



660 PENNSYLVANIA AVE., SE, SUITE 302, WASHINGTON, DC 20003
(202) 547-9359 • FAX (202) 547-9429
WWW.CENTERFORFOODSAFETY.ORG

March 15, 2006

Docket No. APHIS-2006-0016; Comments on Tall Fescue and Italian Ryegrass Environmental Assessment (EA), Permit Numbers 05-278-01r and 05-278-02r

The Center for Food Safety (CFS) appreciates the opportunity to comment on an EA prepared by APHIS/BRS for a field trial for transgenic tall fescue (*Festuca (Lolium) arundinaceae*) and Italian or annual ryegrass (*Lolium multiflorum*) for Love County, Oklahoma (permit numbers 05-278-01r and 05-278-02r). Both grasses are wind pollinated and highly outcrossing, and both are considered to be serious agricultural weeds, and invasive in some habitats.

The proposed fescue/ryegrass field trial consists of three groups of 360 GE plants containing either the hygromycin resistance gene, the β -glucuronidase marker gene, or an antisense gene that reduces expression of the pollen allergen gene, *lol p 1*. An isolation distance of 400 m (1312 ft) was accepted by USDA, with additional requirements to limit gene flow by seed escape, and with a cleared border of 10 feet to allow survey for vegetative escape.

The accepted isolation distance is incrementally greater than the 900 ft isolation distance for the turf grasses of the AOSCA-based performance standards. The proposed isolation distance was based largely on a single study of transgenic tall fescue by the crop developer that found no pollination beyond 150 m, and secondarily on a study of perennial ryegrass that found pollination at the longest distance measured, 144 m.^{1,i,ii}

Although these studies provide useful preliminary data, they are insufficient to determine adequate isolation from wild relatives. The tall fescue

¹ About 1% pollination was found in the downwind direction at 150 m. USDA mistakenly indicated pollination at 200 m, which was instead the maximum distance tested in one of the experiments.

study contained only one year of data where the number of pollen-donor plants was comparable to the number of plants in the proposed field trial (225 in the published study, and 360 in the proposed trial). An earlier plot contained only 49 donor plants, and had considerably less pollen flow at shorter distances than the 225 plant plot the following year.

A significant limitation of the experiment was its relative insensitivity for detecting low levels of pollination. This limits the ability to draw general conclusions from this experiment about gene flow at longer distances. For example, a total of 1405 seedlings from recipient plants from the longest distance, 200 m, were analyzed for hybridization. At the gene flow rates minimally acceptable for conventional foundation seed, about 0.02% - 0.1%, only about 0.3 to 1.5 out of 1405 hybrid seedlings would be expected at 200 m. Furthermore, only four of the eight directions around the donor plants contained the majority of pollen flow. In the other four directions, no pollen flow was detected at 150 m. If only seedlings from the 200 m distance from the four principle directions where hybridization was detected are considered, only 0.125 or 0.626 seedlings would be expected on average based on 0.02% or 0.1% hybridization frequencies, respectively (based on 626 seedlings from those four directions).

Random variation in detection could easily cause these low frequencies to be missed. Even higher levels of gene flow at 200 m could easily have been missed in this experiment. Since even low levels of pollination can lead to gene escape if the transgenes are not deleterious to the wild relative, this experiment was not sensitive enough to reliably detect environmentally meaningful levels of gene flow.

USDA also noted that gene flow was not detected at up to 2294 m (7,526 ft) in this study. However, this experiment was even less sensitive in detecting gene flow than the experiment described in the preceding paragraphs. The recipient plants were placed only in the upwind direction from the field trial, where less gene flow is expected than downwind, based on the data from this and other studies. The closest recipient plants in this experiment were located about 890 m (2,920 ft) from the donor plants, leaving an untested gap of about 690 m (2,263 ft) between the 200 m limit of the primary experiment and this longer-distance experiment.

The cited study of gene flow in perennial ryegrass showed a leptokurtic pollen distribution, unmentioned in the EA, suggesting that low-level pollen flow may occur at distances considerably farther than measured in that study.

The EA also suggested that, because of its larger size, the 400 acre creeping bentgrass field trial in Oregon that allowed long-distance gene flow may not accurately represent gene flow distances for the smaller fescue/ryegrass trial. However, the EA failed to mention a creeping bentgrass field trial of a size similar

(286 plants) to the proposed fescue/ryegrass trial which produced pollination at 1400 ft, and estimated pollination of at least 1300 m (4,265 ft).ⁱⁱⁱ Although this latter study was performed with a different turfgrass species, it should not be dismissed until more adequate studies with tall fescue are available, or other data show that pollination distances are likely to differ substantially between the two species. Such data could include demonstration of significant differences in pollen characteristics such as size, density, longevity, etc., but such data were not discussed. On the other hand, research on tall fescue and creeping bentgrass pollen viability shows similar survival curves, and although methods were not identical, suggests that pollen survival of both species may be similar.^{iv,v} Therefore, the impact of this parameter on gene flow may be similar between the two grass species, and suggests that pollination distances could also be similar.

The EA also claims that the fields around the proposed trial site contain Bermudagrass or are unsuitable for fescue or ryegrass, because the area is too dry to support these cool-season grasses. There is no description of the size of these fields, however, and no indication that they were actually surveyed for wild tall fescue or ryegrass. Both species (as well as the closely related and sexually compatible perennial ryegrass) are known to be widespread in parts of Oklahoma, and there are water sources in the immediate area that would provide for a suitably irrigated habitat.^{vi,vii,viii,ix,x} The Nobel Foundation website indicates that plant surveys have been conducted for the ranch where the proposed field trial would be grown. These surveys should be consulted for the presence of conspecific wild relatives, as long as they carefully examined the riparian or other wetland areas where wild tall fescue or ryegrass would most likely be found.

Although USDA believed the data suggested that "...tall fescue pollen should be effectively contained," it also acknowledged that "the bentgrass studies raise some uncertainty with regard to the confinement of field releases of flowering transgenic grasses."^{xi} Therefore, additional data and arguments were presented that the transgenes would not cause environmental harm or increase the fitness of wild relatives, should gene flow occur. Reference was made to the safety of the beta-glucuronidase and hygromycin genes in previous USDA risk assessments, as well as other arguments beyond the scope of this report. The assessment of the *lol p 1* antisense gene is based on lack of expected harm from the loss of a major allergen.

There is no explicit assessment of unintended or pleiotropic effects, which could potentially be harmful. Sections on weediness and susceptibility to pests implicitly address this issue. However, although USDA cites a paper by the crop developer based on previous field trials as having addressed these issues, only two sentences, and no data, were devoted to the analysis of harmful and beneficial insects and diseases in the cited work. The paper is predominantly devoted to assessing agronomic properties of the transgenic grasses, and there is no indication that an adequate assessment of insects and diseases was performed.^{xii} Although several vegetative and seed production properties were

assessed, several other properties that are often relevant to fitness, such as seed dormancy and survival, were not evaluated. Overall, the risk assessment in the cited paper was minimal

Finally, although the genes in this field trial do not present any obvious environmental impacts or increased fitness, USDA did not consider the possibility that the genes may be fitness-neutral, and therefore persist in wild relatives. Combined with inadequate support for the isolation distance, and inadequate determination of whether wild relatives may exist nearby, the EA cannot assure that permanent gene flow to wild relatives will not occur. In addition, although there is not an *a priori* reason to believe that these genes would cause harm to the environment if gene flow occurred, the current risk assessment for the *lol p 1* gene amounts to little more than the kind of “lack of evidence” argument that the National Research Council criticized APHIS for in its 2002 report. Either additional risk assessment should be performed to ensure no environmental harm if gene flow occurs, or better confinement should be required so that the lack of gene flow can be ensured.

Conclusions

The pollination biology of tall fescue and Italian ryegrass have much in common with creeping bentgrass, which has pollinated plants beyond standard AOSCA isolation distances in at least two previous field trials. Therefore CFS believes that this field trial of transgenic tall fescue and Italian ryegrass should proceed only under stringent confinement that substantially exceeds AOSCA recommendations. Instead, the proposed isolation distance, 1312 ft, only incrementally exceeds the typical 900 ft isolation distance for conventional turfgrasses. Careful analysis of the data presented in the EA leads us to conclude that the confinement methods proposed for this field trial are inadequately supported, and that the risk assessment of the *lol p 1* mutation was cursory and inadequate.

Given the lack of adequate confinement these permits would not meet the conditions required under 7 CFR 340.4(f)(1), in that the permitted plantings would lead to the foreseeable dissemination and establishment of regulated articles that meet the definition of plant pests; further, it would violate 340.4(f)(4) by allowing viable forms of the regulated article to escape from the specified field trial area. The EA is inadequate under NEPA. It cannot provide the basis for a finding of No Significant Impact or justify the issuance of the proposed permits. APHIS is

urged to issue a revised EA that addresses the deficiencies discussed above, and to make that EA available for public comment.

Sincerely,

Doug Gurian-Sherman, Ph.D.
Senior Scientist

and

Peter Jenkins
Attorney/Policy Analyst

ⁱ Wang Z-Y et al, 2004, Pollen-mediated transgene flow in the wind-pollinated grass species tall fescue (*Festuca arundinaceae* Shreb), *Molec. Breeding* 14:47-60

ⁱⁱ Cunliffe KV et al, 2004, Assessment of gene flow using tetraploid genotypes of perennial ryegrass (*Lolium perenne*), *Aust. J. Agric. Res.* 55:389-396

ⁱⁱⁱ Wipff JK and Fricker C, 2001, Gene flow from transgenic creeping bentgrass (*Agrostis stolonifera* L.) in the Willamette Valley, Oregon, *Internatl. Turfgrass Soc. Research J.*, 9: 224-242

^{iv} Wang Z-Y, et al, 2004, Viability and longevity of pollen from transgenic and non-transgenic tall fescue (*Festuca arundinaceae*) (Poeaceae) plants, *Amer. J. Bot.* 91(4):523-530

^v Fei S and Nelson E, 2003, Estimation of pollen viability, shedding pattern, and longevity of creeping bentgrass on artificial media, *Crop Sci.* 43:2177-2181

^{vi} Tall Fescue, U.S. Plants Database, www.nps.gov/plants/alien/factsloar1.htm

^{vii} Nobel Foundation, Plant Image Gallery: Tall Fescue, www.noble.org/imagegallery/Grasshtml/TallFescue.html

^{viii} U.S. Plants Database, PLANTS Profile for *Lolium perenne*, <http://plants.usda.gov/java/profile?symbol=LOPEM2>

^{ix} Nobel Foundation, Plant Image Gallery: Ryegrass, www.noble.org/imagegallery/Grasshtml/Ryegrass.html

^x Aljoe H et al., Coffey Resource Management and Demonstration Ranch: Ten Years of Management and its Impact, <http://www.noble.org/ag/coffey/Ranch.HTM>

^{xi} USDA/APHIS Environmental Assessment, op. cit.

^{xii} Wang Z-Y et al., 2004, Field performance of transgenic tall fescue (*Festuca arundinaceae* Schreb) plants and their progenies, *Theor. Appl. Genet.* 107:406-412