GE Free New Zealand

In Food and Environment Inc.

PO Box 13402, Wellington, NZ

1 November 2023

Licence Application No. DIR 199

Re: Commercial release of banana plants genetically modified for resistance to *Fusarium* wilt tropical race 4 (TR4)

Dear OGTR chair and Committee members,

We oppose the Commercial release of banana plants genetically modified for resistance to *Fusarium* wilt tropical race 4 (TR4) on the ground of a lack of environmental information.

The DIR199 application should not be approved.

Gene Technology Act 2000[1]

1. The object of the regulatory framework is to act with a precautionary approach where there is scientific uncertainty.

   (aa) provides that where there are threats of serious or irreversible environmental damage, a lack of full scientific certainty should not be used as a reason for postponing cost-effective measures to prevent environmental degradation. (Gene Technology Act 2000, Sec:4)

2. We outline our concerns below over the lack of scientific data into the potential serious threats to the environment and health of people from the DIR199 GM banana.

1. The *fusarium* wilt is a problem of monocultures, soil contamination and climate factors that allow the fungus to persist in the soil and infect the roots of the plants as well as infecting the sucker/ratoons directly *via* vascular connection with diseased parent plants. Mutations of the *Fusarium oxysporum* f. sp. *Cubense* renamed *Fusarium odoratissimum* (*F.O*) wilt or Panama disease,

[1] https://www.legislation.gov.au/Details/C2016C00792

have not been studied on the GM Banana.

2. This poses a threat to the environment and livelihoods for any country that is importing banana cultivars. New Zealand is developing a small banana industry, and any transgenic contamination would pose threats to the emerging industry. [2]

3. Alternative solutions provide proven benefits over the unproven GM Banana, which lacks adequate data. These have made a significant impact on the suppression and control of *Fusarium oxysporum* f. sp. *Cubense* fungus that attacks bananas.

4. These existing solutions have not been implemented, and until they are the GM banana should not be approved for commercialisation.

5. We are concerned that prior to submissions on the consultation of the Food Standards application A1274 being closed, the OGTR has stated "*that the risks are negligible… as the introduced proteins are not expected to be toxic or allergenic".*

6. This is merely an assumption, with no experimental data to back it up. Assumptions of safety are not scientifically valid or necessarily accurate. They must be backed up by a convincing data set, that confirms food safety, based on reliable standard parameters.

Risks posed to the health and safety of people, and the environment.

7. We also have concerns about the risk assessment of the environmental effects of the banana and the level of risk having been assessed as "negligible."

8. Commercial banana plants are usually grown for around 10 years. In the 1930's *'Gros Michel'*, the dominant commercial banana was infected by *Fusarium* wilt. The Cavendish (*Musa cavendishii)* banana cultivars replaced the '*Gros Michel*' to become the dominant variety due to their resistance to *Fusarium* wilt in the 1950's.

9. The GM bananas will be sourced from pups/ratoons (clones), not seeds, the clones of the plants are identical. The GM banana ratoons have been shown to inherit the engineered genetic constructs through generations.

10. Over time the diversity and resistance to disease becomes weaker over the generations the clone pups are re planted.

[2] https://www.youtube.com/watch?v=dHNJdk52bGw

11. There is potential to pass on unknown new transgenic proteins to the soil and to become established in the microbial reservoir. This may result in resistance to the TR-4 trait, as the soil-borne fungus can take up root exudates of the transgenic banana, with possible mutations occurring. No studies have been carried out on the persistence threats of the *new unintended proteins, CaMV35S or, nptII* transgenes in the soil from exudates of GM banana plants on the environment or health and safety of people.

12. Intensive monocultures and the application of herbicide/s have denuded the soil, resulting in a loss of biodiversity, including that of beneficial soil microbes such as mycorrhizal fungi. Such a loss of soil microbial life has resulted in the persistence of *Fusarium* in the soil.

13. The TR4 banana could easily succumb to a mutated form of *Fusarium oxysporum* (*F.O.*) in a few generations if the growing conditions are not addressed. The intensive monocultures, herbicide/s have denuded the soil leading to the loss of biodiversity of beneficial mycorrhiza and rhizobia. This has led to the persistence and mutation of *fusarium spp:* in the soil. The TR4 banana will succumb to a mutation of the *F. oxysporum* in a few generations if the growing conditions are not addressed.

14. *F. oxysporum* has over 300 species and mutations are common. Moussa *et al* (2017) reported that six novel species had been found in Australian soil in 2015 and a totally new strain *Fusarium terricola* discovered in 2017.[3] The applicant has provided no evidence on the effects these new mutations will have on the GM Banana. Studies have shown that weeds[4] and pests[5] have evolved resistance or tolerance to the pesticides used on genetically engineered crops within 17 years. The resistance of these pests has become a serious threat to farmers livelihoods.

15. The GM bananas will have to survive any *Fusarium* mutation pressure over the 10 years they are in production. Due to the length of time a banana plant is in the soil, rotation is not feasible. In addition, *Fusarium* propagules persist in soil and will cause disease if the same plant genus is replanted at that site. New banana pups must be planted in a new site and the old site re-planted using unrelated species that are not susceptible to *Fusarium.*

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[3] Moussa TAA, Al-Zahrani HS, Kadasa NMS, Ahmed SA, de Hoog GS, Al-Hatmi AMS. Two new species of the Fusarium fujikuroi species complex isolated from the natural environment. Antonie Van Leeuwenhoek. 2017 Jun;110(6):819-832. doi: 10.1007/s10482-017-0855-1. Epub 2017 Mar 16. PMID: 28303400; PMCID: PMC5427105.

[4] International Herbicide-resistant database https://www.weedscience.org/Pages/Species.aspx

[5] Tabashnik, B., Carrière, Y. Surge in insect resistance to transgenic crops and prospects for sustainability. *Nat Biotechnol* **35**, 926–935 (2017). https://doi.org/10.1038/nbt.3974

16. There is an omission of scientific data considering the mutations which must be provided before license of the application.

Environmental impacts have not been adequately considered.

17. Studies with a variety of different plant species showed that they could "fumigate" the soil and suppress the growth of *F.oxysporum*. Zhang H., et al (2013) found that crop rotation with growing allium species namely, Chinese chives, was a way of controlling the *F.O* fungus. [6]

18. A study by Zhang N., et al (2014) studies on organic bio fertiliser consisting of matured compost mixed with *Paenibacillus polymyxa* SQR-21 and *Trichoderma harzianum* T37, *Bacillus amyloliquefaciens* N6, *Bacillus subtilis* N11, and the combination of N6 and N11. The authors reported that -

    " the application of BIOs significantly decreased the incidence rate of Fusarium wilt by up to 80% compared with the control. BIOs also significantly promoted plant growth, and increased chitinase and β-1,3-glucanase activities by 55%–65% and 17.3%–120.1%, respectively, in the banana roots. The population of FOC in the rhizosphere soil was decreased significantly to about 10^4 colony forming units g^{-1} with treatment of BIOs."[7]

19. The use of bio-organic solutions as a deterrent to F. oxysporum has also been described Fu. et al (2017)[8]

20. Hao Y., et al (2023) found that denuded soils become a reservoir for the *Fusarium* spp. fungus and that Australian soils now have fusarium at high levels in the soil. Where bananas are grown in Australia, they have hot weather and soil temperatures approach 26˚C. Dry weather and low soil moisture encourage this plant disease. Hao found that thymol showed effective inhibitory activity against *Fusarium oxysporum* by inhibiting mycelial growth, conidial production, and germination of the fungus.

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[6] Zhang H., Mallik A., Zeng R.S.(2013) Control of Panama disease of banana by rotating and intercropping with Chinese chive (Allium tuberosum Rottir): role of plant volatiles J Chem Ecol, 39, pp. 243-252

[7] Zhang N., He X., Zhang J., Raza W., Yang X-M., Ruan Y-Z, Shen Q-R., Huang Q-W. (2014) Suppression of Fusarium Wilt of Banana with Application of Bio-Organic Fertilizers, Pedosphere, Volume 24, Issue 5, 2014,pages 613-624, ISSN 1002-0160, https://doi.org/10.1016/S1002-0160(14)60047-3. (https://www.sciencedirect.com/science/article/pii/S1002016014600473

[8] Fu L., Penton C.Y., Ruan Y.Z., Shen Z.Z., Shen Q.R. (2017) Inducing the rhizosphere microbiome by biofertilizer application to suppress banana *Fusarium wilt* disease Soil Biol Biochem, 104 (2017), pp. 39-48.

Pesticide effects on soil and minerals

21. The banana is grown in barren soils sprayed with herbicides. The main one being Roundup - a glyphosate-based herbicide (GBH) known to chelate vital micronutrients and minerals like Ferrum, Boron, Manganese and Zinc.

22. These minerals and nutrients are vital for the health of the banana plant and if unable to access them water and *photosynthesis will be heavily affected. Fusarium spp: are relatively tolerant to Roundup* and can have a stimulated response to the herbicide.

23. Martinez DA et al[9] (2009) concluded that "GBHs have the potential to undermine crop health in a number of ways. These include:
    i. impairment of the innate physiological defenses of glyphosate-sensitive (GS) cultivars by interruption of the *shikimic acid* pathway.
    ii. impairment of physiological disease defenses has also been shown to occur in some glyphosate-resistant (GR) cultivars, despite their engineered resistance to glyphosate's primary mode of action.
    iii. interference with rhizosphere microbial ecology (in particular, GBHs have the potential to enhance the population and/or virulence of some phytopathogenic microbial species in the crop rhizosphere); and finally,
    iv. the as yet incompletely elucidated reduction in the uptake and utilisation of nutrient metals by crops.

24. It is noteworthy that, in contrast, some potentially phytopathogenic species express relatively glyphosate-tolerant forms of the EPSPS enzyme (including some *Fusarium, Pythium* and *Rhizoctonia* spp.) and are unharmed or even stimulated in response to treatment of plants with GBHs.

25. These differential impacts are of considerable concern, since they may cause alterations to microbial community dynamics that may, in turn, impact negatively upon the health and productivity of crops.

26. Ulrich *et al* (2023) Recently published information on the chronic kidney disease on the public from the drinking water contaminated with glyphosate-based

[9] Martinez DA, Loening UE, Graham MC. Impacts of glyphosate-based herbicides on disease resistance and health of crops: a review. Environ Sci Eur. 2018;30(1):2. doi: 10.1186/s12302-018-0131-7. Epub 2018 Jan 16. PMID: 29387519; PMCID: PMC5770481.

herbicides. In areas where irrigation is needed any glyphosate residue in the water would affect the plant if irrigated.

27. Ismaila *et al* (2023)[10] meta-analysis looked at solutions to the inhibition of F.O and found that crop rotation over two years, the application of silica as well as a detailed range of essential oils has a significant impact and reduction on the wilt in the soil and the health of the plants.
    a. "*crop rotation, and application of silicon (Si) have played an important role in minimizing the disease. Currently, the healthier and safer method to manage this tropical plant disease is the use of natural products from plants such as essential oils from ginger and other plants which already has gained wider recognition globally."*

28. Plants treated with combinations of a high content of Fe and B show increased resistance to *F. oxysporum* infection. High Fe and B contents in the plant correlated with decreased conidial germination rate, fungal growth, and FA production. F.O causes depletion of Fe and essential trace elements like boron and manganese. When these were supplemented, the plants were able to overcome the deleterious effects of the *F.O*.

29. Further plants infected with natural cauliflower Mosaic Virus (CaMV) were found to silence the expression of the transgenic CaMV35S promoter that allows the expression of the gene of interest and that "*transgene phenotypes can be modified by pathogen invasion"* (Al-Kaff 2000[11]) The CaMV promoter is influenced by the length of day or photoperiod. They reported -

    > *"Although the expression of transgenes using the CaMV 35S promoter may help elucidate their function, results from these experiments must be interpreted with caution due to several considerations. First, it should be noted that the CaMV 35S promoter has considerable variability in its expression patterns across different plant species, among and within tissues, and under different environmental conditions" (*Amack *et al* 2020.p. 4)[12]

30. Over time it is possible that the soil borne *F.O* and daylength could also silence the CaMV 35S promoter altering the function of the transgene and its

[10] Ismaila AA, Ahmad K, Siddique Y, et al. *Fusarium* wilt of banana: Current update and sustainable disease control using classical and essential oils approaches. *Horticultural Plant Journal*, 2023, 9(1): 1-28. https://doi.org/10.1016/j.hpj.2022.02.004

[11] Al-Kaff NS, Kreike MM, Covey SN, Pitcher R, Page AM, Dale PJ. (2000) Plants rendered herbicide-susceptible by cauliflower mosaic virus-elicited suppression of a 35S promoter-regulated transgene. Nat Biotechnol. 18(9):995-9. doi: 10.1038/79501. PMID: 10973223.

[12] Schnurr, J., Guerra, D. The CaMV-35S promoter is sensitive to shortened photoperiod in transgenic tobacco. *Plant Cell Reports* **19**, 279–282 (2000). https://doi.org/10.1007/s002990050012

susceptibility to *F.O*. As detected in table after 4 years there is a loss of resistance that if continues annually in ratoons then in 10 years the resistance will be negligible and new mutations will have evolved.

Unintended Gene Construct*s:*

31. We received under the FOI the "Compilation of Study Reports Studies Submitted in Support of the Food Safety Assessment of Fusarium Wilt Tropical Race 4 Resistant Banana Event QCAV-4"[13] These reports also included the environmental studies of the GM Banana. These reports show that the GM Banana poses serious and irreversible threats to the environment and economic livelihoods through the omission of supporting data that are greater than the existing threats from *F.O*.
32. The document "*QUT2023-1: Nucleotide sequence analysis"* of the inserted DNA and host genomic flanking regions in event QCAV state -. [14]
    a. The relative amounts of transcripts originating from the *nptII* selectable marker gene were high especially in the fruit of QCAV-4 consistent with the expected transgene controlled by the strong, constitutive CaMV35S promoter in banana. (QUT2023-4, 5.4, p.39)
    b. Multiple attempts to decipher the structure of the insert using conventional PCR and sequencing methods were unsuccessful due to the nature of the large, inverted rearrangement (QUT2023-1, p.5)
    c. Comparative analysis of the insertion locus in QCAV4 with its parent non-GM line (GN212-12) showed that a 116 bp deletion resulted from the integration of the insert into one of the three chromosome 6 loci of QCAV-4. (QUT2023-1, 1.3.2, p.6)
    d. Seven new and unintended ORFs resulting from the presence of the insert in QCAV-4 were identified from this analysis. (QUT2023-1, 1.3.3, p.7)
    e. The expression of the three intact copies present on the insert have been wrongly allocated by Geneious. (QUT2023-6, p.44).
33. These unintended deletions and insertions have not been scientifically understood as to their effects on the environment or health of people. It is not adequate to state that they have no biological significance, without scientifically conducted supporting evidence.

[13] https://www.gefree.org.nz/assets/Uploads/3-Event-QCAV-4-Compilation-of-Study-Reports-Redacted.pdf
[14] QUT2023-1: Nucleotide sequence analysis of the inserted DNA and host genomic flanking regions in event QCAV-4 https://www.gefree.org.nz/assets/Uploads/3-Event-QCAV-4-Compilation-of-Study-Reports-Redacted.pdf

34. Further, the expression of the three intact copies have been allocated wrongly (QUT2023-1 p.44) If this is incorrect, where are they supposed to be allocated and will this affect the expression of the TLR-4 or produce threat to the environment?

35. Regarding the 7 new and unintended ORFS. (QUT2023-6: Safety assessment of the seven new ORFs identified in event QCAV-4) The OGTR should not assume that there are no threats in the absence of any scientific investigation. The ORF proteins are new unintended constructs are not in the database to be able to confirm safety.

36. There is no data or studies on the persistence of the genetically engineered gene constructs in the banana peel to the micro-organisms when disposed of to landfill, home and commercial compost digesters.

37. What is the effect of the high expression of the *nptII* gene? There is no data, studies, or details on the persistence of disposal of banana skin into the environment. Banana skins are disposed of in many households, the *nptII,* kanamycin resistance gene, could persist and cause a buildup of antibiotic resistance bacteria adversely affecting people.

38. Compost material is used as nutrient dense fertilizer and conjugation of microorganisms are able through horizontal gene transfer uptake DNA and transfer it around their microbial community. This is made worse due to the lack of understanding as to the potential adverse harm shown in the unsuccessful attempt to decipher the insert structure, the new and unintended proteins or the 116bp deletion.

39. *The banana does show a weakening of the GM banana's resistance by the 4-stage ratoon generation*. Initially in the first three generations the resistance was zero but by generation 4 it became more susceptible to *F. oxysporum* and there was a 2% loss of resistance. (QUT2023-3: Field performance of banana event QCAV-4, Figure SR3-3 p.22)[15] The applicant provided QUT Compilation of Study Reports mention that there were six ratoon generations produced but only record the resistance data for 4 generations. There is a lack of information on whether there was a further loss of resistance in the 5 and 6 ratoon generation. It has also been noted that with each ratoon generation there is less diversity. So, over time there could be a *F. oxysporum* mutation that would be detrimental

[15] QUT2023-3: Compilation of Study Reports Studies Submitted in Support of the Food Safety Assessment of Fusarium Wilt Tropical Race 4 Resistant Banana Event QCAV-4
https://www.gefree.org.nz/assets/Uploads/3-Event-QCAV-4-Compilation-of-Study-Reports-Redacted.pdf

to the GM banana. If this occurs the genetic modification found in all the cells will persist, but the bananas will be heavily affected.

In Summary:

40. DIR199 has omitted to carry out scientifically supported studies to show that the GM Banana will not cause the spread of further fungal diseases.

41. There is an omission of environmental data on the long term threats the GM banana poses.

42. This application cannot be approved until further environmental testing is conducted on the transgene and on the new and unintended proteins. These studies on the GM Banana should confirm that there will be no adverse effects in the long term or lose resistance in the short term.

43. The alternative existing non-GM options should be tried first, and the GM banana must be only considered once the necessary data has been obtained on environmental and health safety, and if all other options do not work.

44. We ask that the OGTR undertake or commission independent research into risk assessment and the biosafety of the GM banana, before approving a license.

45. Until this has been conducted and the made data available to submitters, the application should be re submitted for public comment.

Yours sincerely,

Jon Muller
Secretary GE Free NZ in Food and Environment.

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