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October 5th 2015

Dr. Michael J. Firko  
Acting Deputy Administrator  
Biotechnology Regulatory Services  
4700 River Rd, Unit 98  
Riverdale, MD 20737

**Re: Confirmation of regulatory status of transgenic bioluminescent *Nicotiana tabacum***

Dear Dr. Firko,

My company, Glowing Plant, Inc, is developing a range of novel ornamental plants for the consumer market. Last year, on October 1st, we sent you an 'Am I regulated' letter confirming the status of a transgenic bioluminescent *Arabdiopsis thaliana*. In December you replied with a determination that that plant was not a regulated article. This letter concerns a very similar product, a bioluminescent *Nicotiana tabacum* plant, which has been genetically engineered to emit a pleasant, dim glow upon the addition of a proprietary formula.

Early customer testing of the previous strain, *Arabdiopsis thaliana*, indicated our target customers found it too difficult to grow well due to the long day/short day cycle. Consequently we have developed a new version of the plant based on *Nicotiana tabacum*. In addition to the change in plant species our subsequent research has also identified a novel promoter which enhances the glowing effect.

This letter includes confidential business information and so as per APHIS instructions I've included an attachment with the confidential information marked as deleted.

Because *Nicotiana tabacum* is not a plant pest or invasive species, the genetic elements introduced are all sourced from fully classified organisms, and the transformation process does not introduce any plant pest DNA components, there is no valid basis for concluding that our transgenic *Nicotiana tabacum* is or will become a plant pest within the meaning of the Plant Protection Act (PPA). Therefore under current regulations, our bioluminescent *Nicotiana tabacum* plant is not a regulated article within the meaning of 7 C.F.R § 340.1 because there is no scientifically valid basis that it satisfies any of the regulatory criteria that would subject it to the oversight of the USDA's Animal Plant Health and Inspection Service (APHIS).

We kindly request that APHIS confirm that our bioluminescent *Nicotiana tabacum* plant is not considered a regulated article within the meaning of the current regulations. If the agency does not concur with our interpretation of the current regulations then we ask you to provide us with the scientific rationale for how our bioluminescent *Nicotiana tabacum* is or will become a plant pest. The rest of this letter details the method for creating our plant, details of the inserted construct and our understanding of APHIS regulations which support our analysis by which bioluminescent *Nicotiana tabacum* is not a "regulated article".

## **I. Description of transgenic bioluminescent *Nicotiana tabacum* and methods used to create it**

To further assist APHIS in understanding the origin of our transgenic bioluminescent *Nicotiana tabacum*, a summary of information on the recipient plant, as well as the genetic and technical elements used to modify the recipient plant to make it glow, is provided below as per the instructions on APHIS' website.

### **A. Bioluminescent *Nicotiana tabacum* Transformation**

Transformation of *Nicotiana tabacum*, using synthetic DNA that is transferred by biolistic (gene gun) methods, results in stably integrated DNA. Our DNA transfer does not involve *Agrobacterium* transformation nor any other plant pest that is currently regulated under the Plant Protection Act. Selectable markers used in the transformation are eliminated via selfing and segregation. Using the genetic elements described in Table 1 below, the genetically enhanced materials express luciferase which causes the *Nicotiana tabacum* plant to glow with the addition of our proprietary chemical additive. Table 1 below describes each genetic element and identifies its respective sources and functions.

**Table 1: Genetic Elements in Bioluminescent Luciferase Construct for Biolistic Transformation of *Nicotiana tabacum***

<b>Element type</b>	<b>Name of Element</b>	<b>Organism from which element is derived</b>	<b>Description of the elements function</b>
<b>Promoter</b>	Ubq10	<i>Nicotiana tabacum thaliana</i>	Constitutive promoter
<b>Gene</b>	Atffluc2	Synthetic construct modified from <i>Photinus pyralis</i> origin	Luciferase enzyme
<b>Terminator</b>	THsp18.2	<i>Nicotiana tabacum thaliana</i>	Heat shock terminator
<b>Promoter</b>	Ubq3	<i>Nicotiana tabacum thaliana</i>	Constitutive promoter
<b>Gene</b>	GFP	Synthetic construct modified from <i>Aequorea victoria</i>	Green Fluorescent Protein
<b>Terminator</b>	TUbq3	<i>Nicotiana tabacum thaliana</i>	Ubiquitin 3 terminator
<b>Promoter</b>	PMYb10-R6	<i>Malus x domestica</i> 'Red Field' OP	Highly expressed promoter from red-fleshed apples
<b>Gene</b>	Atffluc2	Synthetic construct modified from <i>Photinus pyralis</i> origin	Luciferase enzyme
<b>Terminator</b>	THsp18.2	<i>Nicotiana tabacum thaliana</i>	Heat shock terminator

## **B. Recipient *Nicotiana tabacum***

*Nicotiana tabacum* (cultivated tobacco) is not a federal noxious weed<sup>1</sup>. It's an annually-grown herbaceous plant. It is found only in cultivation, where it is the most commonly grown of all plants in the *Nicotiana* genus, and its leaves are commercially grown in many countries to be processed into tobacco. It grows to heights between 1 to 2 meters. Research is ongoing into its ancestry among wild *Nicotiana* species, but it is believed to be a hybrid of *Nicotiana sylvestris*, *Nicotiana tomentosiformis*, and possibly *Nicotiana otophora*<sup>1</sup>.

## **C. Intended Engineered trait/phenotype**

Our transgenic *Nicotiana tabacum* has been engineered to emit light emit a pleasant, dim glow upon the addition of a proprietary formula (which we call 'Glowing Plant fuel').

## **D. Contact information**

If you have any questions or would like to discuss any of the contents of this letter, please contact us (email or phone preferred) at:

- Antony Evans
- Glowing Plant, Inc
- 665 3rd Street, Suite 250
- San Francisco, CA 94107
- Email: antony@glowingplant.com
- Tel: +1-415-779-6333

## **II. APHIS' Interpretation of Regulation 7 C.F.R § 340 dictates a finding that our bioluminescent transgenic *Nicotiana tabacum* is not a regulated Article**

### **A. APHIS has been clear that not all transgenic plants are subject to regulatory oversight**

APHIS defines a "regulated article" as (Part 340.1):

“Any organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest. Excluded are recipient microorganisms which are not plant pests and which have resulted from the addition of genetic material from a donor organism where the material is well characterized and contains only non-coding regulatory regions.”

Consistent with the PPA's definition of a plant pest, APHIS further defines a "plant pest" as:

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<sup>1</sup> [https://en.wikipedia.org/wiki/Nicotiana\\_tabacum](https://en.wikipedia.org/wiki/Nicotiana_tabacum)

“Plant pest: Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants.”

APHIS further claims that its regulations are consistent with the Coordinated Framework, because they apply "only to genetically engineered organisms or products which are plant pests or for which there is a reason to believe are plant pests, and not to an organism or product merely because of the process by which it was produced. APHIS has further stated that its concern arises only "when an organism or product is altered or produced by genetic engineering and one or more of its constituents (donor, vector/vector agent or recipient) comes from a family or genus of organisms known to contain plant pests. This is because there is a risk that certain undesirable traits may be transferred to the new organism and may survive when the organism is released into the environment."

APHIS reiterated this policy on several occasions, first when it introduced its notification and permit process for the confined release of transgenic organisms, and again during the proposed revision to its regulations. It has been clear that not all transgenic plants are to be regulated, and those that are belong to the limited group of "plant pests" as defined in the regulations.

#### **B. Bioluminescent transgenic *Nicotiana tabacum* does not fall within the regulatory definition of a "Regulated Article"**

Under APHIS regulations, a transgenic organism is considered a "regulated article" if the donor organism, recipient organism or vector agent(s) belongs to a genera or taxa designated in 7 C.F.R § 340.2 and the organism meets the definition of a plant pest. The language of the regulation requires that both criteria must be met to satisfy the definition of a regulated article.

For our bioluminescent transgenic *Nicotiana tabacum* none of the donor organisms, the recipient organism nor the vectors used to transform the plant belong to any taxa identified in § 340.2. Further, none of the genetic elements identified above are sourced from any plant pest. In addition, the recipient organism, *Nicotiana tabacum*, is not a plant pest. Therefore our bioluminescent *Nicotiana tabacum* does not satisfy either of the criteria set forth to qualify as a "regulated article".

Another definition of a "regulated article" includes transgenic organisms that are unclassified or whose classification is unknown. The genetic donor element sources and our bioluminescent *Nicotiana tabacum* itself are well classified and do not relate to the types of organisms that could raise concerns, such as pathogens, predators or parasites, or weeds or commercially available pollinators such as honeybees, bumble bees etc.

#### **III. Finding that transgenic bioluminescent *Nicotiana tabacum* is not a regulated article is consistent with previous precedents of APHIS determinations**

APHIS has previously made a number of determinations that transgenic plants, analogous to our proposed bioluminescent *Nicotiana tabacum*, are not "regulated articles". A number of these examples are posted and available on USDA's website.<sup>2</sup> A finding that bioluminescent *Nicotiana tabacum* is not a regulated article is consistent with these other determinations.

#### **IV. Conclusions**

In summary, our bioluminescent *Nicotiana tabacum* is not itself a plant pest and there are no plant pest elements involved in the genetic transformation, and further all sources for the genetic elements to be used have been fully classified. Therefore we respectfully ascertain that there is no basis to assume our plant is or will become a plant pest within the meaning of the Plant Protection Act.

We thank you in advance for your consideration and prompt confirmation that our transgenic bioluminescent *Nicotiana tabacum* plants are not a "regulated article" for the reasons described above. If you have any further questions please don't hesitate to contact us via the contact information supplied above.

Sincerely,

Glowing Plant, Inc.



By \_\_\_\_\_  
Antony Evans  
Chief Executive Officer

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<sup>2</sup>[http://www.aphis.usda.gov/wps/portal/aphis/home/?1dmy&urile=wcm%3apath%3a%2Faphis\\_content\\_library%2Fsa\\_our\\_focus%2Fsa\\_biotechnology%2Fsa\\_regulations%2Fct\\_reg\\_loi](http://www.aphis.usda.gov/wps/portal/aphis/home/?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_biotechnology%2Fsa_regulations%2Fct_reg_loi)