Plant & Food RESEARCH
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Dear Ms Bleakley,

The Environmental Protection Authority recently transferred to our organisation aspects of a request for information you made to that entity under the Official Information Act. Specifically, Plant & Food Research was requested to consider whether a section in an historic application made to ERMA by HortResearch (ref GMD99090), which was marked confidential at the time of application, could be released to you.

Information in this section of the application was considered commercially sensitive at the time of the application, however this is no longer the case and I have attached to this letter the relevant text. It may be of interest to you that the work proposed in the application to transform *Beauveria bassiana* with genes encoding biotin-binding proteins was never carried out.

Yours Sincerely,

Roger Bourne

Corporate Communications Manager

THE FOLLOWING SECTION IS CONFIDENTIAL

The moulting inhibitor protein is a biotin-binding protein. The genes for avidin (Gope *et al.*, 1987) and streptavidin (Thompson and Weber, 1993) have been cloned and prepared synthetically and fully characterized. These genes have been expressed as transgenes in bacteria (*E. coli*) (Airenne, *et al.*, 1994; Sano and Cantor, 1990), baculovirus (Airenne, *et al.*, 1997; Nagarajan *et al.*, 1993), and plants (tobacco) (Christeller *et al.*, 1998) without affecting the phenotype of the organism. We have found that these proteins, both as dietary supplements and in transgenic plants, prevent both lepidopteran larvae and orthoptera nymphs from completing a moult (unpublished data). Previous research with overexpressed fungal transgenes in the insect hemocoel have reduced the time to mortality by 0-20% (St Leger *et al.*, 1996). These are not considered economically significant.

These biotin-binding proteins, under long term experimentally-induced conditions, can have antinutritional effects on vertebrates by causing vitamin defficiency. Under normal dietary situations, biotin defficiency is never observed. It can be reversed quickly and easily by feeding or injection of excess biotin. Vertebrates commonly excrete 5-7 times more biotin than their daily requirement. There is no suggestion in 8 Material Safety Data Sheets, from a range of sources, examined that these proteins are toxic. Neither the fungus nor insect cadavers represent a food source for vertebrates. Since the expression is placed under organism specific signals, expression following gene transfer to a biotin-requiring organism would either not occur or would cause inviability. The likelihood of gene transfer is addressed elsewhere.

Airenne KJ, Oker-Blom C, Marjomaki VS, Bayer EA, Wilchek M, Kulomaa MS (1997) Production of biologically active recombinant avidin in baculovirus-infected insect cells. *Prot. Express. Purific.* **9**: 100-108.

Airenne KJ, Sarkkinen P, Punnonen E-L, Kulomaa MS (1994) Production of recombinant avidin in *Escherichia coli*. *Gene* **144**: 75-80

Christeller JT, Sutherland P, Murray C, Markwick NP, Phung MM (1998) Improvements in or relating to chimeric polypeptides of biotin binding proteins. New Zealand Provisional patent 389947, pp 39.

Gope ML, Keinanen RA, Kristo PA, Conneely OM, Beattie WG, Zarucki-Schultz T, O'Malley BW, Kulomaa MS (1987) Molecular cloning of the chicken avidin cDNA. *Nucleic Acids Res.* 15: 3595-3606.

Nararajan V, Ramaley R, Albertson H, Chen M (1993) Secretion of streptavidin from *Bacillus subtilis*. *Appl. Environ. Microbiol.* **59**: 3894-3898.

Sano T, Cantor CR (1990) Expression of a cloned streptavidin gene in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **87**: 142-146.

Thompson LD, Weber PC (1993) Construction and expression of a synthetic streptavidin-encoding gene in *Escherichia coli*. *Gene* 136: 243-246.