Journal of Invertebrate Pathology 136 (2016) 68–73

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Hygienic food to reduce pathogen risk to bumblebees

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ARTICLE INFO

Article history:
Received 22 September 2015
Revised 14 February 2016
Accepted 9 March 2016
Available online 9 March 2016

Keywords:
Commercially produced bumblebees
Disease
Pollination services
Bombus
Apicystis
Pollen

ABSTRACT

Bumblebees are ecologically and economically important pollinators, and the value of bumblebees for crop pollination has led to the commercial production and exportation/importation of colonies on a global scale. Commercially produced bumblebee colonies can carry with them infectious parasites, which can both reduce the health of the colonies and spillover to wild bees, with potentially serious consequences. The presence of parasites in commercially produced bumblebee colonies is in part because colonies are reared on pollen collected from honey bees, which often contains a diversity of microbial parasites. In response to this threat, part of the industry has started to irradiate pollen used for bumblebee rearing. However, to date there is limited data published on the efficacy of this treatment. Here we examine the effect of gamma irradiation and an experimental ozone treatment on the presence and viability of parasites in honey bee pollen. While untreated pollen contained numerous viable parasites, we find that gamma irradiation reduced the viability of parasites in pollen, but did not eliminate parasites entirely. Ozone treatment appeared to be less effective than gamma irradiation, while an artificial pollen substitute was, as expected, entirely free of parasites. The results suggest that the irradiation of pollen before using it to rear bumblebee colonies is a sensible method which will help reduce the incidence of parasite infections in commercially produced bumblebee colonies, but that further optimisation, or the use of a nutritionally equivalent artificial pollen substitute, may be needed to fully eliminate this route of disease entry into factories.

1. Introduction

Insect pollinators are essential for sustainable food production. While most of the major human food crops are not reliant on pollinators, insect pollinators are necessary for the production of a wide diversity of other food crops that contribute important micronutrients to human diets, and there is consequently great concern about declines in the wild populations of many pollinator species (Biesmeijer et al., 2006; Potts et al., 2010; Vanbergen et al., 2013). The economic importance of pollination has led to the commercial utilisation of bees for the pollination of many crops. Although the western honey bee *Apis mellifera* is the best known managed pollinator species, bumblebees are more efficient pollinators of certain plant species and hence several species of bumblebees (*Bombus* spp.) are also produced commercially for the pollination of a variety of fruit and vegetable crops in glasshouses, polytunnels and open fields (Velthuis and van Doorn, 2006). The bumblebee colonies are reared by a small number of companies, with over a million colonies now being produced and used on a global scale (Goulson and Hughes, 2015). Increasingly, local bumblebee species are being produced in local factories, but still a significant number of colonies are exported.

As with the production of any animal, the commercial production of bumblebees has to deal with the threat of disease. Bumblebees suffer from three main microbial parasites, the neogregarine *Apicystis bombi*, the trypanosome *Crithidia bombi*, and the microsporidian *Nosema bombi* (Schmid-Hempel, 2001). In addition, they can also be infected by the parasites *Nosema ceranae* and deformed wing virus, which are best known from honey bees but are now realised to have multi-host dynamics and to be widespread in bumblebees (Plischuk et al., 2009; Evison et al., 2012; Li et al., 2012; Fürst et al., 2014; Manley et al., 2015; McMahon et al., 2015). All of these parasites can have significant effects on

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http://dx.doi.org/10.1016/j.jip.2016.03.007
0022-2011/

bumblebees, reducing lifespan, fat stores, learning ability, and capacity to deal with other stresses (Schmid-Hempel, 2001; Graystock et al., 2013a; Fürst et al., 2014; Graystock et al., 2016). Parasite infections are therefore very likely to reduce the pollination services that a commercially produced bumblebee colony will provide to farmers, in addition to presenting a threat of parasite spillover to wild bees. Many studies have shown that commercially produced bumblebee colonies are often infected by a diversity of parasites (Goka et al., 2000; Whittington and Winston, 2003; Gegear et al., 2005; Colla et al., 2006; Otterstatter and Thomson, 2007; Manson et al., 2010; Singh et al., 2010; Meeus et al., 2011; Graystock et al., 2013b; Murray et al., 2013; Sachman-Ruiz et al., 2015). There is correlative evidence that parasites from commercially produced bumblebees have spilled over to wild bumblebees in at least North America, South America and Japan, and of there being concordant declines of wild bumblebees in North America and Argentina (Goka et al., 2001; Colla et al., 2006; Otterstatter and Thomson, 2008; Plischuk and Lange, 2009; Plischuk et al., 2011; Szabo et al., 2012; Arbetman et al., 2013; Maharramov et al., 2013; Schmid-Hempel et al., 2014).

One of the major reasons why commercially produced bumblebee colonies continue to carry parasites is that the colonies are reared on pollen collected from honey bees (Goulson and Hughes, 2015). Honey bee pollen is often contaminated with a diversity of bee parasites, both of honey bees and bumblebees (Singh et al., 2010; Graystock et al., 2013b), which may be because the honey bees themselves were diseased or because the flowers they visited have been contaminated by previous pollinator visits (Graystock et al., 2015). Feeding commercially produced bumblebees with pollen contaminated with bumblebee parasites is problematic enough, but there is also growing evidence that some of the honey bee parasites found in pollen can infect bumblebees too, notably *N. ceranae* and deformed wing virus (Graystock et al., 2013a; Fürst et al., 2014; Meeus et al., 2014a; Manley et al., 2015; McMahon et al., 2015). There are two solutions to the problem of rearing commercially produced bumblebees on parasite-contaminated pollen food: (1) replace the pollen with a hygienic, artificial pollen substitute, or (2) sterilise the pollen in some way to kill any parasites that it contains. To date, there is no commercially available artificial pollen substitute for rearing bumblebees over multiple generations, and the challenge for sterilising pollen is developing a method which is effective at killing all parasites without negatively affecting the nutritional value of the pollen for the bees.

Irradiation and ozone (O_3) treatment are two methods commonly used to kill microbes on food for human consumption that have been considered for sterilising pollen, with gamma irradiation having been shown to reduce the viability of the Israeli acute paralysis virus in pollen (Yook et al., 1998; Meeus et al., 2014b). At least one major producer of bumblebees (Biobest) now exclusively uses irradiated pollen in its factories. However, the effectiveness of irradiation against the full diversity of bee parasites that can be present in pollen is not known. Here we examine the effectiveness of gamma irradiation, as well as an experimental method of ozone treatment and an artificial pollen substitute, for providing parasite-free food for bumblebees.

2. Materials and methods

In order to compare the effectiveness of pollen sterilisation methods, our experiment tested six treatments: (1) irradiated, fresh pollen (processed for the experiment immediately upon receipt from the pollen supplier), (2) ozone-treated, fresh pollen, (3) untreated, fresh pollen, (4) untreated pollen that had been stored frozen for >2 years, or (5) Nutri-bombus artificial pollen substitute control, in each case made up as 40% w/v suspensions in sucrose solution, or (6) sterile 40% sucrose solution control. The pollen for Treatments 1, 2 and 3 was provided by Biobest. While Biobest only uses irradiated pollen on its premises, the pollen for these treatments was taken at delivery from a batch before it was irradiated. As a consequence, Treatments 1, 2, and 3 came from the same batch of pollen, allowing direct comparisons between the treatments. Pollen for Treatment 1 underwent gamma irradiation of 16.9 kGy in the GAMMIR irradiation cell of Sterigenics (Fleurus, Belgium), while pollen for Treatment 2 received ozone (O_3) treatment from an external contractor under a nondisclosure agreement; both methods are used to remove to kill microbes on human food and have previously been shown to have potential for sterilising pollen (Yook et al., 1998; Meeus et al., 2014b). The pollen for Treatment 4 was purchased from a major distributor of commercially produced bumblebee colonies, had been dehydrated and stored for at least two years, and was hard and grainy compared to the fresh pollen, which was soft and fluffy in texture. The Nutri-bombus pollen substitute is a new experimental diet for bumblebees that was developed and provided by Nutrifeed Canada Inc. Four samples of pollen from the same batch provided by the supplier were collected randomly for each treatment and checked by PCR or RT-PCR (see below) for the presence of 13 parasites: *C. bombi*, *A. bombi*, *N. bombi*, *N. apis*, *N. ceranae*, deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), black queen cell virus (BQCV), sacbrood virus (SBV), *Ascosphaera* fungi, American foulbrood and European foulbrood bacteria.

The experiment used 15 *Bombus terrestris terrestris* colonies that were provided by Biobest. Colonies were queenright, each with ~120 workers, and appeared in good health. Initially, 16 workers from each colony were screened for disease using PCR (see below), representing about 13% of the colony population. This screening identified four colonies that appeared to be uninfected by any parasites and which were selected for use in the experiment. Three of these colonies were confirmed by the additional bees screened during the experiment to have been genuinely free of parasite infections (see Section 3). However, one of the four 'uninfected' colonies (Colony 3) was subsequently found to have low prevalence (<10%) infections with *A. bombi* and *N. ceranae*, and the experimental results were therefore analysed both including and excluding this colony (see Section 3). Each of the six treatments was fed to 64 bees from the four selected colonies (16 bees per colony). For this, bees were initially starved for 8 h, then placed individually into an Eppendorf tube with a small hole at the end through which they were hand-fed a 5 µl dose of the treatment. All treatment solutions were thoroughly vortexed immediately before use to ensure pollen or Nutri-bombus particles were fully in suspension. The bees were then placed in groups of 8 like-treated nestmates in 10 × 6 × 6 cm plastic boxes, provided with 40% sucrose solution *ad libitum*, and their survival checked daily for 14 days. Any bees that died during the experiment were stored at −80 °C. All of the experimental bees which survived at the end of 14 day period, as well as all bees which died during the experiment, were screened by PCR or RT-PCR for seven parasites that infect adult bees: *C. bombi*, *A. bombi*, *N. bombi*, *N. apis*, *N. ceranae*, DWV and IAPV.

2.1. Parasite screening

In order to check for the presence of parasites in the pollen used for the experiment, four samples of each of the six treatments were screened prior to the experiment for the parasites *A. bombi*, *C. bombi*, *N. bombi*, *N. ceranae*, *N. apis*, *Ascosphaera*, American foulbrood and European foulbrood using conventional PCR, and for the DWV, IAPV, Kashmir bee virus, black queen cell virus, and sacbrood virus using RT-PCR. In order to check whether the bumble-