mode of virus resistance based upon gene silencing (Scorza, Callahan et al. 2001; Scorza 2005).

4. Mechanism of resistance:

Post-transcriptional gene silencing (PTGS) has been the subject of intense investigation in recent years and has also been described as an effective means of resistance to plant viruses (Angell and Baulcombe 1997; Jan, Pang et al. 1999; Savenkov and Valkonen 2001; Lacomme, Hrubikova et al. 2003; Lu, Martin-Hernandez et al. 2003; Baulcombe 2004; Chang, Chen et al. 2005). A number of analyses were performed on C5 plum to further elucidate the mechanism of resistance in C5 plum including: RNA and protein expression; DNA sequencing; nuclear run-on analysis; analysis of transgene methylation; and analysis of the presence of short interfering RNA (siRNA).

Results of the nuclear run-on analysis showed that both C4 and C5 clones had similar levels of PPV-CP RNA transcript (Figure 8, page 32 of petition). This suggests that the low levels of mRNA and non-detectable levels of PPV coat protein found in C5 plum, as described earlier, resulted from post-transcriptional gene silencing.

Another characteristic of PTGS is evidence of transgene methylation (Gonzalez-Zulueta 1995; Elbashir 2001; Turfarelli 2003). Results of restriction digest and Southern blot analysis suggest that the PPV-CP gene sequence in C5 plum is methylated. This determination is based upon larger than expected fragments of *Sau*3A digest probed with a PPV-CP probe. Based upon the results for C3 and C5 plum samples, there appears to be specific methylation of the PPV-CP insert in the C5 plum (Figure 9 and Figure 10, pages 33 & 34 of petition).

Finally, production of siRNA is considered to be diagnostic of PTGS (Angell and Baulcombe 1997; Jan, Pang et al. 1999; Savenkov and Valkonen 2001; Lacomme, Hrubikova et al. 2003; Lu, Martin-Hernandez et al. 2003; Baulcombe 2004; Chang, Chen et al. 2005). Total RNA from C3-2 and C5 was used in northern blot analysis to determine the presence of siRNAs. Samples of inoculated and non-inoculated C5 plum showed the presence of small RNAs of approximately 22 and 25-26 nucleotides (nt), Figure 11, page 35 of petition). These results indicate not only the presence of siRNA in C5 plum, but also that inoculation is not required to induce production of these siRNAs. No siRNAs were detected in either the C3-2 or the non-transgenic plum (Figure 11, page 35 of petition) as expected.

Seeds from C5 progeny fruit that resulted from open-pollination experiments conducted at the USDA-ARS research facility in Kearneysville, WV were collected and analyzed. Results of these analyses showed that at one month post-germination, the PPV-CP gene in leaves of seedlings was specifically methylated and produced a similar pattern to the C5 parent (Figure 22, page 56 of petition). In addition, siRNA was detected in

ungerminated through four-week post-germination embryo samples. (Figure 25, page57 of petition).

The cumulative RNA analysis data presented, in addition to data collected over multiple years of field trials support the conclusion that the mechanism of resistance for the C5 plum is PTGS. The presence of siRNAs and the lack of detectable protein production are consistent with published literature on gene silencing and the mechanism described for other virus resistant plants.

5. Stability and resistance of C5 plum to PPV

Field trials were performed under appropriate European permits in Poland, Spain and Romania beginning in 1996-1997. The experimental design is described in Section X of the petition and the results of this work are thoroughly described in published literature (Ravelonandro, Monsion et al. 1992; Malinowski, Zawadzka et al. 1998; Hily, Scorza et al. 2004). Briefly, results from the field trials in Poland, conducted with plum lines C2-C6 and a non-transformed control plum, show that the C5 plum was highly resistant to PPV via aphid inoculation, and tolerant to chip bud inoculation with PPV. Despite signs of mild symptoms in chip bud inoculated C5 plum beginning in the second year of the field trial, by year seven of the trial, none of the C5 trees showed symptoms of PPV infection. In contrast, all trees from the other transformed lines (C2-C4 & C6), as well as the non-transformed plum, were infected by year seven. Infection in these other lines started in year one of the trial and increased yearly through year four where there was 95% infection, and finally at year seven when there was 100% infection. Visual symptoms of PPV infection or non-infection were confirmed by use of ELISA, reverse transcription polymerase chain reaction (RT-PCR) and immunocapture RT-PCR (IC-RT-PCR). The IC-RT-PCR test conducted in 2000 revealed the presence of PPV in some leaves of chip bud inoculated C5 trees, but very few if any symptoms. Figures 13 (A) and (B) of the petition (pages 38 & 39) provide details of the plot design and results of the PPV infection analysis.

Further analysis was performed on samples collected from the Poland field trials which compared transgene RNA produced by C3 and C5 plum. Consistent with earlier results, C5 plum produced very small amounts of detectable transgene RNA compared to C3 plum, providing confirmation for the stability of PTGS in C5 plum field trials (Figure 15, page 42 of petition).

Results from both the Spain and Romania field trials corroborated the data obtained in Poland. In both of these trials, both PPV inoculum and aphid vectors were present. Despite adequate virus pressure from two PPV serotypes, and from aphid vectors as evidenced from nearly 100% infection of non-C5 plum trees, none of the C5 trees were infected by PPV (see Figure 17, page 44 of petition). In Spain, the C4 plum showed good initial resistance against aphid-vectored infection, but once the protection broke down, virus was able to spread throughout the C4 tree.

Data provided and reviewed by APHIS demonstrate stable integration and inheritance of the PPV-CP gene and its associated regulatory sequences over several years of field trials

conducted in the United States and Europe. Analyses of inheritance showed the expected Mendelian segregation as a single gene dominant trait and stability of the trait through subsequent generations in the breeding program (Table 6, page 55 of petition).

6. Gene Flow from Transgenic Plum

Pollen flow experiments were performed with the C5 plum at the USDA-ARS Kearneysville research facility. Very low levels of pollen flow were seen from transgenic to non-transgenic *P. domestica* trees both within a transgenic trial block and between a transgenic block and a non-transgenic block (Figure 26, page 61 of petition). Pollen flow between the transgenic and non-transgenic plum occurred at a distance of 520 m at a rate of 0.067% (2 out of 2,950 seeds) over a six year period.