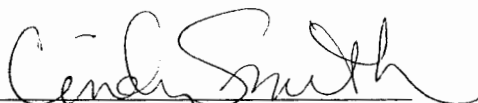


FINDING OF NO SIGNIFICANT IMPACT

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) is proposing to issue a permit in response to an application (APHIS number 03-279-01r) received from Oregon State University to conduct field tests with genetically engineered non-pathogenic strains of *Erwinia amylovora* (fireblight). In support of our permitting decision, we have prepared an environmental assessment (EA) under APHIS regulations at 7 CFR Parts 340 and 372 and the National Environmental Policy Act, 42 U.S.C. 4321. The field tests are scheduled to begin in April 2004 in Benton and Jackson Counties, Oregon.

Based on the analysis documented in its environmental assessment, APHIS concludes that the preferred alternative, Alternative III., is to issue the field test permit with the addition of supplemental permit conditions for conducting the test and the filing of field tests reports with APHIS. APHIS' conclusions regarding this permit application can be found in Section VI. of its EA. We conclude that the field test will not present a significant impact, either individually or cumulatively, on the quality of the human environment. Before reaching this decision, APHIS requested, received and considered comments on the EA from the public. A response to the comment received is included as an attachment to this FONSI statement.



Cindy Smith

Deputy Administrator

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

Date: MAY 11 2004

Attachment
Finding of No Significant Impact
Response to comments
APHIS permit no. 03-279-01r

In response to a notice published in the *Federal Register* on March 22, 2004 (69 FR 13280-13281), Docket No. 04-012-1), APHIS received one comment on the environmental assessment (EA) prepared for APHIS Permit No. 03-279-01r during the designated 30-day comment period which ended April 21, 2004. The comment, which was from a private individual, asserted that the product proposed for testing could be worse than the non-engineered fire blight and was not safe, but provided no supporting data, information, analysis, or explanation. We do not agree with the commenter's assertions. The strains of *Erwinia amylovora* bacterium to be used in the proposed field test have been genetically engineered to be avirulent, or incapable of causing disease. In contrast, the non-engineered strains of the subject bacterium are the causal agent of one of the most destructive diseases of apple and pear trees. The attached EA provides additional detail establishing the safety of the engineered strains of *E. amylovora*.

USDA/APHIS Environmental Assessment in response to permit application (03-279-01r) received from Oregon State University for field testing of a genetically engineered non-pathogenic bacterium, *Erwinia amylovora*

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Appendix I.	Threatened and Endangered Plant and Animal Species in Oregon
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Appendix III.	Supplemental Permit Conditions

I. Summary

USDA/APHIS has prepared an environmental assessment in response to a permit application (APHIS Number 03-279-01r) received from Oregon State University for controlled field tests in Benton and Jackson counties, Oregon of two genetically engineered non-pathogenic (avirulent) strains of a bacterium, *Erwinia amylovora*, the causal agent of fire blight disease on apple and pear trees. The purpose of the field trials is to determine whether these engineered strains of *E. amylovora* are effective as biocontrol/ disease suppression agents of phytopathogenic *E. amylovora*. Fire blight, one of the most destructive diseases of apple and pear trees, occurs sporadically and can cause extensive tree damage when outbreaks occur. The introduction of these avirulent strains, alone and in combination with other non-pathogenic bacteria, is expected to protect susceptible plants from infection by indigenous, phytopathogenic *E. amylovora*.

The *E. amylovora* that was subsequently engineered was isolated from infected trees in Oregon. The bacteria have been genetically engineered using the neomycin phosphotransferase (*nptII*) gene from transposon 10 (Tn10) from *Escherichia coli* strain DH5 α using molecular biology techniques as detailed in the permit application. Insertion of this transposon into the specific *hrp* (hypersensitive reaction on non-host plants and pathogenesis on host plants) gene (HrpS- or HrpL-) results in inactivation of the gene and disruption of the disease-causing mechanism within the bacterium, thereby rendering the bacterium avirulent/ non-pathogenic. The *nptII* gene, which confers resistance to the antibiotic kanamycin (neomycin), has been safely used in many genetically engineered organisms.

Monitoring will be conducted to determine persistence of the recombinant bacterium on plants. Data provided in the permit indicate that *E. amylovora* does not typically survive on surfaces of trees or fruit for longer than 1-2 weeks, thus the engineered bacteria are not expected to survive. Data submitted shows that the engineered bacteria are genetically stable. Even if the transposon is deleted by a classical genetic mechanism, the resulting bacteria would be a virulent *Erwinia* strain virtually identical to the strain that is already widely prevalent in Oregon.

II. Purpose and Need

II.1 Proposal:

USDA/APHIS is proposing to issue a permit for confined field release/testing in Benton and Jackson Counties, Oregon of two strains of genetically engineered avirulent (non-pathogenic) *Erwinia amylovora* as a biological control agent of fire blight disease caused by native/wild type *E. amylovora*.

Fire blight is a bacterial disease of apple, pear and other trees in the family Rosaceae that kills blossoms, shoots, limbs and sometimes entire trees. The disease was first observed in New York prior to 1800 and can now be found throughout most of the U.S. and Canada. The disease can be both costly and quite difficult to control. Management and control methods include pruning out of diseased tissues, copper sprays, streptomycin or oxytetracycline sprays, management of tree vigor, insect control and planting of resistant cultivars (http://www.caf.wvu.edu/kearneysville/disease_month/fireblight.html). Infection often results in canker development on twigs, branches or limbs that can be a source of inoculum for subsequent infections. A primary site for new infections is through open blossoms. Infected tissues die and eventually turn black. Bacteria may ooze from infected tissues and serve as new inoculum for

disease spread. Further disease description and management practices may be in a University of California publication (UC Publication 3340, 1991).

Two non-pathogenic biocontrol agent bacteria have previously been identified that are useful in spray preparations to reduce fire blight infection through either direct competition for nutrient resources or production of antimicrobial compounds; *Pseudomonas fluorescens* A506 (Frostban® or Bliteban®) and *Pantoea agglomerans* (syn. *Erwinia herbicola*) C9-1 (Blossom Bless®). Although these organisms have been shown to be effective, their effectiveness is limited under certain environmental conditions. The use of the avirulent genetically engineered *E. amylovora* is designed to more closely match growth and survival niches of virulent *E. amylovora* to better compete and control infection.

A permit application was submitted to USDA/APHIS pursuant to regulations in 7 CFR Part 340 which are entitled "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests." The regulations govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A permit must be obtained or a notification acknowledged before a regulated article may be introduced into the U.S.

A genetically engineered organism is considered a regulated article if it is being introduced and if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the 7 CFR 340 and is also a plant pest, or if there is reason to believe that it is a plant pest. In this submission, the recipient organism is in the genus *Erwinia*, which is one of the listed taxa, and it has been genetically engineered using recombinant DNA techniques. Thus, the genetically engineered microorganism in this Oregon State University submission is deemed a regulated article.

Generally, permitting for field trials of regulated articles is categorically excluded from requirements for an EA under APHIS NEPA implementing procedures (7 C.F.R. Section 372.5(c)(3)(i)). However, when APHIS determines that a confined field release of genetically engineered organisms involves new species or organisms or novel modifications that raise new issues, APHIS prepares an EA under an exception to the categorical exclusion (7 C.F.R. Section 372.5(d)(4)). APHIS is preparing this EA because this is the first request for a field test of a genetically engineered plant pathogen, *Erwinia amylovora*, which APHIS considers a novel modification that raises new issues. This documents that the analysis is in compliance with the National Environmental Policy Act (NEPA) of 1969 and the pursuant implementing regulations published by the Council on Environmental Quality and APHIS (42 U.S.C. 4331 et seq.; 40 C.F.R. 1500-1508; 7 C.F.R. part 1b; and 60 FR 6000-6005, February 1, 1995).

II.2. Description of regulated article

The wild type (native) *Erwinia amylovora* strain 153 is indigenous to Oregon and was originally isolated from an apple orchard there in 1990. It has been studied and used in experimental trials for 13 years. Its virulence is typical of other indigenous *E. amylovora* strains in the U.S. Its nature is epiphytic and it grows well on flowers but does not reproduce or persist well on other plant surfaces. As is common with many bacterial plant pathogens, *E. amylovora* induces a hypersensitive response (rapid cell death) when injected into leaves of tobacco, a non-host. *E. amylovora* strains 153 HrpS- and 153 HrpL- have been developed through a marker-exchange

mutagenesis process to contain transposable elements (Tn10-mini-kan) from *E. coli* and the corresponding *hrp* (h_{ypersensitive} r_{eaction} on non-host plants and p_{athogenesis} on host plants) genes from *E. amylovora* strain Ea321. The *E. amylovora* genome contains a number of *hrp* genes which are responsible for production of proteins required for pathogenesis on susceptible plants. Insertion of the transposable element Tn10-mini-kan within the coding region of these genes in *E. amylovora* effectively disrupts production of the HrpS or HrpL proteins thereby disrupting the pathogenesis (disease) process. Selected derivative strains are no longer able to induce disease on pear or apple blossoms or elicit a hypersensitive reaction in tobacco (see permit application and Tharaud, et al, 1997). The insertions in the newly developed avirulent strains have been stable for ~100 generations in laboratory cultures and no reversions to wild type/antibiotic sensitivity have been detected over the same time period.

Marker Genes Used as Experimental Controls: The marker gene, neomycin phosphotransferase (*nptII*), from Tn10 from *E. coli* serves three purposes: 1) to allow the initial selection of the recombinant bacterium, 2) to follow the fate of the recombinant bacterium after release into the environment, and 3) to differentiate the recombinant from the non-recombinant bacterium, both indigenous and introduced, after release into the environment. The proposed experiments use resistance to the antibiotic kanamycin as an experimental marker. The introduction of these resistance genes even in the event that they were transferred to new organisms would not be expected to present a significant risk for the following reasons: (1) Tn10 occurs naturally in enteric bacteria such as *E. coli* (<http://jb.asm.org/cgi/content/full/180/23/6408?view=full&pmid=9829956>) and can transfer its resistance determinants to related microorganisms (Wachsmuth, et al., 1983); (2) a variety of kanamycin resistant genes are known to occur in nature (Tenover and Elvrum, 1988, Flamm, et al., 1993); and (3) creating new bacterial strains resistant to the antibiotic kanamycin will not have any clinical significance since their use as medical therapeutics is limited (<http://vm.cfsan.fda.gov/~dms/OPA-ARMG.HTML#BACK6>).

III. Alternatives Including the Proposed Action

APHIS has considered the following three alternatives in response to the applicant's request for a permit:

Alternative 1: Deny the permit: release of the regulated organism would not be authorized.

Alternative 2: Issue the permit: the test conditions proposed by the applicant would be authorized, or

Alternative 3: Issue the permit with additional conditions required by APHIS for conducting the field test.

III.1 Discussion of the alternatives:

Alternative 1: No Action/ denial of permit application--- Under this alternative, release would not be authorized and current management and control practices for *E. amylovora* would continue at the proposed testing locations. Costs associated with additional labor required for pruning of diseased tissue would continue. Spraying of copper products, streptomycin and/or oxytetracycline antibiotics, non-pathogenic competing biocontrol bacteria would continue as deemed necessary. Other research options using non-genetically engineered organisms would likely be explored and implemented.

Alternative 2: Issue the permit for the field testing under the conditions proposed by the applicant--- Under this alternative, field release of the microorganisms would be authorized at the specified locations with no additional conditions outside of what the applicant provided in his request. Standard permit conditions under 7 CFR 340.4 would be required (see appendix 2). Standard management practices, including use of some antibiotic sprays, will be included as part of the experimental design.

Alternative 3: Issue the permit with additional conditions for conduction of the field test. Supplemental permit conditions, based on APHIS analysis, comments from U.S. Fish and Wildlife Service, the State of Oregon and public comment from this environmental assessment, would be required. If warranted based on environmental risk of escape of the engineered bacteria, APHIS will require mitigating measures to prevent spread of the organism outside the test area. These measures could include any of the following: spraying of the test site with antimicrobial compounds to kill the engineered bacteria, removal and destruction of treated limbs or trees, and/or any other method deemed effective by APHIS.

IV. Description of the Field Test/ Affected Environment

Colonization and infection of apple and pear blossoms by *Erwinia amylovora* are processes that are tremendously influenced by weather conditions (Kim, JF and Beer, S, 2000). Trials are proposed that will be conducted over 3 bloom seasons (typically between March 15 and May 15) in order to determine if the variety of bacteria used alone and in combination with other non-pathogenic bacteria can control or suppress fire blight disease.

Screenhouse and field trials will be conducted in Benton County, Oregon and/ or Jackson County, Oregon. Access to test sites is restricted by fences and/or chained gates that are expected to provide adequate physical containment and security.

Screenhouse studies: Pear and apple trees blossoms will be inoculated multiple times with a variety of bacterial strains over a 5 week period (including both engineered and non-engineered bacteria). A total of 120 trees may be used in the trials. The screenhouse has been used for multiple years strictly for purposes of fire blight research. It will serve to protect the trees from rain, UV radiation/sunlight and insect visitation. Coexistence and competition interactions will be evaluated among all the bacteria used by measuring starting and final bacterial populations over a wide range of normally occurring and temperature controlled regimes. Non-pathogenic bacterial strains of *Pseudomonas fluorescens* and *Pantoea agglomerans* will be included in this testing.

Field efficacy studies: Treatment bacterial strains and strain mixtures will be applied to pear and apple trees at 30% and 70% of full bloom. Treatment plots consist of 50 to 100 trees that are at least 15 years old each. Inoculation consists of spraying of a bacterial suspension onto tree blossoms under calm wind conditions during sunrise hours. At full bloom, pathogenic *E. amylovora* will be inoculated onto blossoms to ensure adequate disease causing exposure. Experimental details are described in the permit application. Standard agricultural practices of using streptomycin or oxytetracycline sprays to control disease will also be included as part of the testing. Bacterial populations on blossoms and incidence of fire blight disease will be determined at regular intervals during the experiments.

The applicant expects that using avirulent *Hrp*- strains of *E. amylovora* will lower the incidence of fire blight disease over a broad range of temperatures and environmental conditions (Tharaud, et al, 1997, Stockwell, et al, 2001). Both *P. fluorescens* and *P. agglomerans* (syn. *Erwinia herbicola*) have been shown to suppress pathogen growth at lower temperatures. Conducting these experiments at locations in Jackson and Benton counties over several years is expected to provide adequate data to evaluate this hypothesis.

Spraying of the bacterial suspensions will take place between sunrise and 8 a.m. under calm wind conditions to minimize spray drift. These applications will take place only during the bloom period of the pear and apple trees (estimated between March 15 and May 15) in the trials. Due to the epiphytic nature of the organism, bacteria are not expected to survive on plant surfaces past mid-summer. Applicators will wear disposable spray suits that will be autoclaved and discarded after use. Following application, spray equipment will be disinfected with a chlorine bleach solution to kill residual bacteria (see permit).

Field Observation, Monitoring, and Final Disposition of the Test Plants: Data on bacterial populations and incidence of disease will be collected throughout the testing periods. Site monitoring and containment protocols have been designed to limit dispersal of the recombinant bacterium and are expected to provide the necessary degree of both biological and physical containment. In 13 years of similar testing using non-recombinant organisms, the applicants have not detected *E. amylovora* strains overwintering on trees and none is expected (see permit).

V. Potential Environmental Impacts

Alternative I: No Action/ denial of permit request:

Field release research would not be allowed. Environmental impacts associated with current management practices (spraying of streptomycin and/or oxytetracycline and/ or competitive/antagonistic bacteria) would continue. Streptomycin resistant strains of *E. amylovora* exist currently and it could be expected that other antibiotic resistant strains would develop.

Alternative II: Issue the permit with no additional conditions:

The proposed field test is a controlled release of the regulated article into the environment. The bacteria, *E. amylovora*, have been mutagenized by marker exchange mutagenesis and the avirulent *hrp*- mutant strains Ea153 HrpS- and Ea153 HrpL- were selected for their inability to induce disease reactions in pear, apple and tobacco. Insertion of Tn10-mini-kan into the chromosome of each derivative also conferred resistance to the antibiotic kanamycin. Reversion of these strains would not pose any additional environmental risk because reverted mutants will be similar to the other *E. amylovora* strains that are commonly present on these plants. The risks associated with the introduction of genetically engineered organisms are the same kind as those associated with the introduction into the environment of unmodified organisms and organisms modified by other genetic techniques.

V.1. Impact on Native Floral and Faunal Communities: *E. amylovora* strains 153 HrpS- and HrpL- are identical to the indigenous strain except for their inability to induce a disease reaction in a number of plant species and the expression of certain antibiotic resistance marker genes. The applicant provided data addressing the possible extension of the host range of the engineered *E. amylovora* to other species resulting from insertion of the new

genes. Experimental data submitted included host range information on the wild type *E. amylovora* and the two engineered avirulent mutants on eleven (11) non-host species (including strawberry, corn, tomato, potato, *Arabidopsis*, wheat, and others). The wild type/ native bacterium elicited a hypersensitive response in eight (8) of the species. The engineered bacteria elicited no response in any of the eleven (11) species. The host response data were consistent with what is expected based on our understanding of gene-for-gene relationship between host and pathogens. Data submitted in the application supports that the engineering has not altered the bacterium host range.

The mutants do not have any selective ability to persist in the environment. Physical factors that influence the behavior of the bacteria in the environment have been identified. *E. amylovora* is capable of growth on flowers but does not persist well on other plant surfaces (Tharaud, et al, 1994). Additionally, multiple years of testing have demonstrated that these wild type/ native *Erwinia* bacteria do not overwinter on plant surfaces in these locations (see permit).

V.2. Impact on Existing Agricultural Practices: This small field test will not have any significant impact on existing agricultural practices because this test is solely for research purposes.

V.3. Impacts on Human Health: These experiments use resistance to the antibiotic kanamycin as an experimental marker. The introduction of these resistance genes, even in the event that they were transferred to new organisms, would not be expected to present a significant risk because Tn10 is naturally occurring in enteric bacteria such as *E. coli* (<http://jb.asm.org/cgi/content/full/180/23/6408?view=full&pmid=9829956>) and the antibiotic kanamycin is not a risk since this antibiotic has limited therapeutic use due to its somewhat toxic nature (<http://vm.cfsan.fda.gov/~dms/OPA-ARMG.HTML#BACK6>). The Food and Drug Administration has developed documents addressing use of neomycin phosphotransferase as genetic material and its presence as a protein in genetically engineered organisms (<http://vm.cfsan.fda.gov/~dms/OPA-ARMG.HTML#BACK6>). The Environmental Protection Agency has granted the associated gene (*nptII*) and the protein exemptions from tolerance in or on raw agricultural products when used as plant-pesticide inert ingredients (http://a257.g.akamaitech.net/7/257/2422/08aug20031600/edocket.access.gpo.gov/cfr_2003/julqtr/pdf/40cfr180.1134.pdf). These reviews include both toxicity and allergenicity safety assessments.

Erwinia species are not known as animal or human pathogens. As stated in Bergey's Manual of Systematic Bacteriology, *Erwinia* species are "Associated with plants as pathogens, saprophytes, or as constituents of the epiphytic flora." *E. amylovora* "causes a necrotic disease (fireblight) of most species of the Pomoideae and of some species in other subfamilies of the Rosaceae." There are no references to association with human or animal disease even though farm workers have been exposed to *Erwinia* for decades. No risk to university personnel handling the colonized pear and apple trees is expected. No potential impact of this experiment on people living in the area of the field trial test plot or any other human population can be identified.

V.4. Horizontal transfer of kanamycin resistance gene from *Erwinia* to other species.

Neomycin and kanamycin are infrequently used antibiotics, neither is unique for any use, nor rarely are administered orally (Food and Drug Administration, <http://vm.cfsan.fda.gov/~dms/opa-armg.html>). Resistance to the antibiotic kanamycin

(neomycin) is already widely prevalent in soil-borne bacteria (Cole and Elkan, 1979). Gene transfer from *Erwinia* to animals and plants is highly unlikely under the conditions of this field test (Syvanen, 1999; Syvanen and Kado, 1998).

V.5. Effects on Threatened and Endangered Species: The proposed field test is a controlled release of the regulated article into the environment in Benton and Jackson counties in Oregon. Neither the engineered *Erwinia* nor the *nptIII* gene will affect any non-target organism including any threatened and endangered species listed in Oregon. An analysis of TES distribution in these counties using the U.S. Fish and Wildlife ECOS database (<http://ecos.fws.gov/ecos/index.do>, and Appendix I) indicated that seven threatened or endangered plant species exist or once existed in these two counties. These species are in the Asteraceae, Liliaceae, Limnanthaceae, Apiaceae, Fabaceae and Malvaceae families. None of the listed species is in the Rosaceae family. Only species in the Rosaceae family are known to be susceptible to *E. amylovora*. (Bergey's Manual, 1984). In addition, none of these species grows in habitats similar to those found in fruit tree orchards. Also, none of the threatened and endangered species listed is within the known host range of *E. amylovora* and therefore it is unlikely that any impact will occur (see also information under **V.1.** above regarding no extended host range).

Examination of threatened or endangered animals listed for Oregon in the ECOS database listed thirty-six species. Most are fresh and saltwater fish, amphibians and marine mammals, which would not be impacted by this test. Examination of potentially impacted species such as birds and insects showed that the two butterfly species listed are confined to a narrowly defined habitat due to their dependence on a lupine and a violet species for their lifecycle. All the bird species listed are either marine, estuary, or forest species whose habitats do not occur in or near these field test sites. The applicant consulted with two OSU faculty members and three other individuals knowledgeable about the proposed test sites. Each indicated that there were no known threatened or endangered species or species habitats adjacent to the testing locations (emails to Ken Johnson and submitted as part of the permit application). Therefore these field tests should not impact any threatened or endangered species.

Consistent with BRS procedures, APHIS has sent this EA to Fish and Wildlife Service for their input on our no effect decision on TES.

V.6. Cumulative Environmental Effects: Two primary factors should prevent persistence of the bacteria at the test sites: 1) the nature of the *hrp*- mutants of *E. amylovora* is that they grow well on flower surfaces but not other plant surfaces therefore will likely be undetectable by mid-summer or earlier, and 2) in 13 years of testing at these sites, bacterial epiphytes inoculated onto trees have not been found to overwinter successfully. Thus, the engineered bacteria are not expected to persist in the environment.

V.7 Special Considerations. Because *Erwinia* is not a human pathogen and the small scale and research nature of the field test, this experiment will not pose disproportionately high or adverse human health or environmental effects to any specific minority or low-income group (Executive Order (EO)12898, "Federal Actions To Address Environmental Justice in Minority Populations and Low-Income Populations," and EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks).

Alternative III: Issue the permit with additional conditions.

The potential environmental impacts under this alternative include all those noted under Alternative II. This alternative was chosen because the additional supplemental conditions (see Appendix III) allow BRS to add inspections of the site as needed to ensure that the applicant is following all procedures and conditions described in the application, and to monitor the disposal of regulated material. In addition, the applicant must provide BRS with a written summary of the data from the field test which will aid BRS in evaluating the potential risk of future field tests. APHIS received concurrence for this field test from the State of Oregon without the imposition of additional permit conditions from the State.

VI. Conclusions

- The test bacterium, *Erwinia amylovora*, has been rendered incapable of causing disease
- Virulent strains of this bacterium are indigenous to the area of the test
- Dissemination of the bacteria will be prevented through physical methods, normal site security, the small size of the trials and decontamination or appropriate disposal of application equipment
- The host range of the engineered bacteria has not changed
- The bacterium has never been associated with animal or human disease and will not therefore pose a health risk
- Neomycin phosphotransferase (from the marker gene) does not confer any plant pest characteristics to *E. amylovora*
- Native floral and faunal communities, including threatened and endangered species, are not in the host range of *E. amylovora* and therefore will not be affected by the trials.

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Appendix I. Threatened and Endangered Plant and Animal Species in Oregon
http://ecos.fws.gov/tess_public/TESSWebpageUsaLists?usMap=1&status=listed&state=OR

COMMON NAME	LATIN NAME	COUNTY LOCATION - OREGON*	FAMILY	HABITAT OF SPECIES IN COUNTIES WHERE FIELD TESTS ARE PROPOSED (I.E. BENTON AND JACKSON)
RED MOUNTAIN ROCKCRESS	<i>Arabis macdonaldiana</i>	Curry (41015), Josephine (41033)	BRASSICACEAE	
MARSH SANDWORT	<i>Arenaria paludicola</i>	No Oregon location given (www.natureserve.org) Locations: CA Riverside (06065), San Bernardino (06071), San Francisco (06075), San Luis Obispo (06079), Santa Cruz (06087), WA Grays Harbor (53027), King (53033), San Juan (53055)	CARYOPHYLLACEAE	
APPLEGATE'S MILK-VETCH	<i>Astragalus applegatei</i>	Klamath (41035)	FABACEAE	
GOLDEN PAINTBRUSH	<i>Castilleja levisecta</i>	Linn (41043), Marion (41047), Multnomah (41051)	SCROPHULARIACEAE	

WILLAMETTE VALLEY DAISY	<i>Erigeron decumbens</i> var. <i>decumbens</i>	Benton (41003), Clackamas (41005), Lane (41039), Linn (41043), Marion (41047), Polk (41053), Washington (41067), Yamhill (41071)	ASTERACEAE	Species occurs on alluvial soils (deposited by flowing waters). The species is known to have been extirpated (destroyed or no longer surviving) from an additional 19 historic locations. Willamette daisy populations are known mainly from bottomland but one population is found in an upland prairie remnant.
GENTNER'S FRITILLARIA	<i>Fritillaria gentneri</i>	Jackson (41029), Josephine (41033)	LILIACEAE	Gentner's fritillary typically grows in or on the edge of open woodlands at elevations from 180 to 1,360 m (60 to 450 feet) with Oregon white oak (<i>Quercus garryana</i>) and Pacific madrone (<i>Arbutus menziesii</i>) as the most common overstory plants. Western yellow pine (<i>Pinus ponderosa</i>) and Douglas fir (<i>Pseudotsuga menziesii</i>) are also frequently present. Gentner's fritillary can also grow in open chaparral/grassland habitat, which is often found within or adjacent to the mixed hardwood forest type, but always where some wind or sun protection is provided by other shrubs. It does not grow on very dry sites.
WATER HOWELLIA	<i>Howellia aquatilis</i>	Clackamas (41005), Marion (41047), Multnomah (41051)	CAMPANULACEAE	
WESTERN LILY	<i>Lilium occidentale</i>	Coos (41011), Curry (41015)	LILIACEAE	
LARGE-FLOWERED WOOLLY MEADOWFOAM	<i>Limnanthes floccosa</i> ssp. <i>Grandiflora</i>	Jackson (41029)	LIMNANTHACEAE	Woolly meadowfoam occurs at the edge of vernal pools at elevations of 375-400 m (1230-1310 feet), generally near the wetter, inner edges as opposed to the drier outer fringes which harbor the sympatric ssp. <i>floccosa</i> . Associated species include small-flowered lupine (<i>Lupinus micranthus</i>), poverty clover (<i>Trifolium depauperatum</i>), and least mouse-tail (<i>Myosurus minimum</i>).
BRADSHAW'S LOMATIUM	<i>Lomatium bradshawii</i>	Benton (41003), Lane (41039), Linn (41043), Marion (41047)	APIACEAE	The majority of Bradshaw's lomatium populations occur on seasonally saturated or flooded prairies, adjacent to creeks and small rivers in the southern Willamette Valley. Soils at these sites are dense, heavy clays, with a slowly permeable clay layer located 15-30 cm (6-12 in) below the surface. Bradshaw's lomatium occurs on alluvial (deposited by flowing water) soils.
AGATE DESERT LOMATIUM	<i>Lomatium cookii</i>	Jackson (41029), Josephine (41033)	APIACEAE	This plant occurs only where soil types have a hard pan or clay pan layer close to the soil surface, creating seasonally wet soils and vernal pools. The Agate Desert is characterized by shallow, Agate-Winlow soils, a relative lack of trees, sparse prairie vegetation, and agate on the soil surface.

KINCAID'S LUPINE	<i>Lupinus oregonus</i> <i>var. kincaidii</i>	Benton (41003), Douglas (41019), Lane (41039), Linn (41043), Marion (41047), Polk (41053), Yamhill (41071)	FABACEAE	Kincaid's lupine is found mainly in the Willamette Valley, Oregon where it occupies native grassland habitats. Kincaid's lupine is typically found in native upland prairie with the dominant species being red fescue (<i>Festuca rubra</i>) and/or Idaho fescue (<i>Festuca idahoensis</i>). These dry, fescue prairies make up the majority of habitat for Kincaid's lupine. Although Kincaid's lupine is occasionally found on steep, south-facing slopes and barren rocky cliffs, it does not appear capable of occupying the most xeric oatgrass communities on these south facing slopes. The plant's distribution implies a close association with native upland prairie sites that are characterized by heavier soils and mesic to slightly xeric soil moisture levels. At the southern limit of its range, this species occurs on well-developed soils adjacent to serpentine outcrops (high in magnesium, iron and certain toxic metals) where it is often found under scattered oaks.
MACFARLANE'S FOUR-O'CLOCK	<i>Mirabilis macfarlanei</i>	Wallowa (41063)	NYCTAGINACEAE	
ROUGH POPCORN-FLOWER	<i>Plagiobothrys hirtus</i>	Douglas (41019)	BORAGINACEAE	
NELSON'S SIDALCEA	<i>Sidalcea nelsoniana</i>	Benton (41003), Clackamas (41005), Linn (41043), Marion (41047), Polk (41053), Tillamook (41057), Washington (41067), Yamhill (41071)	MALVACEAE	Within the Willamette Valley, Nelson's checkermallow most frequently occurs in Oregon ash (<i>Fraxinus latifolia</i>) swales and meadows with wet depressions, or along streams. The species also grows in wetlands within remnant prairie grasslands. Some populations occur along roadsides at stream crossings where non-native plants, such as reed canarygrass (<i>Phalaris arundinacea</i>), blackberry (<i>Rubus</i> spp.), and Queen Anne's lace (<i>Daucus carota</i>), are also present. Nelson's checkermallow primarily occurs in open areas with little or no shade and will not tolerate encroachment of woody species.
SPALDING'S CAMPION	<i>Silene spaldingii</i>	Wallowa (41063)	CARYOPHYLLACEAE	
MALHEUR WIRE-LETTUCE	<i>Stephanomeria malheurensis</i>	Harney (41025)	ASTERACEAE	
HOWELL'S SPECTACULAR THELYPODY	<i>Thelypodium howellii</i> ssp. <i>Spectabilis</i>	Baker (41001), Malheur (41045), Union (41061)	BRASSICACEAE	

*data from www.natureserve.org -via ecos.fws.gov				
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Common name	Latin name	Group	
Albatross, short-tailed	<i>Phoebastria (=Diomedea) albatrus)</i>	Bird	Marine bird - unlikely contact with orchards
Butterfly, Fender's blue	<i>Icaricia icarioides fenderi</i>	insect	Feeds on Kincaid's Lupin in upland prairies. Larva dependent on this species for survival. Habitat is native grassland habitats. Kincaid's lupine is typically found in native upland prairies.
Butterfly, Oregon silverspot	<i>Speyeria zerene hippolyta</i>	insect	Primary source of food is the western blue violet. Adults and larvae both depend on the violet for nectar but the adults may use other nectar sources as well when the larvae are completely dependent on the violet. Other adult food sources include: yarrow (<i>Achillea millefolium</i>), pearly everlasting (<i>Anaphalis margaritacea</i>), Canada goldenrod (<i>Solidago canadensis</i>), and Aster subspicatus. All of these food sources are native to the Silverspot's habitat. The silverspot requires one of three types of grasslands with nearby meadows. These are: coastal salt spray meadows, stabilized dunes, montane meadows.
Chub, Borax Lake	<i>Gila boraxobius</i>	Fish	
Chub, Hutton tui	<i>Gila bicolor ssp.</i>	Fish	
Chub, Oregon	<i>Oregonichthys crameri</i>	Fish	
Dace, Foskett speckled (Foskett)	<i>Rhinichthys osculus ssp.</i>	Fish	
Deer, Columbian white-tailed Columbia River DPS	<i>Odocoileus virginianus leucurus</i>	mammal	
Eagle, bald (lower 48 States	<i>Haliaeetus leucocephalus</i>	Bird	Unlikely contact with orchards. Habitat is close to lakes and rivers
Fairy shrimp, vernal pool	<i>Branchinecta lynchi</i>	crustacean	
Murrelet, marbled (CA, OR, WA)	<i>Brachyramphus marmoratus marmoratus</i>	Bird	Pelagic bird only coming ashore to breed
Owl, northern spotted	<i>Strix occidentalis caurina</i>	Bird	Habitat is primarily old growth forests
Pelican, brown	<i>Pelecanus occidentalis</i>	Bird	Marine bird - unlikely contact with orchards

Plover, western snowy (Pacific coastal pop.)	<i>Charadrius alexandrinus nivosus</i>	Bird	Coastal species - unlikely contact with orchards
Salmon, chinook (fall Snake R.)	<i>Oncorhynchus (=Salmo) tshawytscha</i>	Fish	
Salmon, chinook (spring/summer Snake R.)	<i>Oncorhynchus (=Salmo) tshawytscha</i>	Fish	
Salmon, chinook (lower Columbia R.)	<i>Oncorhynchus (=Salmo) tshawytscha</i>	Fish	
Salmon, chinook (upper Willamette R.)	<i>Oncorhynchus (=Salmo) tshawytscha</i>	Fish	
Salmon, chum (Columbia R.)	<i>Oncorhynchus (=Salmo) keta</i>	Fish	
Salmon, coho (OR, CA pop.)	<i>Oncorhynchus (=Salmo) kisutch</i>	Fish	
Salmon, sockeye U.S.A. (Snake River, ID stock wherever found.)	<i>Oncorhynchus (=Salmo) nerka</i>	Fish	
Sea turtle, green (except where endangered)	<i>Chelonia mydas</i>	amphibian	
Sea turtle, leatherback	<i>Dermochelys coriacea</i>	amphibian	
Sea turtle, loggerhead	<i>Caretta caretta</i>	amphibian	
Sea-lion, Steller (eastern pop.)	<i>Eumetopias jubatus</i>	mammal	
Steelhead (Snake R. Basin)	<i>Oncorhynchus (=Salmo) mykiss</i>	Fish	
Steelhead (lower Columbia R.)	<i>Oncorhynchus (=Salmo) mykiss</i>	Fish	
Steelhead (middle Columbia R.)	<i>Oncorhynchus (=Salmo) mykiss</i>	Fish	
Steelhead (upper Willamette R.)	<i>Oncorhynchus (=Salmo) mykiss</i>	Fish	
Sucker, Lost River	<i>Deltistes luxatus</i>	Fish	
Sucker, shortnose	<i>Chasmistes brevirostris</i>	Fish	
Sucker, Warner	<i>Catostomus warnerensis</i>	Fish	
Trout, bull (U.S.A., conterminous, lower 48 states)	<i>Salvelinus confluentus</i>	Fish	
Trout, Lahontan cutthroat	<i>Oncorhynchus clarki henshawi</i>	Fish	
Whale, humpback	<i>Megaptera novaeangliae</i>	marine mammal	
Wolf, gray Western Distinct Population Segment	<i>Canis lupus</i>	mammal	

Appendix II. Standard Conditions for APHIS 2000 permits

(f) Permit conditions. A person who is issued a permit and his/her employees or agents shall comply with the following conditions, and any supplemental conditions which shall be listed on the permit, as deemed by the Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packing material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner so as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit;

(4) The regulated article shall be maintained only in areas and premises specified in the permit;

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article;

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation;

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article;

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the Administrator to be necessary to prevent the spread of plant pests;

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment;

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Verbally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

Appendix III. Supplemental Permit Conditions

[Note: Any regulated article introduced not in compliance with the requirements of 7 CFR Part 340 or of any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary to prevent the introduction of such plant pests, and the responsible party may be subject to fines or penalties as authorized by the Plant Protection Act.]

1. **The permittee is required to notify the State regulatory official and APHIS's Chief of Biotechnology Risk Assessment at least one week prior to the start of these trials.** APHIS's Biotechnology Regulatory Services (BRS) and/or an APHIS PPQ Regional Biotechnologist or APHIS State Plant Health Director may conduct inspections of the test site, facilities, and/or records at any time. APHIS may invite the FDA, EPA or State Regulatory Officials to participate in these inspections. Confidential Business Information (CBI) will be handled according to the APHIS policy statement at 50 F.R. 38561-63.

2. The procedures, processes, and safeguards which will be used to prevent escape, dissemination, and persistence of the transgenic organism and its progeny at each of the intended destinations as described in the permit application and in these supplemental permit conditions must be strictly followed. The permittee must maintain records sufficient to verify compliance with these procedures, including information regarding who performed the activity.

3. Within 28 calendar days after release, submit a report that includes the following information for each field test site:

- A. A diagram of the sites, with sufficient information to locate it.
- B. The total acreage of the test plots.

Fax the report to the following APHIS personnel:

- 1. The Chief, Biotechnology Risk Assessment Staff at Area Code (301) 734-8669
- 2. The PPQ Regional Biotechnologist (fax number enclosed)
- 3. The State Regulatory Official (CBI-Deleted copy only)

4. Consistent with standard permit conditions at 7 CFR 340.4(f) (9), **field test data reports must be submitted within 6 months after the end of the test.** For purposes of these trials, the final data report should include results of post application monitoring for presence of the transgenic organism. Monitoring should continue at least every 2 months or until the organism can no longer be identified at the test sites. **Failure by an applicant to provide data reports in a timely manner for a field trial may result in the withholding of permissions by APHIS for future field trials.**

5. This Permit (APHIS form 2000) does not eliminate the permittee's legal responsibility to obtain any and all other Federal and State approvals that might be required.

7. Consistent with standard permit conditions at 7 CFR 340.4(f) (10), APHIS shall be notified verbally immediately upon discovery and in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article. For immediate verbal notification, contact the following APHIS staff in the order indicated below.

1. APHIS BRS Deputy Administrator's office [phone numbers: (301) 734-7324; (301) 734-5716; (202) 720-4383]. Indicate that you wish to report an unauthorized or accidental release of a regulated article to the BRS Regulatory Division Director; or in that person's absence, to the Chief of either the BRS Biotechnology Permit Program Operations staff or the Biotechnology Risk Assessment staff, or the permit reviewer. In the event that one of these persons cannot be reached, contact:

2. The appropriate APHIS PPQ Regional Biotechnologist.

3. The appropriate APHIS State Plant Health Director.

Contact information is maintained at the APHIS Biotechnology Regulatory Services website at <http://www.aphis.usda.gov/brs>.

Unless otherwise directed, written notification should be sent to:

Animal and Plant Health Inspection Service (APHIS)
BRS Regulatory Division (2) Director, Rm. 5B54
4700 River Rd. Unit 147
Riverdale, MD 20737.

When the regulated article or associated host organism is found to have characteristics substantially different from those listed in the permit application, or suffers an unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms), APHIS shall be notified as soon as possible but no later than within 5 working days. In such cases, notice should be sent to:

Animal and Plant Health Inspection Service (APHIS)
Chief, Biotechnology Permit Program Operations, Rm. 5B53
4700 River Rd. Unit 147
Riverdale, MD 20737.