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## DEPARTMENT OF AGRICULTURE

### Animal and Plant Health Inspection Service

[Docket No. 97-067-2]

#### Bejo Zaden BV; Availability of Determination of Nonregulated Status for Genetically Engineered Radicchio Rosso

**AGENCY:** Animal and Plant Health Inspection Service, USDA.

**ACTION:** Notice.

**SUMMARY:** We are advising the public of our determination that Bejo Zaden BV's Radicchio rosso lines designated as RM3-3, RM3-4, and RM3-6, which have been genetically engineered for male sterility and tolerance to the herbicide glufosinate as a marker, are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by Bejo Zaden BV in its petition for a determination of nonregulated status and an analysis of other scientific data. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

**EFFECTIVE DATE:** November 7, 1997.

**ADDRESSES:** The determination, an environmental assessment and finding of no significant impact, the petition, and any written comments received regarding the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are asked to call in advance of visiting at (202) 690-2817 to facilitate entry into the reading room.

**FOR FURTHER INFORMATION CONTACT:** Dr. Subhash Gupta, Biotechnology Evaluation, BSS, PPQ, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 734-8761. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-4885; e-mail: mkpeterson@aphis.usda.gov.

#### SUPPLEMENTARY INFORMATION:

##### Background

On May 28, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 97-148-01p) from Bejo Zaden BV (Bejo) of Warmenhuizen, The Netherlands, seeking a determination that Radicchio rosso (red-hearted chicory) lines designated as RM3-3, RM3-4, and RM3-6, which have been genetically engineered for male sterility and tolerance to the herbicide glufosinate as a marker, do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On August 27, 1997, APHIS published a notice in the *Federal Register* (62 FR 45387-45388, Docket No. 97-067-1) announcing that the Bejo petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency, and the Food and Drug Administration in regulating Radicchio rosso lines RM3-3, RM3-4, and RM3-6 and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether these Radicchio rosso lines posed a plant pest risk. The comments were to have been received by APHIS on or before October 27, 1997. APHIS received no comments on the subject petition during the designated 60-day comment period.

##### Analysis

Radicchio rosso (*Chichorium intybus* L.) lines RM3-3, RM3-4, and RM3-6 have been genetically engineered with a *barnase* gene from *Bacillus amyloliquefaciens* encoding a ribonuclease which inhibits pollen formation and results in male sterility of the transformed plants. The subject Radicchio rosso lines also contain the *nptII* selectable marker gene and the *bar* gene isolated from the bacterium *Streptomyces hygroscopicus*. The *bar*

gene encodes a phosphinothricin acetyltransferase (PAT) enzyme, which, when introduced into a plant cell, inactivates glufosinate. Linkage of the *barnase* gene, which induces male sterility, with the *bar* gene, a glufosinate tolerance gene used as a marker, enables identification of the male sterile line for the production of pure hybrid seed. The subject Radicchio rosso lines were transformed by the *Agrobacterium tumefaciens* method, and expression of the introduced genes is controlled in part by gene sequences derived from the plant pathogen *A. tumefaciens*.

Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain regulatory gene sequences derived from a plant pathogen. However, evaluation of field data reports from field tests of the subject Radicchio rosso lines conducted in Europe since 1993 and under an APHIS permit since 1995, indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of these Radicchio rosso lines.

##### Determination

Based on its analysis of the data submitted by Bejo and a review of other scientific data and field tests of the subject Radicchio rosso lines, APHIS has determined that Radicchio rosso lines RM3-3, RM3-4, and RM3-6: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become a weed than Radicchio rosso lines developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed; (4) will not cause damage to raw or processed agricultural commodities; and (5) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture. Therefore, APHIS has concluded that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 and any progeny derived from hybrid crosses with other nontransformed Radicchio rosso varieties will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the subject Radicchio rosso lines already field tested, or those observed for Radicchio rosso in traditional breeding programs.

The effect of this determination is that Bejo's Radicchio rosso lines designated as RM3-3, RM3-4, and RM3-6 are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of Bejo's Radicchio rosso lines RM3-3, RM3-4, and RM3-6 or their progeny. However, the importation of the subject Radicchio rosso lines or seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

#### **National Environmental Policy Act**

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 14th day of November 1997.

**Craig A. Reed,**

*Acting Administrator, Animal and Plant Health Inspection Service.*

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**USDA/APHIS Petition 97-148-01p for Determination of Nonregulated Status for  
Radicchio Rosso Lines Designated as RM3-3, RM3-4, and RM3-6**

**Environmental Assessment and  
Finding of No Significant Impact**

**November 1997**

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has prepared an environmental assessment before issuing a determination of nonregulated status for genetically engineered male sterile and glufosinate-tolerant chicory (*Chichorium intybus*) plants called Radicchio rosso lines RM3-3, RM3-4, and RM3-6. APHIS received a petition from Bejo Zaden BV, The Netherlands regarding the status of these Radicchio rosso lines as regulated articles under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition, supporting documentation, and other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment for its determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 shall no longer be regulated articles.

A handwritten signature in cursive script, reading "John H. Payne", written over a horizontal line.

**John H. Payne, Ph.D.**  
**Director**  
**Biotechnology and Scientific Services**  
**Animal and Plant Health Inspection Service**  
**U.S. Department of Agriculture**  
**Date: NOV 07 1997**

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## I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U. S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) before deciding on the regulated status of genetically engineered lines of male sterile and glufosinate-tolerant chicory (*Chichorium intybus*) designated hereafter as Radicchio rosso lines RM3-3, RM3-4, and RM3-6. The developer of Radicchio rosso lines RM3-3, RM3-4, and RM3-6, Bejo Zaden BV of Warmenhuizen, The Netherlands, petitioned APHIS requesting a determination on the regulated status of these Radicchio rosso lines that have been regulated articles under APHIS regulations. Interstate movements and field tests of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have been conducted under permits issued by APHIS. Bejo Zaden BV has petitioned APHIS for a determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 do not present a plant pest risk and should, therefore, no longer be regulated articles under APHIS regulations at 7 CFR Part 340.

The Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have been developed to provide a more reliable system to generate hybrid seeds by genetically engineering a plant to be male sterile. To generate a male sterile plant, a *barnase* gene from *Bacillus amiloliquefaciens* is engineered into the plant. The *barnase* gene encodes a ribonuclease protein which, when produced in the tapetum cell layer of the anther, disturbs the metabolism and functioning of this cell layer, resulting in male sterility of the plant (Mariani et al., 1990; Seurinack et al., 1990). Expression of *barnase* gene in these cells apparently results in degradation of host RNAs, thus blocking pollen cell development. Two selectable genetic marker genes are linked to the *barnase*: the *neo* gene conferring kanamycin resistance to facilitate selection of transformed cells in the laboratory (Flavell et al., 1992); and the *bar* gene conferring phosphinothricin tolerance for identification of male fertile plants during plant breeding.

The genes were introduced into the plant genome via a well-characterized transformation method using disarmed Ti-plasmid of *Agrobacterium tumefaciens* (see reviews by Klee and Rogers, 1989; Zambryski, 1988). Although some of the DNA sequences used in the transformation process were derived from the plant pathogen *A. tumefaciens*, the genes which cause crown gall disease have been removed, and therefore the recipient plant does not have crown gall disease. Once inserted into the chromosome of the recipient, the introduced genes are maintained in the same manner as any other genes.

Field trials have been carried out in The Netherlands, Italy, United Kingdom, France, Belgium and the U.S. One field trial was conducted in the United States under APHIS permit number 95-205-01 for which an environment assessment (EA) was prepared. The EA addressed questions pertinent to plant pest risk issues concerning the conduct of field trial under physical and reproductive confinement, but the EA did not address several issues that are of relevance to the unconfined growth of Radicchio rosso lines

RM3-3, RM3-4, and RM3-6. With respect to these new issues, APHIS concludes the following:

1. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 exhibit no plant pathogenic properties. Although components of pathogenic organisms were used in their development, these Radicchio rosso plants are not infected by these organisms nor can these plants incite disease in other plants.
2. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are no more likely to become a weed than those developed by traditional breeding techniques. Radicchio rosso plant is not a serious, principal, or common weed pest in the U.S. and there is no reason to believe that male sterility would enable Radicchio rosso to become a weed pest.
3. Multiple barriers, including sterility of this line, insure that gene introgression from Radicchio rosso lines RM3-3, RM3-4, and RM3-6 into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.
4. Seeds of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are substantially equivalent in composition, quality and other characteristics to nontransgenic Radicchio rosso plants and should have no adverse impacts on raw or processed agricultural commodities.
5. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 exhibit no significant potential to either harm threatened or endangered species or organisms beneficial to the agricultural ecosystem.

Therefore, after a review of the available evidence, APHIS believes that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 will be just as safe to grow as traditionally bred Radicchio rosso varieties not subject to regulation under 7 CFR Part 340. APHIS concludes that there will be no significant impact on the human environment if Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are no longer considered regulated articles under regulations at 7 CFR Part 340.

## II. BACKGROUND

Bejo Zaden BV has submitted a "Petition for Determination of Non-regulated Status" for Radicchio rosso plants that contain a gene that blocks pollen development, thereby producing a male sterile plant. Bejo Zaden BV requested a determination from APHIS that Radicchio rosso lines RM3-3, RM3-4, and RM3-6, and any progeny derived from hybrid crosses between these lines and other non-transformed Radicchio rosso varieties, no longer be considered regulated articles under 7 CFR Part 340.

**Development of Radicchio rosso lines RM3-3, RM3-4, and RM3-6.** Up to 10 years ago breeding activities focussed on Radicchio rosso were very low. Growers in European production areas mainly in Italy produced their own seeds using grower-selection methods. These methods result in poor seed quality, low productivity and insufficient uniformity. But about 10 years ago Bejo Zaden BV decided to improve this crop by means of intensive breeding program emphasizing the development of hybrid varieties. These hybrid varieties have the following advantages: pure seed quality with germination rate, high productivity, and uniformity of the crop. Until now the breeding of hybrid Radicchio rosso crops was dependent upon the self incompatibility characteristics of parental lines. The alleles that regulate self incompatibility often appear to be unstable resulting in seed lots contaminated with seeds from parental lines. With the availability of genetically engineered male sterile lines hybrid seeds can be produced by cross-pollinating new lines with conventional male fertile parental line. Seed is harvested on the male sterile plants only resulting in a 100% pure hybrid seed lot.

To generate a male sterile plant, a *barnase* gene from *Bacillus amiloliquefaciens* is engineered into the plant. The *barnase* gene encoding a protein ribonuclease which, when produced in the tapetum cell layer of the anther, disturbs the metabolism and functioning of this cell layer, resulting in male sterility of the plant. Expression of *barnase* in these cells apparently results in degradation of host RNAs blocking cell development. Two selectable genetic marker genes are linked to the *barnase* gene: *neo* gene conferring kanamycin resistance and *bar* gene encoding phosphinothricin acetyltransferase have also been introduced into the Radicchio rosso chromosome to facilitate selection of transformed cells in the laboratory and identification of male fertile plants during plant breeding respectively. The genes were introduced into the plant genome via a well-characterized transformation method using disarmed Ti-plasmid of *Agrobacterium tumefaciens*. The genes of interest are localized between the T-DNA border sequences of the Ti-plasmid and the DNA fragment transferred to the plant genome is restricted to this part. Details of the genetic vectors and sequences used to produce Radicchio rosso lines are given in the petition on pages 15-24.

Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have been field tested by Bejo Zaden BV and its partners in several European countries since 1993 and in the United States since 1995 under APHIS permit 95-205-01. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that they exhibit the desired agronomic characteristics and do not pose a plant pest risk. Although the field tests of Radicchio rosso RM3-3, RM3-4, and RM3-6 have been conducted in agricultural settings, the permit conditions have stipulated physical and reproductive confinement.

**APHIS Regulatory Authority.** APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority under the Federal Plant Pest Act, (7 U.S.C. 150aa-

150jj) as amended, and the Plant Quarantine Act, (7 U.S.C. 151-164a, 166-167) as amended, affect the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

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 there is reason to believe that it is a plant pest. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 described in the Bejo Zaden BV petition have been considered regulated articles because they contain noncoding DNA regulatory sequences derived from a plant pathogen.

Section 340.6 of the regulations, entitled "Petition Process 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 should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. Thereafter, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

**Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority.** Radicchio rosso lines RM3-3, RM3-4, and RM3-6 may also be subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered before distribution or sale, unless exempt by EPA regulation. In cases in which the genetically modified plants allow for a new use of an herbicide or involve a different use pattern for the herbicide, the EPA must approve the new or different use. In making such an approval, the EPA considers the possibility of adverse effects to human health and the environment from the use of this herbicide. When the use of the herbicide on the genetically modified plant would result in an increase in the residues of the herbicide in a food or feed crop for which the herbicide is currently registered, or in new residues in a crop for which the herbicide is not currently registered, establishment of a new tolerance or a revision of the existing tolerance would be required. Residue tolerances for pesticides are established by the EPA under the Federal Food, Drug and Cosmetic Act (FFDCA) as amended (21 U.S.C. 301 *et seq.*). The Food and Drug Administration (FDA) enforces tolerances set by the EPA under the FFDCA.

FDA's 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.



### III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 as regulated articles under APHIS regulations. The developer of Radicchio rosso lines RM3-3, RM3-4, and RM3-6, Bejo Zaden BV, has submitted a petition to USDA, APHIS requesting that APHIS make a determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 shall no longer be considered regulated articles under 7 CFR Part 340. This EA was prepared in compliance with: (1) the National Environmental Policy Act of 1969 (NEPA)(42 U.S.C. 4321 *et seq.*), (2) Regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372; 60 FR 6000-6005, February 1, 1995).

### IV. ALTERNATIVES

#### A. **No Action.**

Under the Federal "no action" alternative, APHIS would not come to a determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are no longer regulated articles under the regulations at 7 CFR Part 340. Permission from APHIS would still be required for introductions of Radicchio rosso lines RM3-3, RM3-4, and RM3-6. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6.

#### B. **Determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are no longer regulated articles.**

Under this alternative, Radicchio rosso lines RM3-3, RM3-4, and RM3-6 would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits from APHIS would no longer be required for introductions of Radicchio rosso lines RM3-3, RM3-4, and RM3-6. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969 (42 USC 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

### V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 should no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. The previous EA prepared by

APHIS with the issuance of the permit for field tests of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 has addressed various attributes of this Radicchio rosso. This EA discusses the genetic modification, and the potential environmental impacts that might be associated with the unconfined cultivation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6.

Additional technical information is included in the determination document appended to this EA, and incorporated by reference. This includes detailed discussions of the biology of Radicchio rosso, the genetic components used in the construction of Radicchio rosso lines RM3-3, RM3-4, and RM3-6, and the analyses that lead APHIS to conclude that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have no potential to pose plant pest risks.

**A. Potential impacts based on increased weediness of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 relative to other male sterile Radicchio rosso.**

Although various definitions of the term "weed" have been proposed in the scientific literature, the salient point is that a plant can be considered a weed when it is growing where humans do not want it (Baker 1965; de Wet and Harlan 1975; Muenscher 1980). Baker (1965) lists 12 common attributes that can be used to assess the likelihood that a plant species will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants. Both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

The cultivated Radicchio rosso is not considered a weed pest and is unlikely to become a weed pest. Radicchio rosso is considered a highly inbred, well-characterized crop plant that is not persistent in undisturbed environments without human intervention. Although Radicchio rosso volunteers are not uncommon, they are easily controlled using herbicides or mechanical means. Radicchio rosso also possess few of the characteristics of plants that are notably successful weeds (e.g., it does not produce abundant, long-lived seed; it does not propagate vegetatively; it does not compete well with other plant species in the environment).

In the United States, Radicchio rosso is not listed as a weed in the major weed references (Holm et al. 1979; Muenscher 1980), nor is it listed as a noxious weed species by the Federal Government (7 CFR Part 360).

The product, Radicchio rosso, is grown by farmers and growers for the production of vegetable chicory crops. After harvest the product will be put up for sale by the growers and subsequently distributed to supermarkets and vegetable-traders. The crop will be consumed by humans, mainly as a component of fresh mixed salads. The parent plant of the Radicchio rosso lines RM3-3, RM3-4, and RM3-6 exhibits no appreciable weedy

characteristics. The relevant introduced trait, male sterility, is unlikely to increase weediness of Radicchio rosso lines RM3-3, RM3-4, and RM3-6. There is no indication that the presence of *barnase* gene encoding ribonuclease protein will convert Radicchio rosso plant into a weed. The Radicchio rosso plants have also been transformed with *bar* gene encoding phosphinothricin acetyltransferase, which confers resistance to the herbicide glufosinate. One more gene *neo* codes for a third protein (neomycin phosphotransferase II) which, when present in the tissue of the plant, renders the plant tissue easily identifiable from other plant tissues which do not contain this protein. The genes have no involvement in plant disease or damage. Also, the presence of *bar* does not result in the presence of the herbicide in Radicchio rosso and does not indicate that glufosinate will be used in the cultivation of the Radicchio rosso. No other attributes of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 suggest that it be any more "weedy" than the present Radicchio rosso cultivars that are the result of traditional breeding. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have retained the agronomic characteristics of the parental Radicchio rosso. Bejo Zaden BV did not find any irregularities in morphology or inflorescence in the new lines. In field trials in Europe and in California, insect behavior did not differ from the transgenic Radicchio rosso lines in cross-pollination experiments. The seed production was comparable with the nontransgenic control lines and the seed quality was good. Thus, Bejo Zaden BV has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other test reported in its application that support APHIS' conclusion that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 is just are safe to grow as any other Radicchio rosso plants.

**B. Potential impacts on the sexually-compatible relatives of Radicchio rosso arising from pollination by Radicchio rosso lines RM3-3, RM3-4, and RM3-6**

Radicchio rosso plants can exchange genetic information with other lines and species of the same genus (*Cichorium*) due to cross pollination by insects. Fecundation occurs essentially by insect cross pollination. Self pollination is rather exceptional, but occurs, depending on genotype. Asexual reproduction does not occur. Radicchio rosso has been selected for bolting tolerance. Therefore, lower temperature is needed to induce flowering. It is possible that cross-pollinations occur with other, non modified vegetable chicory plants grown in the proximity or with wild relatives which possibly emerge in the surroundings of the cultivation areas. However, no dispersal can be expected by the male sterile plants. Accordingly, transfer of the genetic material from the transgenic plants to wild relatives is unlikely to happen, the opposite is possible. However, the probability for such outcrossings to occur is considered rather small, since for hybrid seed production those sites are selected where emergence of wild relatives is as little as possible, which is indispensable due to the high demands upon the purity of the seed. Throughout the practical cultivation of Radicchio rosso, the crop will be in the

vegetative stage and not attain the flowering stage, unless inappropriate flowering occurs due to climatical circumstances.

**C. Consideration of potential environmental impacts associated with the cultivation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 outside the United States.**

In accordance with Executive Order 12114, January 4, 1979, entitled "Environmental effects abroad of major federal actions," APHIS has also considered potential environmental impacts associated with the cultivation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 outside the United States and its territories.

Our analysis of the biology of cultivated male sterile Radicchio rosso and its relatives leads to the conclusion that the environmental impacts of cultivation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 anywhere in the world would be no different from impacts attributable to similar varieties produced with traditional breeding techniques.

Any International trade in Radicchio rosso lines RM3-3, RM3-4, and RM3-6 would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC) of the Food Agricultural Organization. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (105 countries as of October 1996). The treaty, now administered by a Secretariat housed within the United Nations Food and Agriculture Organization in Rome, came into force on April 3, 1952. It establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering such legislation or have prepared guidelines. These signatories includes Mexico, which has in place a regulatory process requiring a full evaluation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 before it can be introduced into the environment. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) such as the North American Plant Protection Organization (NAPPO). Our trading partners will be kept informed of our regulatory decisions through NAPPO and other fora. Our decision in no way prejudices regulatory action in any other country.

It should be noted that all the existing national and international regulatory authorities and phytosanitary protocols that currently apply to introductions of new male sterile chicory varieties will apply to Radicchio rosso lines RM3-3, RM3-4, and RM3-6.

**D. Potential impacts on nontarget organisms, including beneficial organisms such as bees and earthworms, and threatened or endangered organisms**

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for Radicchio rosso lines RM3-3, RM3-4, and RM3-6, and plant products derived from them, to have damaging or toxic effects directly or indirectly on nontarget organisms, particularly those that are recognized as beneficial to agriculture and to those that are recognized as threatened or endangered in the United States.

Bejo Zaden BV's analysis of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 identified no toxic components that are present in concentrations significantly different from concentrations in nontransgenic Radicchio rosso. The genetic modification in Radicchio rosso lines RM3-3, RM3-4, and RM3-6 does not result in the production of new proteins, enzymes, or metabolites in the plant that are known to have toxic properties. The plants also do not exhibit any pathogenic properties.

APHIS concludes that the unconfined growth of Radicchio rosso lines RM3-3, RM3-4, and RM3-6, and products derived from these lines will not have any deleterious effects on organisms recognized as beneficial to agriculture (e.g., earthworms, honey bees) or on other organisms, including any species recognized as threatened or endangered in the United States.

Because the *bar* gene would only be in half of the hybrid seeds sold to farmers, use of this male sterility system does not necessarily provide farmers herbicide tolerant Radicchio rosso plants. However, if the other parent in the hybrid breeding system was phosphinothricin-tolerant, then plants derived from that hybrid seed would be herbicide tolerant and the farmers could potentially apply the herbicide. The use of phosphinothricin-class of herbicides in the cultivation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 or their offspring will be regulated by the EPA under its existing regulations for the registration of pesticide use. As part of the pesticide registration process, EPA considers the impacts on the environment, including nontarget organisms.

**E. Potential impacts on biodiversity.**

It was determined from our analysis that genetically engineered male sterile and glufosinate-tolerant Rosso lines RM3-3, RM3-4, and RM3-6 are no more likely to become weed than chicory lines developed by traditional breeding techniques, are unlikely to increase the weediness potential of any other cultivated plant or native wild species with which these lines can interbreed, and will not harm threatened and endangered species and non-target organisms. Based on this analysis, APHIS concludes that there is no potential impact of these chicory lines on biodiversity.

**F. Potential impacts on agricultural and cultivation practices.**

Based on its analysis, APHIS concludes that there is unlikely to be any significant verse impact on agricultural practices associated with the use of Radicchio rosso lines RM3-3, RM3-4, and RM3-6.

**G. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 will not cause damage to processed agricultural commodities.**

In APHIS' opinion, the components and processing characteristics of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

**VI. CONCLUSION**

APHIS has evaluated information from the scientific literature as well as data submitted by Bejo Zaden BV that characterized Radicchio rosso lines RM3-3, RM3-4, and RM3-6. After careful analysis, APHIS has made a finding of no significant impact to the environment from issuance of a determination that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 should no longer be regulated articles under APHIS regulations at 7 CFR Part 340. That finding is supported by the following conclusions:

1. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 exhibit no plant pathogenic properties. Although components of pathogenic organisms were used in their development, these Radicchio rosso plants are not infected by these organisms nor can these plants incite disease in other plants.
2. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are no more likely to become a weed than those developed by traditional breeding techniques. Radicchio rosso plant is not a serious, principal, or common weed pest in the U.S. and there is no reason to believe that male sterility would enable Radicchio rosso to become a weed pest.
3. Multiple barriers, including sterility of this line, insure that gene introgression from Radicchio rosso lines RM3-3, RM3-4, and RM3-6 into wild or cultivated sexually-compatible plants is extremely unlikely, and such rare events should not increase the weediness potential of any resulting progeny or adversely impact biodiversity.
4. Seeds of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are substantially equivalent in composition, quality and other characteristics to nontransgenic Radicchio rosso and should have no adverse impacts on raw or processed agricultural commodities.

5. Radicchio rosso lines RM3-3, RM3-4, and RM3-6 exhibit no significant potential to either harm threatened or endangered species or organisms beneficial to the agricultural ecosystem.

Therefore, after review of the available evidence, APHIS concludes that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 will be just as safe to grow as traditionally bred Radicchio rosso varieties that are not subject to regulation under 7 CFR Part 340. APHIS concludes that there should be no significant impact on the human environment if Radicchio rosso lines RM3-3, RM3-4, and RM3-6 were no longer considered a regulated article under its regulations (7 CFR Part 340).

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**Response to Bejo Zaden BV's Petition 97-148-01p for a Determination of  
Nonregulated Status for Male Sterile Radicchio Rosso Lines designated as RM3-3,  
RM3-4, and RM3-6**

**Prepared by  
United States Department of Agriculture  
Animal and Plant Health Inspection Service  
Biotechnology and Scientific Services**

## I. SUMMARY

In a petition dated May 20, 1997, Bejo Zaden BV, The Netherlands requested a determination from the Animal and Plant Health Inspection Service (APHIS), U. S. Department of Agriculture that male sterile and glufosinate-tolerant Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 and any progeny derived from these lines, should no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. The male sterile chicory (*Chichorium intybus*) designated hereafter as Radicchio rosso lines RM3-3, RM3-4, and RM3-6 have been considered regulated articles because they were engineered with DNA sequences derived from the plant pathogen *Agrobacterium tumefaciens*.

Based on a review of available scientific information, APHIS has determined that Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 do not present plant pest risks and therefore are no longer regulated articles under the regulations at 7 CFR Part 340. Because of this determination, oversight under these regulations will no longer be required from APHIS for field testing, importation, or interstate movement of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 or their progeny.

This determination has been made based on an analysis that revealed that Radicchio Rosso lines RM3-3, RM3-4, and RM3-6: (1) exhibit no plant pathogenic properties; (2) are no more likely to become weed than chicory lines developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential of any other cultivated plant or native wild species with which Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 can interbreed; (4) will not cause damage to processed agricultural commodities; and 5) will not harm threatened and endangered species and other organisms, such as bees, which are beneficial to agriculture. APHIS has also concluded that there is no reason to believe that new chicory varieties derived from Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 lines already field tested, or those observed for chicory in traditional breeding programs.

## II. BACKGROUND

**APHIS Regulatory Authority.** APHIS regulations found at 7 CFR Part 340 (hereafter referred to as the regulations) were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA)(7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended. The regulations pertain to the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

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 there is reason to believe that it is a plant pest. Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 have been considered "regulated articles" under Part 340 of the regulations because they have been engineered with certain noncoding regulatory sequences derived from the plant pathogen *Agrobacterium tumefaciens*.

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted information and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. As a consequence of such a determination, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

APHIS' decision on the regulatory status of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 under APHIS' regulations at 7 CFR 340, do not release these transgenic lines and their progeny from EPA and FDA regulatory oversight. The regulation of herbicide use is under the jurisdiction of the EPA.

### III. COMMENTS

On August 27, 1997, APHIS published a notice in the Federal Register (62 FR 45387-45388, Docket No. 97-067-1) announcing that the Bejo Zaden BV petition had been received and was available for public review. In the notice, APHIS solicited written comments from the public on any plant pest risk issues associated with the subject Radicchio rosso lines. The comments were to have been received by APHIS on or before October 27, 1997. APHIS received no comments on the subject petition during the designated 60-day comment period.

### IV. Analysis of Radicchio Rosso Lines RM3-3, RM3-4, and RM3-6

Biology of Chicory. Chicory (*Cichorium intybus* L.) belongs to the family of the Compositae together with other species like *Lactuca sativa* (lettuce) and *Helianthus annuus* (sunflower). *Cichorium intybus* L. var. *silvestre* BISCHOFF is the wild-type form which is the origin of all cultivated varieties. The genus has a diploid number of 18 chromosomes.

The identification of *Cichorium* species is determined by phenotypical characteristics. The plants have a typical composite morphology with ligulate, mostly blue corollas and herbaceous bracts in two rows. The flowers are grouped to form blue colored

flowerheads. They include 5 stamens and 2 styles. Radicchio rosso (red hearted chicory) is phenotypically easy to identify due to the typical formation of the head and the red turning to green-red color of the leaves with striking thick, white veins.

In general only phenotypic aspects are utilized to distinguish the source organism from its nearest relatives. Each individual (hybrid) variety is hereby described by means of the following characteristics, and differs in at least one of them from other (hybrid) varieties.

**Characteristics :**

- Formation of anthocyanin in the leaf
- Color of the leaf, shape, etc.
- Heading
- Earliness / Harvest time
- Bolting resistance
- Flower color

Radicchio rosso plants can exchange genetic information with other lines and species of the same genus (*Cichorium*) due to cross pollination by insects. Fecundation occurs essentially by insect cross pollination. Self pollination is rather exceptional, but occurs, depending on the genotype. Asexual reproduction does not occur. Radicchio rosso has been selected for bolting tolerance. Therefore lower temperature is needed to induce flowering.

The parental organism is indigenous to European countries, except the very north. It is also indigenous in the USA. Its natural habitats are waysides and land laying fallow. In Europe, approximately 40,000 acres of Radicchio rosso are grown, of which about 35,000 acres are in Italy. In the USA, Radicchio rosso is a very minor crop with a estimated annual production of approximately 2,400 acres, mainly in California.

**Rationale for Development of Male Sterile Chicory.** Up to 10 years ago breeding activities focussed on Radicchio rosso were very low. Growers in European production areas mainly in Italy produced their own seeds using grower-selection methods. These methods result in poor seed quality, low productivity and insufficient uniformity. But about 10 years ago Bejo Zaden BV decided to improve this crop by means of intensive breeding program emphasizing the development of hybrid varieties. These hybrid varieties have the following advantages: pure seed quality with germination rate, high productivity, and uniformity of the crop. Until now the breeding of hybrid Radicchio rosso crops was dependent upon the self incompatibility characteristics of parental lines. The alleles that regulate self incompatibility often appear to be unstable resulting in seed lots contaminated with seeds from parental lines. With the availability of genetically engineered male sterile lines hybrid seeds can be produced by cross-pollinating new lines

with conventional male fertile parental line. Seed is harvested on the male sterile plants only resulting in a 100% pure hybrid seed lot.

Development of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6. To develop a male sterile chicory plant, a ribonuclease encoded by *barnase* gene, has been engineered into chicory. The ribonuclease, which is expressed only in the tapetum cells of the pollen sac during anther development, blocks pollen development, thus producing a male sterile plant. To direct the expression of *barnase* to pollen cells, a pollen specific promoter, pTA 29 (Seurinck et al., 1990) was used to direct the synthesis of ribonuclease enzyme. Expression of *barnase* in pollen cells apparently results in degradation of host RNAs and blocking development of mature viable pollen. *Barnase* gene encodes for an extracellular ribonuclease protein in *Bacillus amyloliquefaciens*, a common soil microorganism. *B. amyloliquefaciens* also contains an intracellular protein called barstar which specifically inhibits barnase by combining with it in a one-to-one complex (Hartely 1989). Thus, barstar is produced intracellularly by the same organism that secretes barnase (Hartely and Smeaton 1973).

The second gene (*bar*) inserted into the Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 genome encodes for phosphinothricin acetyltransferase (PAT) enzyme which inactivates phosphinothricin, the active component in the herbicide glufosinate. The *bar* gene is produced in Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 under the direction of *Arabidopsis thaliana* PssuAra promoter and nopaline synthase termination/polyadenylation sequences. Although Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 are tolerant to the herbicide, hybrid progeny (the seeds that would be available to the farmers) developed from these lines may or may not be tolerant to the herbicide. If the female parent is not herbicide tolerant, then only half of the progeny hybrid chicory would carry the tolerance gene. This would mean that farmers could not apply the herbicide because 50% of the plants would die. However, if the female parent in the hybrid selection scheme was glufosinate tolerant, all the progeny would herbicide tolerant and farmers could apply the herbicide (assuming the herbicide is registered by EPA for use on chicory).

The third gene *neo* encodes for a protein (neomycin phosphotransferase II) which, when present in the tissue of the plant, renders the plant tissue easily identifiable from other plant tissues which do not contain this protein. All three genes (male sterility and two selectable markers) were introduced into the plant genome via a well-characterized transformation method using disarmed Ti-plasmid of *Agrobacterium tumefaciens* (see reviews by Klee and Rogers, 1989; Zambryski, 1988).

- A. The introduced genes, their products, and the added regulatory sequences controlling their expression do not present a plant pest risk in Radicchio Rosso lines RM3-3, RM3-4, and RM3-6.**

As summarized above, the genetic construct was introduced into the plant genome via a well-characterized transformation method using disarmed Ti-plasmid of *Agrobacterium tumefaciens*. Once inserted into the chromosome of the chicory plant, the introduced genes are maintained and transmitted in the same manner as any other genes as demonstrated by Mendelian inheritance data (data presented by applicant).

Many of the noncoding DNA regulatory sequences, sequences attached to the coding regions that control expression of the introduced coding sequences, were derived from a plant pathogen *A. Tumefaciens*). The regulatory sequences can not cause plant disease by themselves or in conjunction with the coding regions which they regulate in the constructs. Although these regulatory sequences are derived from a plant pathogen *A. tumefaciens*), there is no evidence to suggest that they pose a plant pest risk. These sequences do not code for a protein and are not implicated in disease pathogenesis.

- B. Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 have no significant potential to become successful weed.**

The cultivated Radicchio rosso is not considered a weed pest and is unlikely to become a weed pest. Radicchio rosso is considered a highly inbred, well-characterized crop plant that is not persistent in undisturbed environments without human intervention. Although Radicchio rosso volunteers are not uncommon, they are easily controlled using herbicides or mechanical means. Radicchio rosso also possess few of the characteristics of plants that are notably successful weeds (e.g., it does not produce abundant, long-lived seed; it does not propagate vegetatively; it does not compete well with other plant species in the environment).

In the United States, Radicchio rosso is not listed as a weed in the major weed references (Holm et al. 1979; Muenscher 1980), nor is it listed as a noxious weed species by the Federal Government (7 CFR Part 360).

Bejo Zaden BV has provided data regarding seed germination rates, yield characteristics, disease and pest susceptibilities, compositional analyses, and numerous other test reported in its application that support APHIS' conclusion that Radicchio rosso lines RM3-3, RM3-4, and RM3-6 are just as safe to grow as any other male sterile Radicchio rosso plants.

- C. Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 will not increase the weediness potential of any other plant with which they can interbreed.**

APHIS considered whether the movement of the male sterility gene and the other genes from Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 to other cultivated chicory or wild relatives might result in offspring that would present problems as weeds. First, Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 are male sterile, thus significantly reducing the likelihood of outcrossing. Second, the genetic integrity of commercial cultivated chicory lines and varieties is carefully controlled through established plant breeding practices. These standard practices make it unlikely that this trait will be inadvertently incorporated into the germplasm of cultivated chicory lines.

Our analysis of the biology of cultivated male sterile Radicchio rosso and its relatives leads us to predict that the environmental impacts of cultivation of Radicchio rosso lines RM3-3, RM3-4, and RM3-6 anywhere in the world would be no different from impacts attributable to similar varieties produced with traditional breeding techniques.

**D. Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 will not harm organisms beneficial to agriculture or organisms that are designated as threatened or endangered.**

APHIS evaluated the potential for Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 plants to harm organisms either directly or indirectly, particularly those organisms that are recognized as beneficial to agriculture. There is no reason to believe that the cultivation of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 or their progeny will exert any deleterious effects on organisms recognized as beneficial to agriculture. Likewise, cultivation of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 will not harm any species designated as threatened or endangered. Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 produce two enzymes, ribonuclease and phosphinothricin acetyltransferase, that are not produced in nontransgenic chicory. There is no indication that these enzymes are toxic to beneficial organisms or result in the production of toxic constituents. In addition, APHIS can envision no plausible mechanism whereby Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 would be injurious or pathogenic to beneficial organisms such as bees and earthworms.

The definition of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 encompasses not only the chicory lines that have already been field tested, but also new chicory lines produced through conventional breeding using Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 as one or both the parents. APHIS believes that the analysis applied to the Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 plants already field tested will apply equally well to these new chicory lines, and that the data provided by Bejo Zaden BV justify the conclusion that such new lines derived from Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 will not present a plant pest risk. The variation in agronomic characteristics among the Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 plants that have been field tested does not differ significantly from that seen in commercial cultivars of chicory that have never been considered regulated articles. Therefore, there is no



reason to believe that any of the progeny of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 will possess plant pest properties.

**E. Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 should not cause damage to processed agricultural commodities.**

The characteristics of Radicchio Rosso Lines RM3-3, RM3-4, and RM3-6 have no apparent attributes that could have an indirect plant pest effect on any processed plant commodity. During extensive testing in the laboratory, greenhouse and in the field, plants of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 exhibited the typical agronomic characteristics of the parent chicory. In APHIS' opinion, the components and processing characteristics of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

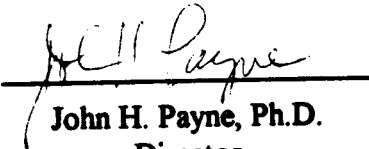
#### IV. CONCLUSION

APHIS has determined that Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 that have previously been field tested under APHIS permit will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permission under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those chicory lines or their progeny. However, the importation of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 and vegetative plant material or seeds capable of propagation are still subject to the restrictions in foreign quarantine notices in 7 CFR Part 319.

This determination has been made based on information from field trials, laboratory analyses, and literature references presented herein which demonstrate that Radicchio Rosso lines RM3-3, RM3-4, and RM3-6:

(1) exhibit no plant pathogenic properties; (2) are no more likely to become a weed than chicory lines developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential of any other cultivated plant or native wild species with which Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 can interbreed; (4) will not cause damage to processed agricultural commodities; and 5) will not harm threatened and endangered species and other organisms, such as bees, which are beneficial to agriculture.

APHIS has also concluded that there is a reasonable certainty that new progeny of Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 or varieties bred from these lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for Radicchio Rosso lines RM3-3, RM3-4, and RM3-6 plants already field tested, or those observed for chicory in traditional breeding programs.

  
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Date: NOV 07 1997

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