Prevalence and persistence of deformed wing virus (DWV) in untreated or acaricide-treated Varroa destructor infested honey bee (Apis mellifera) colonies

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Stephen J Martin
University of Salford

Norman Carreck
University of Sussex

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ORIGINAL RESEARCH ARTICLE

Prevalence and persistence of deformed wing virus (DWV) in untreated or acaricide-treated *Varroa destructor* infested honey bee (*Apis mellifera*) colonies

Stephen J Martin[1*], Brenda V Ball[2] and Norman L Carreck[2,3]

[1]Department of Animal and Plant Sciences, Sheffield University, Western Bank, Sheffield, S10 2TN, UK.
[2]Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK.
[3]Present address: Department of Biological and Environmental Science, University of Sussex, Falmer, Brighton, East Sussex, BN1 9QG, UK.

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*Corresponding author: Email: S.J.Martin@sheffield.ac.uk

Summary

The ectoparasitic mite *Varroa destructor* is a serious pest of the honey bee *Apis mellifera*. The naturally occurring virus known as deformed wing virus (DWV) has long been linked with the collapse of mite infested honey bee colonies. We therefore surveyed the prevalence and persistence of DWV in four heavily infested untreated colonies (Survey 1), and five heavily infested colonies that were treated with an acaricide (Survey 2). The presence of DWV in samples of adult bees, sealed brood and mites was detected using an Enzyme Linked Immunosorbent Assay (ELISA). Twenty individuals of each type were sampled monthly from each colony over the course of the study. During the summer, the proportion of adults, sealed brood and mites in which DWV was detected increased until either the colony died or was treated. When colonies were treated, thus removing mites from the colony, DWV became undetectable in the sealed bee brood at a similar rate to the loss of mites. The speed at which DWV became undetectable in adult workers depended, however, on the season, reflecting differences in life span between adult workers emerging in summer or winter. If treatment was delayed until October, DWV was still detected in adult bees during the winter even in the absence of mites. To reduce the viral load of the colony, therefore, mite treatment should be started no later than the end of August in order to remove the mites before production of the overwintering bees begins.

Prevalencia y persistencia del virus de alas deformadas (DWV) en colonias tratadas con acaricidas y no tratadas de abejas (*Apis mellifera*) infestadas con *Varroa destructor*

Resumen

El ácaro ectoparásito *Varroa destructor* es una seria plaga de la abeja *Apis mellifera*. El virus conocido como virus de las alas deformadas (DWV son su siglas en inglés) se ha relacionado con el colapso de las colonias de abejas infestadas con el ácaro. Por lo tanto examinamos el predominio y la persistencia del DWV en cuatro colonias no tratadas fuertemente infestadas (encuesta 1), y cinco colonias fuertemente infestadas que fueron tratadas con un acaricida (encuesta 2). La presencia de DWV en muestras de abejas adultas, de cría sellada y de ácaros fue detectada usando un análisis inmunosorbente de enzima ligado (ELISA). Se muestrearon mensualmente veinte individuos de cada tipo de cada colonia durante el estudio. Durante el verano, la proporción de adultos, de cría sellada y de ácaros en los que se detectó DWV fue aumentando hasta que la colonia murió o fue tratada. Cuando las colonias fueron tratadas, eliminando así los ácaros de la colonia, el DWV llegó a ser indetectable en la cría de la abeja sellada a una tasa similar a la pérdida de ácaros. La velocidad a la cual DWV llegó a ser indetectable en obreras adultas dependió, sin embargo, de la estación, reflejando diferencias en el rango de vida entre las obreras adultas que emergían en verano o en invierno. Si el tratamiento se retrasaba hasta octubre, el DWV todavía fue detectado en abejas adultas durante el invierno incluso en ausencia de ácaros. Por tanto para reducir la carga viral de la colonia, el tratamiento acaricida debe comenzar antes del final de agosto para eliminar los ácaros antes de que empiece la producción de las abejas para la invernada.

Keywords: *Varroa destructor*, deformed wing virus, *Apis mellifera*, virus prevalence, virus persistence, threshold

Introduction

During the last 50 years, the ectoparasitic mite *Varroa destructor* (Anderson and Trueman), has become the most serious pest of the honey bee *Apis mellifera* L. As eradication of the mite, even in a small closed honey bee population has not proved to be possible (Sampson and Martin, 1999), beekeepers must control mite populations in order to ensure the long term survival of their colonies. For any effective control strategy, the size of the mite population which either causes the collapse of the colony (the "damage threshold") or significantly affects the honey production or pollination efficiency of the colony (the "economic threshold") needs to be known. The "economic threshold" has been determined to be around 3,000-4,000 mites per colony in the southern USA (Delaplane and Hood, 1999) and the "damage threshold" to be around 2,500-3,000 mites per colony in the UK (Martin, 1999). Both the colony size and the presence of certain naturally occurring bee viruses may, however, affect these threshold values, and this may help to explain why variations in threshold values have been found in previous studies (Delaplane and Hood, 1999; Martin, 1999). A better understanding of the prevalence and persistence of certain mite transmitted viruses in the honey bee colony is needed. Although around 14 honey bee RNA viruses are known (Bailey and Ball, 1991) only a small sub-set are associated with the presence of *V. destructor* (Todd *et al.*, 2007; Carreck *et al.*, 2010a). These include deformed wing virus (DWV), acute bee paralysis virus (ABPV), slow paralysis virus (SPV) and Kashmir bee virus (KBV), whereas others such as cloudy wing virus (CWV) (Carreck *et al.*, 2010b) and black queen cell virus (Declan Schroeder, personal communication) appear to be entirely independent of the mite. Although ABPV, KBV and SPV are known, or strongly suspected, to be vectored by *V. destructor* (Todd *et al.*, 2007), modelling work (Martin, 2001; Sumpter and Martin, 2004) indicated that very large (10,000+) mite populations are required for these highly virulent viruses to become established within a colony. This may help to explain their poor association with collapsing colonies that are infested by *V. destructor*. Overt DWV infections can, however, be maintained in asymptomatic worker honey bees (Martin, 1991; Bowen-Walker *et al.*, 1999; Nordström *et al.* 1999; Gisder *et al.*, 2009) and may explain why it is currently one of the most widespread honey bee viruses (Allen and Ball, 1996; Ellis and Munn, 2005). The close link between *V. destructor* and DWV is shown by the increased prevalence of DWV after the spread of the mite in: the UK (Carreck *et al.*, 2002; 2010b); France (Tentcheva *et al.*, 2004); central Europe (Berenyi *et al.* 2006) and the USA (Ribière *et al.*, 2008). It has also been shown that DWV can cause reduced longevity of infected bees, which can ultimately lead to colony collapse (Martin, 2001; Nordström, 2003; Sumpter and Martin, 2004; Chen *et al.*, 2004; Yang and Cox-Foster, 2007).

The present study describes the prevalence and persistence of detectable DWV infections in bees and mites from two independent surveys; the first in 1997-8 that studied four untreated mite infested colonies, and the second in 1999-2000 that studied five infested colonies that were later treated with an acaricide. The aim of the two surveys was to test the hypothesis that DWV prevalence and persistence in colonies is dependent on the presence of *V. destructor*.

Materials and methods

Survey 1: untreated colonies

In May 1997, 20 queenright *A. mellifera* colonies naturally infested with *V. destructor* were established at St Albans, Hertfordshire, UK. Each month, the numbers of adult workers and sealed brood cells were estimated using photographic standards (Martin, 1998a). At the same time, 100-300 live adult workers, 100 sealed worker brood cells and 30 fallen mites were collected and stored at –10°C. Mite population estimates were obtained by calculating the proportion of the 100-300 adult workers (by washing) and 100 sealed worker brood (by opening cells) that were infested. These proportions were then combined with the photographic estimates of adult and sealed brood to calculate the total mite population within each colony. In May 1997 virus analysis of pooled mite (n = 10 mites / colony) samples was used to establish which viruses were present in the study colonies (Carreck *et al.*, 2010b). All 20 colonies died during the winter period of 1997-8. Four colonies were then chosen (U1-U4) for detailed DWV analysis of the stored samples. These four colonies were chosen as they represented the range of mite populations and time of colony collapse of the original 20 colonies. That is, amongst the 20 colonies, U1, U2, U3 and U4 had the 6^{th}, 12^{th}, 18^{th} and 19^{th} highest mite populations, and were the 2^{nd}, 9^{th}, 16^{th} and 19^{th} to collapse.

Survey 2: treated colonies

During May 1999, five queenright honey bee colonies (T1-T5) containing no or very few mites were artificially infested with around 1,000 mites by introducing approximately 2,000 infested sealed brood and 500-1,000 adult workers from two heavily (> 40%) infested colonies from an apiary within 3 km of the study site. Virus analysis of a pooled sample (n = 10) of the introduced mites confirmed that DWV was present in both the bees and mites. From May 1999, all colonies were sampled monthly and their mite and bee population sizes estimated as described for the untreated colony survey. The time of treatment was determined according to the general health and size of the colony, together with the proportion of mites testing positive for DWV. We waited until the mite population was close to, or had exceeded, the theoretical "damage threshold" of 2,000-3,600 DWV carrying mites (Martin, 2001). Each colony was then treated with four flumethrin (Bayvarol®) strips, for a period of six weeks. Mite resistance to flumethrin was not reported in the study area until four years after the study was conducted (Martin, 2004). After treatment until July 2000, all fallen mites from the "Varroa floors" (not sticky boards) were counted in addition to 20-50 live adult workers that were collected monthly for DWV analysis. Colony estimates were not carried out during the winter in order to avoid unnecessary colony disturbance.

Analysis of samples

The precise numbers of adult workers and any associated mites in the 100-300 bee samples were counted, and 20 adult workers and 20 mites, if present, were selected for DWV analysis. All brood cells in the sample were carefully opened to determine the proportion of infested cells. Twenty uninfested white-eyed pupae and (if present) twenty infested pupae and their associated mites were collected for DWV analysis. After treatment in Survey 2, ten mites and twenty live workers were tested each month for DWV. All samples were tested individually for the presence of DWV using an enzyme-linked immunosorbent assay (ELISA) developed by Allen *et al.* (1986) and reported in full by Nordström *et al.* (1999). This ELISA has a detection limit of around 10^7 viral particles per ml. In this system, covert or overtly infected mites or bees are normally indistinguishable unless the adult bees have deformed wings. Asymptomatic honey bees i.e. those with normal wings, with DWV infections of > 10^7 particles have, however, a reduced longevity (Martin, 2001; Yang and Cox-Foster, 2007), which leads to colony collapse (Sumpter and Martin, 2004). Yue *et al.* (2007) suggested that a distinction should be made between "true inapparent" and their newly defined "covert infection" based on the long-term nature of DWV infection in honey bee colonies and by the nature of its transmission. Sorrell *et al.* (2009) state, however, that a distinction should be made between asymptomatic infections that are still horizontally transmitted such as DWV, and covert infections. Throughout this study we will therefore simply refer to whether DWV was detectable or not using the ELISA. In all cases this will be a replicating 'overt' infection that can be transmitted horizontally despite the bees often being asymptomatic.

Data Analysis

As the total numbers of adult workers, sealed brood and mites were known from the photographic standards, the total number of *infested* adults and sealed brood can be estimated by:-

Number of infested adults = (proportion of adults infested x total number of workers)

Then using the proportion of individual mites (n = 10) or adults (n = 20) testing positive for DWV the total number of DWV *infected* adults, sealed brood and mites can be estimated by:-

Number of infected mites = proportion of DWV positive mites x total number of mites

The numbers of non-infested, infested and DWV infected adults and sealed workers in each colony at each sampling point were calculated using an Excel spreadsheet.

An independent t-test was used to compare the peak mite and bee populations between the colonies of the two surveys, and a paired t-test was used to investigate differences between infested and DWV infected adults or brood over the summer or autumn / winter periods.

Results

Survey 1: untreated colonies

Nineteen of the 20 colonies died before April 1998, and DWV was detected in all 20 colonies (see Carreck 2010a). The surviving colony, which initially had the lowest mite population of all, died later in 1998. Fig. 1 shows the prevalence and persistence of DWV in the four colonies studied in detail. These died between August 1997 and April 1998 and had estimated peak mite populations of 4,000 to 12,000. All colonies showed a similar pattern, with an increasing proportion of adult workers, sealed brood and mites in which DWV was detected during the year. The most rapid change in the proportion of DWV positive samples (adults, brood and mites) occurred when the bee population began to decline naturally during July and August as the colonies prepared for overwintering.

During the summer (March to August) the number of adult workers in which DWV was detected was significantly greater ($t = -5.08$, $df = 12$, $p < 0.0001$) than the number of adult workers infested by mites, whereas during the autumn and winter (September to February) this difference disappeared ($t = -0.334$, $df = 9$, $p = 075$). During the summer, however, the number of sealed pupae in which DWV was detected was significantly less ($t = 2.619$, $df = 9$, $p = 0.028$) that the number of pupae infested by mites, and again this difference disappeared during autumn ($t = 1.88$, $df = 5$, $p = 0.12$). Among the four colonies the proportion of mites in which DWV was detected increased during the season (Fig. 1).

Colony U4 had the lowest mite population, with an estimated peak mite population of 4000, and was just able to survive through the winter before dying in March 1998. In this colony, adults and mites in which DWV was detected were present throughout the winter.

Table 1. The proportion (%) of fallen mites in which DWV was detected based on analysis of 10 individual mites (if present) per month, per colony. Percentages in bold are values less than 50%. The number of falling mites (N) is given and counts start after colony treatment.

Colony #	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
T1 N	90% 6350	66% 366	90% 124	50% 38	55% 13	100% 1	50% 2	**42%** 12	**0%** 2	50% 2			
T2 N		76% 13317	65% 140	70% 29	67% 18	100% 1	67% 3	60% 5	**20%** 5	**17%** 18	**8%** 13		**30%** 36
T3 N			68% 2808	70% 75	**45%** 43	50% 6	**40%** 17	**45%** 30	**10%** 41	**15%** 20	**20%** 53		**40%** 18
T4 N			88% 6543	95% 1348		70% 123	**0%** 4	66% 3	80% 22	**20%** 20	**35%** 37	**25%** 36	**30%** 47
T5 N				70% 4322	67% 15	**0%** 2	**0%** 1			**0%** 2	**21%** 14	**23%** 13	60% 66
Mean N	90% 6350	76% 13683	82% 9615	76% 5812	55% 89	68% 133	**36%** 27	**47%** 50	**32%** 70	**18%** 62	**24%** 117	**24%** 49	**43%** 167

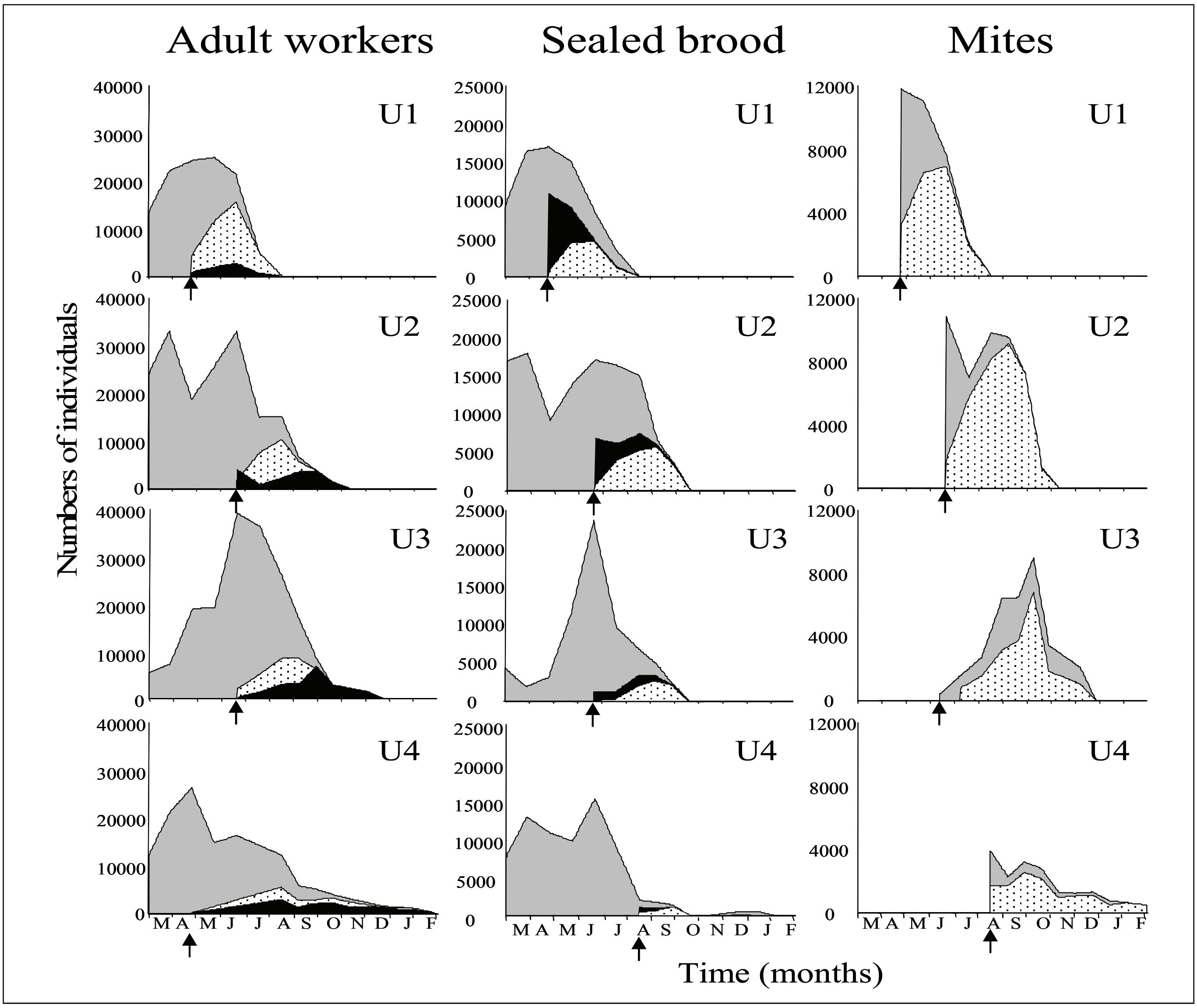

Fig. 1. The four untreated colonies (U1-U4) showing the estimated numbers of adult workers, sealed brood and mites testing negative for DWV (grey); numbers of adults and brood infested by mites (black) and number of adults, brood and mites testing positive of DWV (dotted). The arrow indicates when bee and mite samples were analysed for the presence of DWV.

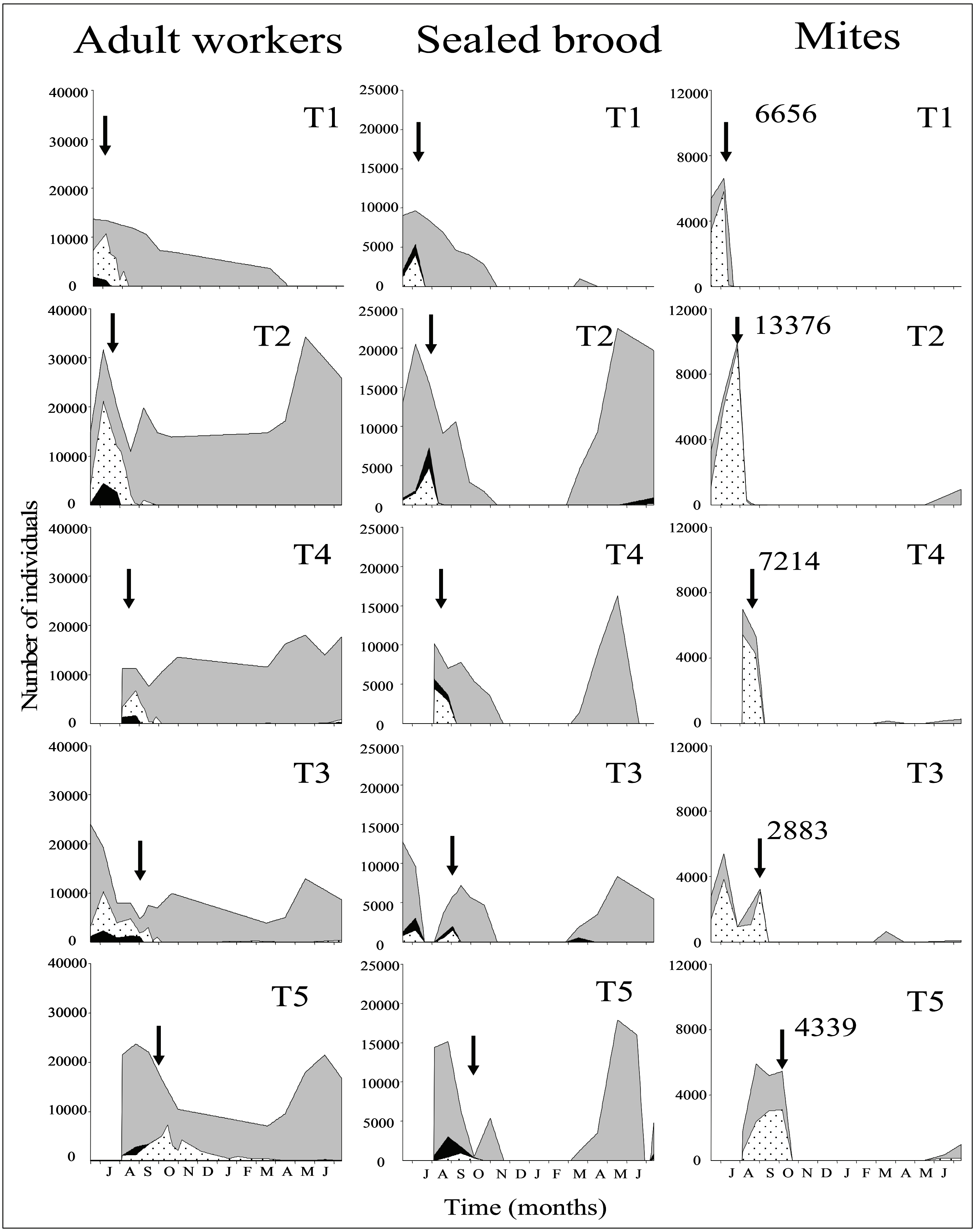

Fig. 2. The five treated colonies (T1-T5) showing the estimated numbers of adult workers, sealed brood and mites testing negative for DWV (grey); numbers of adults and brood infested by mites (black) and number of adults, brood and mites testing positive of DWV (dotted). The arrow indicates the time each colony was treated.

Survey 2: treated colonies

Prior to treatment, DWV was detected in the mite, adult bee and sealed brood populations of each colony (Fig. 2). At the time of treatment the number of mites in which DWV was detected exceeded the theoretical "damage threshold" of 2,000-3,600 (Martin, 2001) (Fig. 2). One month after treatment, infestation levels of the adult bees and sealed brood had dropped to 0% and DWV became undetectable in the sealed brood in all five colonies (Fig. 2). In the adult workers, DWV was not detected after four to six weeks in colonies treated in July,/,August, but persisted for 23 weeks in the colony (T5) treated in October (Fig. 2).

Analysis of the mites which fell after colony treatment showed that at the time of treatment, 68-90% of the mites tested positive for DWV. This proportion declined from summer / autumn (90% in July - 76% in October) through winter (55% in November - 32% in March) and reached its lowest value in the following spring (18% in April - 24% in May) before starting to rise again during the following summer (24% in June - 43% in July) (Table 1). During the winter, live mites were too scarce to be detected, but during the next season the mite populations started to increase again (Fig. 2).

Effect of treatment on colony survival

Although the two surveys were conducted during different years, there was no significant difference in the peak adult bee population (t = -0.762, df = 7, p = 0.78) or maximum mite population (t = -1.94, df = 7, p = 0.32) between the untreated or treated colonies. The survival of the treated colonies was, however, significantly higher (p = 0.047, n = 9, Fishers exact test) than the untreated colonies.

Discussion

In all nine study colonies (four untreated and five treated) the proportion of adult workers, bee sealed brood and mites in which DWV was detected increased until the colony either died or was treated. The number of adult workers testing positive for DWV in all colonies was greater than the number of adults infested by *V. destructor*. This indicates that mites change hosts several times during each phoretic phase. It could also indicate that an oral infection was occurring but this is unlikely since covert DWV infections are known to be transmitted sexually (De Miranda and Fries, 2008) and mites are known to move between bees, especially during the first few days after bee emergence. The number of sealed pupae testing positive for DWV was, however, always less than the number of infested sealed brood cells, which supports the finding that mite to brood transmission of DWV is less than 100% (Martin, 2001; Nordström, 2003). The strong association between the removal of the mite population from a colony and all subsequent brood and adult samples testing negative for DWV supports the finding that *V. destructor* is the primary vector of 'overt' DWV infections (Martin, 1999; Bowen-Walker *et al*., 1999; Nordström, 2003; Gisder *et al*., 2009). Furthermore, if the DWV strain is able to replicate within mites, it appears to cause the deformed wings typical of DWV (Gisder *et al*., 2009). Independently of the mite, inapparent (covert) DWV infections can be vertically transmitted via honey bee eggs (Chen *et al*., 2004; Yue *et al*., 2007), sperm (de Miranda and Fries, 2008) and larval food (Chen *et al*., 2006), but in this study the ELISA would not have been able to detect covert infections.

In our study, once the mites were removed by the acaricide, we were unable to detect DWV in the sealed bee brood at the same rate as the mites disappeared from the colony (two to four weeks). The detection of DWV in adult workers in relation to mite removal depended, however, on the time of treatment, i.e. four to six weeks in summer and up to 23 weeks in the winter. This reflects the differences in life span between adult workers emerging in the summer (weeks) or the winter (months) (Fukuda and Sekiguchi, 1966). The number of mites testing positive for DWV which caused irreversible damage (the "damage threshold") varied throughout the year, i.e. colony T2 had a mite population exceeding 13,000 mites before treatment, although this colony only survived because the treatment was carried out early and its bee population was large. The role of colony size is important, as demonstrated by colony T1, which died despite early treatment and having had a peak mite population of only 6,660 mites, although chalkbrood may have played a role in preventing colony recovery (Medina and Mejia, 1999).

In the absence of natural bee to bee covert transmission of DWV, the interactions between the honey bee and mite population dynamics and the "threshold" will vary during the year as follows: In spring, when the honey bee colony undergoes rapid expansion, those mites which have survived over winter start to reproduce. During this period, the prevalence of DWV in both the mites and bees is at its lowest level. As long as the bee population expands at a rate greater than that of the mite population, the relative proportion of individual bees infected by DWV grows only slowly. This is because the probability of a mite feeding on an infected adult bee is low due to the large numbers of newly emerging bees (Sumpter and Martin, 2004). In temperate regions, honey bee colonies undergo a large reduction in their adult worker population in autumn as they prepare to over-winter. During this period the proportion of the infected adult bees and brood, and virus carrying mites all increase very rapidly. Simultaneously the "damage threshold" falls dramatically since the infestation of both the adult bees and brood is increasing rapidly (Martin, 1998b). This greatly assists the transmission of DWV between mites, since the probability of encountering an infected bee, or entering a cell that already contains a mite carrying DWV greatly increases. It is now that the colony is under serious threat, since a high proportion of the emerging adult workers have a reduced longevity, and so will not survive the winter.

To ensure colony survival in temperate regions, mites must therefore be removed prior to the start of the brood production for over wintering which starts in early September (Fukuda and Sekiguchi, 1966). For example, we were still able to detect DWV in adults during the winter period when treatment was delayed until October (colony T5, Fig. 2). As it takes two to three weeks to remove the majority of the mites from the colony using effective acaricides, the treatment must therefore start in mid August if all the over-wintering bees are to be protected. Although the "threshold" varies with the time of year and colony size, maintaining the mite population below 3,000-4,000 (Martin 1998b; Hood and Delaplane, 2001) remains sound advice, but the treatment should be started no later than the end of August. These figures assume that all the mites are transmitting DWV, which is slightly unrealistic (Martin, 2001; Nordström, 2003), but this cautious approach has benefits when transferring advice derived from research to the beekeeping industry.

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