



Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States

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ABSTRACT

Using standard epidemiological methods, this study set out to quantify the risk associated with exposure to easily diagnosed factors on colony mortality and morbidity in three migratory beekeeping operations. Fifty-six percent of all colonies monitored during the 10-month period died. The relative risk (RR) that a colony would die over the short term (~50 days) was appreciably increased in colonies diagnosed with Idiopathic Brood Disease Syndrome (IBDS), a condition where brood of different ages appear molten on the bottom of cells (RR = 3.2), or with a “queen event” (e.g., evidence of queen replacement or failure; RR = 3.1). We also found that several risk factors—including the incidence of a poor brood pattern, chalkbrood (CB), deformed wing virus (DWV), sacbrood virus (SBV), and exceeding the threshold of 5 Varroa mites per 100 bees—were differentially expressed in different beekeeping operations. Further, we found that a diagnosis of several factors were significantly more or less likely to be associated with a simultaneous diagnosis of another risk factor. These findings support the growing consensus that the causes of colony mortality are multiple and interrelated.

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1. Introduction

Honey bees (*Apis mellifera* L.) play a vital role in modern agriculture. An estimated 35% of the western human diet benefits—directly or indirectly—from honey bee pollination (Klein et al., 2007). While colony numbers have increased globally over the last 60 years (Aizen et al., 2008), this increase has not kept pace with increased acreages planted with pollinator-dependent crops (Aizen and Harder, 2009). Additionally, increases in colony

numbers have not been consistent across all regions, with long-term losses documented in the US and European nations (Potts et al., 2010; NRC, 2006). These trends have raised fears that demand for pollinating units will outstrip supply in the future (NRC, 2006). While some have questioned the basis of these fears (Ghazoul, 2005), researchers agree that there is a need for consistent and reliable enumeration of pollinator populations and focused research investigating the causes of mortality (Neumann and Carreck, 2010; Nguyen et al., 2010b).

In recent years, there has been increased attention paid to documenting overwintering honey bee colony mortality in North America (vanEngelsdorp et al., 2008, 2010a, 2011; Currie et al., 2010) and Europe (Potts et al., 2010; Brodschneider et al., 2010; Nguyen et al., 2010a). While these efforts have not attempted to empirically test

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the causes of mortality, most have accepted self-reports from beekeepers about which factors they believe most likely contributed to colony mortality in their particular operation (vanEngelsdorp et al., 2008, 2010a, 2011; Brodschneider et al., 2010). These factors are generally limited to those with which beekeepers are most familiar and can most readily diagnose. Some factors self-identified by survey respondents as leading causes for increased mortality, such as Varroa mite parasitism, have been corroborated by more systematic empirical surveys (Haubruge et al., 2006; Chauzat et al., 2010b; Guzmán-Novoa et al., 2010). While Varroa mites clearly contribute to colony mortality, other factors (including pesticide exposure, other bee parasites and pathogens, foraging conditions in the fall, and beekeeper management) may also negatively affect colony survival (vanEngelsdorp and Meixner, 2010). There seems little doubt that various factors can interact with one another. In Denmark, for instance, elevated losses were compounded when weather conditions in the fall prevented effective mite treatments, facilitating higher mite loads on bees that may not have had optimal pollen stores (Vejsnæs et al., 2010).

The objective of this study was to identify and quantify risk factors associated with annual colony mortality in migratory beekeeping operations in the eastern U.S. Specifically, we monitored risk factors that are readily identified during colony inspection or quantified by standard diagnostic techniques of sampled bees. These factors included clinical outbreaks of American foulbrood, European foulbrood, chalkbrood, sacbrood, deformed wing virus, queen events, brood pattern quality, and Varroa mite and Nosema spore load. Brood pattern quality is a general measure of queen and colony health and poor brood survival can be linked to reduced honey production (Woyke, 1981). A good brood pattern, as indicated by solid patches of capped brood, indicates the queen is laying viable eggs which are developing into healthy larval and pupal bees. Poor brood quality, indicated by large numbers of empty cells among capped cells, are indicative of a poor queen or disease (hygienic bees typically remove diseased larvae and pupa, leaving empty cells). In addition, we monitored Idiopathic Brood Disease Syndrome (IBDS), a syndrome first described by Shimanuki et al. (1994), but renamed here because its underlying cause is yet unknown. IBDS is diagnosed by the presence of brood at different ages that appear molted on the bottom of cells or have other symptoms reminiscent of, but not caused by, infection with American foulbrood (AFB; *Paenibacillus larvae*), European foulbrood (EFB; *Melissococcus pluton*), or sacbrood virus (SBV). Shimanuki et al. (1994) described this syndrome as part of their study of Parasitic Mite Syndrome (PMS), but unlike the symptoms of PMS in adult bees it is not believed that IBDS is caused by Varroa mites.

We used basic epidemiological methods to calculate and compare the relative risk associated with exposure to these easily-quantified putative risk factors. This risk-factor approach is commonly used in human studies to inform future hypothesis-driven analytical studies designed to elucidate causes of disease and mortality (Koepsell and Weiss, 2003). Just as in human studies, we intend for the results of this study to highlight areas for future research

intended to aid us in understanding and mitigating colony losses.

2. Materials and methods

2.1. Colony selection

This cohort study monitored honey bee, *A. mellifera*, colonies in three migratory beekeeping operations (OP1, $n=20$; OP2, $n=24$; OP3, $n=18$). The study was conducted during a 10-month period (mean=300 days) between March 2007 and January 2008. The selected operations were considered representative of East coast migratory operations, as the beekeepers transported their beehives north and south within the eastern United States to pollinate and/or produce honey on a diverse variety of crops and natural vegetation (Fig. 1). All colonies travelled from the state of Florida to New Jersey or Maine and back to Florida within the year, pollinating various crops en route. Colonies were selected randomly from an apiary within each beekeepers operation that contained colonies that had survived the previous year and were likely to be of similar lineages. Selected colonies were tagged with individually numbered cattle ear tags for tracking purposes. Upon first inspection, each colony's queen was located, marked, and had one of her wings clipped to help monitor queen replacement. Wing clipping is not thought to have a negative impact on queen longevity and was done in addition to marking queens because queen markings are commonly removed from marked queens by her attending workers (Laidlaw and Page, 1997). Surviving colonies were inspected at intervals that varied depending on the frequency that the colonies were moved (Fig. 1).

2.2. Colony measurements

During each inspection, the condition of monitored colonies was first noted. Colonies were considered to be dead when they were found completely depopulated of live adult bees. The strength of surviving colonies was assessed by separately estimating the number of frames covered with adult bees and containing capped brood (DeGrandi-Hoffman et al., 2008). The quality of brood was also assessed by averaging the number of empty brood cells in four randomly-selected patches of contiguous capped brood (100 brood cells per patch). When the four patches had an average of $\geq 20\%$ empty cells, the brood pattern was considered to be 'poor'.

During inspection, clinical symptoms of disease were noted, including chalkbrood (CB) *Ascosphaera apis*; European foulbrood (EFB), *M. pluton*; American foulbrood (AFB), *P. larvae*; sacbrood virus (SBV); and deformed wing virus (DWV). Also clinical symptoms of Idiopathic Brood Disease Syndrome (IBDS) were noted, as described above and by Shimanuki et al. (1994).

During each inspection, the condition of a colony's queen was also assessed. Attempts were always made to find the original marked and clipped queen. In cases where the marked queen was not found (12.5% of the time), it was assumed that she was present if eggs were found in the brood nest. A colony was diagnosed as having experienced

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