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Review

Diversity of Honeybee Behavior Is a Potential Inbuilt Trait for Varroa Tolerance: A Basic Tool for Breeding Varroa-Resistant Strains

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Abstract: The ectoparasitic mite *Varroa destructor* is well known for transmitting a number of viruses that can contribute to the collapse of honeybee colonies. To date, the many control measures put in place to limit the spread of *V. destructor* have yielded no satisfactory results. This is challenging because the effect of the parasite on honeybee colonies is becoming notorious. This has weakened the beekeeping industry and reduced pollination services, which may contribute to global food insecurity in the future. Therefore, it is necessary to put in place possible control measures and outline sustainable approaches to mitigate research efforts against the *Varroa destructor*. Extensive research to elaborate on the best possible solution has revealed that the selective breeding of naturally occurring *V. destructor* immune-related traits of honeybee strains is sustainable. Since the Integrated Pest Management approach was introduced, while still being very unreliable, there are open questions as to what control strategy could be considered effective. After cross-examination of existing strategies, a more practical way could be the adoption of an integrated approach. This approach should involve the association of selective breeding of honeybee colonies with *V. destructor* immune-related traits and the application of soft chemical treatment.

Keywords: *Varroa destructor*; *Apis melliefera*; selective breeding; resistant strain

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1. Introduction

The greatest threat to the honeybee *Apis mellifera* remains the parasitic mite *Varroa destructor*, which causes many health challenges leading to colony collapse [1–3]. The severity of the parasite could be attributed to the susceptibility of honeybees to infectious diseases due to their relative closeness and high frequency of social interactions within their colony [1,4]. However, other factors, including environmental conditions that are related to the life cycle of the parasite, the susceptibility afforded by their genetic constitution, and the haplotype, longevity, and fertility of the mite could increase the severity of the parasite. The detrimental effects of *V. destructor* and the impacts of acaricides on honeybee colonies have prompted beekeepers to seek sustainable approaches to control the mite. In order to achieve this, some beekeepers are trying to breed resistant honeybee stocks against *V. destructor* by using different selection criteria [5–7]. The social behavior of honeybees has called on the attention of beekeepers to address their intra-specific and inter-specific relationships while placing emphasis on their intrinsic behavior to reduce the spread of *V. destructor*.

Nowadays, scientists and beekeepers are faced with the challenges of developing approaches to combat the spread of *V. destructor* because, despite substantial evidence of the need to control the mite, no satisfactory solution has been discovered [8,9]. Although some of the approaches have little proven efficacy, they are harmful to bees. For instance, Rosenkranz et al. [10] reported that the use of strong chemicals (miticides) to reduce populations of *V. destructor* is harmful to bees and is only temporarily effective due to the mites rapidly developing resistance. Also, Warner et al. [11] reported that the use of thymol, oxalic acid, formic acid, hope beta acids, formamidine, and fluvalinate to control the spread of *V. destructor* was not sustainable due to increasing resistance and risk to honeybee health. The application of chemicals to control the spread of *V. destructor* has revealed that the use of synthetic, organic, and inorganic chemicals at low doses may be safer for bees. However, their efficacy is still unknown due to a lack of research evidence [11]. In another study, Dietemann et al. [8] demonstrated that the physical removal of the mite is limited by increasing labor for beekeepers. The failure of chemical control measures and the reluctance to physically remove the mites have left the possibilities for eradicating the mites questionable.

In this respect, the development of natural control methods for the mite through the behavioral output of honeybees is a concern for researchers. This is because natural methods have shown more reliable outputs than the use of synthetic or organic chemicals [11]. Studies have reported the efficacy of some behavioral traits associated with honeybee immunity and their potentials in fighting against *V. destructor*. For instance, Wagoner et al. [9] reported that the hygienic behavior of honeybees has emerged as a social immune trait that can reduce the impact of *V. destructor* on honeybee colonies. Moreover, the hygienic behavior of honeybees is not only stimulated by *V. destructor*-infested broods [12], but also by broods infested with other diseases (e.g., the deformed wing virus) [13]. The predominant threats that contribute to severe loss of managed honeybee colonies are brood diseases [14,15]. Amongst other interacting factors, the parasitic mite *V. destructor* is the central threat [3,16,17]. Recently, the behavior of honeybees toward *V. destructor*-infested broods and their ability to remove diseased broods are of interest to beekeepers for selecting resistant honeybee colonies. Early studies on the grooming and defensive behaviors of honeybees revealed resistant mechanisms against the mite [18,19]. However, the behavioral output of honeybees can be altered by many biological and environmental factors. Therefore, the proportion of chewed mites in the debris of a colony can be used to determine their grooming success under normal field conditions [20–24]. However, Büchler [21] reported that colonies selected for grooming behavior showed significantly more damaged mites and lower infestation rates after several generations of selection. The reliability on the grooming behavior of honeybees in selecting resistant colonies against *V. destructor* is still not very clear. Wagoner et al. [9] suggested that the association of naturally occurring immune behaviors of honeybees could be used as an approach to combat the effects of the mite. Another emerging trait of honeybee colonies against *V. destructor* is their ability to slow down mite reproduction. This could be attributed to the severity of the mite, which depends on the infestation level [25]. As a result, slow mite population growth could be considered a fundamental characteristic of *V. destructor*-resistant colonies. However, this is not accurate because behaviors associated with this characteristic remain unknown [26].

In a more recent study conducted by Bubnič et al. [27], queen caging and the trapping comb technique associated with oxalic acid treatment could be considered effective treatment strategies. They further explained that their long-term effects on viral loads need to be investigated. This approach seems more effective as the number of mites collected from *V. destructor*-surviving bee (VSB) colonies were far lower than those collected from control colonies [28]. Büchler [21] demonstrated that colonies selected for behavioral traits against *V. destructor* showed significantly more damaged mites and lower infestation rates than unselected colonies with unknown behavioral outputs. Despite the implementation of many approaches to combat the spread of the mite, no effective measures have been fully adopted to safeguard the health of honeybees.

Many studies have focused on the biology of *V. destructor* and the history of acaricides used to control the mite [29–31]. However, knowledge on the effect of the combination of naturally occurring behavioral traits of honeybee colonies immune to the mite is scant.

This study aimed to document the behavioral characteristics of honeybee colonies that are immune to *V. destructor*, the effects of the mite on honeybee colonies, and the way forward for increasing the survival of honeybee populations.

2. Evolutionary Biology of *V. destructor* and Their Host–Parasite Interaction

V. destructor is widely spread and known to feed on the fat bodies and hemolymph of honeybees [32] while passing on pathogens and bacteria that infest and lead to the collapse of many commercially managed colonies [3]. The immune system of *A. mellifera* is constantly being weakened because of viruses [3]. The evolutionary trend of *V. destructor* shows a successful shift from its main host (*A. cerana*) to the present host (*A. mellifera).* It is believed that *A. cerana* has developed host defense mechanisms against the mite [33]. The life history of *V. destructor* can be traced back to 1904, when *V. jacobsoni* was first described in *A. cerana* honeybee colonies in Java and later spread to other parts of the world [3]. Until the year 2000, many scientists and beekeepers had believed that *V. jacobsoni* Oudemans was the mite responsible for the collapse of honeybee colonies. In the same year, 2000, taxonomic work and the first microsatellites identified previously unidentified varroa species and found that *V. destructor* reproduced on *A. mellifera* and was responsible for the damages [3,34]. Before Anderson and Trueman described *V. destructor* [34], Meisch had observed *V. destructor* for the first time in Luxembourg in 1985 [35]. Then, it was hard for beekeepers to admit the existence and the damages caused by the parasite proposed by Claude Meisch. Nowadays, the feeding habit (feeding on the fat bodies and hemolymph of honeybees) and reproductive success of *V. destructor* in honeybee colonies has left it undoubtedly the principal threat to *A. mellifera* [36]. The distribution of *V. destructor* is presented in detail in Table S1 (Supplementary Materials).

Evidence suggests the host shift of *V. destructor* took about half a century or a century (50–100 years) and has become nearly worldwide in distribution [37,38] (Figure 1). In Australia, the first case of *V. destructor* was reported in June 2022 at the Port of Newcastle by the national varroa mite management program [39].

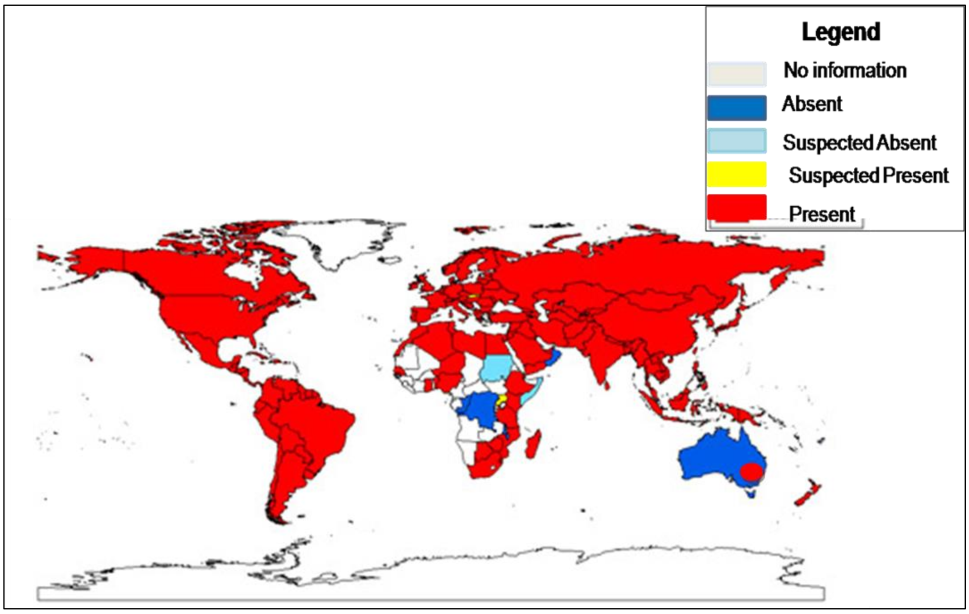

Figure 1. World distribution of *V. destructor* reported by Chase Kimmel, University of Florida [40], with some updates according to the 2024 National Varroa Mite Management Program in Australia.

The rapid wide spread of the parasite is still questionable, as *V. destructor* is eyeless, wingless, and unable to crawl between distant honeybee colonies [41]. However, studies have ascertained that the parasite spreads through drifting, possibly transferring to new colonies when healthy bees exploit resources from unhealthy colonies (known as robbing) [41]. The spread of *V. destructor* could also be accredited to poor colony handling and beekeeping malpractices; for instance, strengthening weak colonies by adding broods from strong colonies may spread the parasite; transporting colonies from one apiary to another without thorough check of the mite may spread *V. destructor*; swarm colonies may spread the parasite to their new site or on moving to their new sites. Until 2001, when colonies of *A. mellifera* were brought to Asia, the devastating effect of the mite was not noticed. A detailed schematic representation of the possible modes of the spread of *V. destructor* is presented in Figure 2.

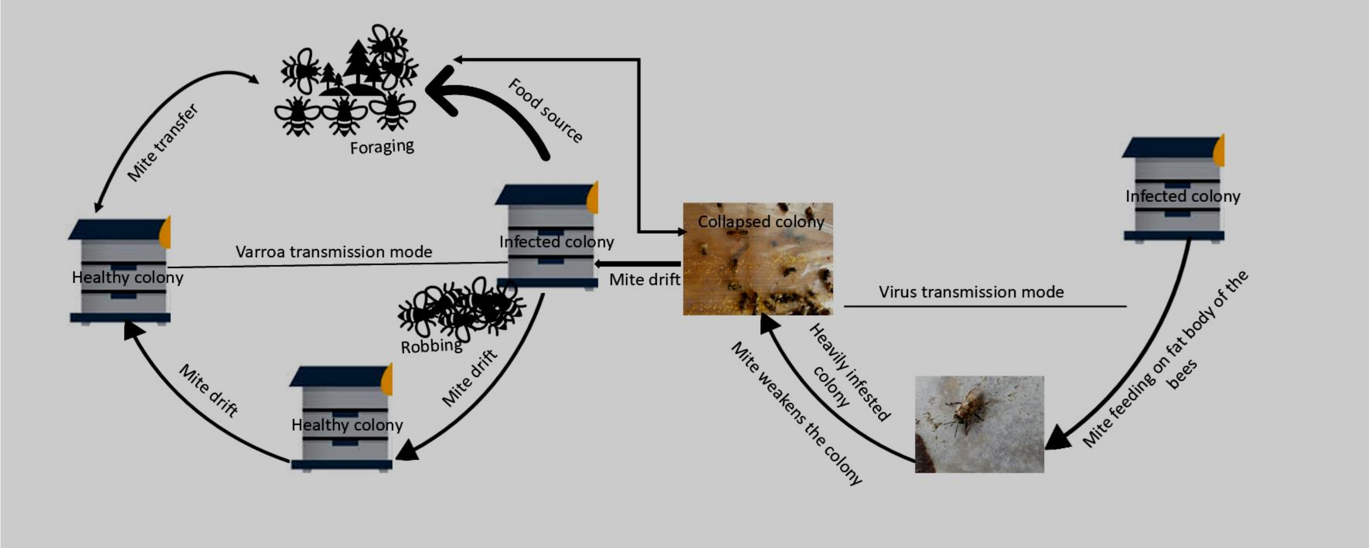

Figure 2. Transmission of *V. destructor* and its associated viruses.

As mentioned earlier, the transmission of *V. destructor* varies from within the hive, to between hives, to within and between foraging sites (Figure 2). The mite can be transferred from one colony to another through robbing, when worker bees from a healthy colony rob with worker bees from an infected colony (Figure 2). Also, during foraging, foragers from an infected colony may transport the mite to the foraging sites which could be carried on by healthy foragers to infest healthy colonies (Figure 2). Due to the small body mass of the mite, fallen mites can be transported (by wind and/or runoff) from infected colonies to the entrance of healthy colonies, which may later infest the healthy colonies. This is known as mite drift (Figure 2). When *V. destructor* infests a colony, the mites begin feeding on the fat body of the bees, passing on viruses such as the deformed wing virus (DWV) (Figure 2). In most cases, these viruses weaken colonies and cause them to collapse (Figure 2). Also, a few worker bees from a collapsed colony may transfer the mite to a healthy colony during foraging (Figure 2).

The behavioral ecology and the adaptability of *V. destructor* to their host under different climates is complex and hard to understand. However, several studies have described their physiology, ecology, reproduction, and host–parasite interactions. Adult females are reddish-brown or dark-brown, with an oval shape measuring about 1–1.77 mm long and 1.5–1.99 mm wide; meanwhile, adult males are yellowish, with a spherical body shape measuring 0.75–0.98 mm long and 0.70–0.88 mm wide [42]. The reproductive cycle of the mite begins from the egg and progressed to the adult stage (Figure S1, Supplementary Materials). Their life cycle involves two phases: the reproductive phase, where adult female mites raise young once they are inside brood cells; the dispersal phase, where female mites feed on the fat bodies of adult bees [43]. During the reproductive phase, adult mites invade prepupa cells at their docile and non-feeding stage, while before brood capping, the mite perforate the cuticle to create a feeding site [44]. The reproductive success of *V. destructor* depends on their feeding habits [32] and the duration of the development of their host [45]. The energy demand for oviposition needs *V. destructor* to extract more nutrients from their host, thus leaving adult bees in poor conditions.

3. Survivability of Honeybee Colonies Under the Infestation of *V. destructor*

Despite the many threats caused by *V. destructor*, some honeybee colonies of *A. mellifera* still survive and can resist the parasite. For instance, the Africanized *A. mellifera scutellata* has been found to survive *V. destructor* in Brazil [46], in Africa [47], and in Mexico [48]. The survivability of honeybee subpopulations of European races under mite infestation has been reported for decades [49–52]. Honeybee response to *V. destructor* is oriented by their immune-related traits that are thought to be heritable, though they could be altered by some environmental factors depending on the ecology. Although environmental pollutants, poor beekeeping practices, climate change, and nutritional stress could reduce bee population, *V. destructor* remains the principal cause of colony losses [25,53]. Dating back to 2001, reports from individuals in the United States stated how honeybee colonies cannot survive under the infestation of *V. destructor* unless treated with miticides [37]. However, some subpopulations of *A. mellifera* can survive under untreated conditions because they express genes that are tolerant to *V. destructor* [50].

As the former host gained resistance to *V. destructor*, it is believed that, irrespective of the severity of the parasite to their present host, some subpopulations are still resistant [29,54,55]. Importantly, the *V. destructor* immune-related responses in honeybees are associated with their social behaviors, which are thought to be anti-pathogenic [56] and can suppress lethal viruses caused by *V. destructor* [57,58]. Many years of research have produced the hypothesis that parasites become less virulent with time; this hypothesis has been driven by Tom Rinderer and co-workers, who have evaluated bees from Far East Russia and reported on the severity of *V. destructor* infestations since the 1950s. After a preliminary evaluation of the bees imported from the Primorsky Region in Russia into the USA in 1997, reports have shown that they are more resistant to *V. destructor* than other available stocks [59]. It is thought that the survival of honeybee colonies under the infestation of *V. destructor* is associated with naturally occurring tolerance that can hinder the reproductive success of the mite and lessen viral load [29,55]. It is still unclear which particular tolerance factor reduces the viral load in untreated colonies. However, in the strains of resistant breeds from Russia, studies have reported their ability to reduce the number of female offspring [60], to remove the mite's ability to produce good broods, to suppress mite reproduction, and to conduct successful grooming [61]. These immune-related responses are still under extensive, critical investigations in pursuit of determining which naturally mediated mechanisms could influence their occurrence and expression; here, it is likely that we will need to rely on each immune-related trait and note that such research depends on individual observation, infestation, and the degree of viral tolerance. An overview of possible *V. destructor* immune-related traits is summarized in Table 1; these will be described in detail later.

Table 1. Immune-related traits of honeybees and their impact on mite development.

Honeybee Immune-Related Traits to *V. destructor*	Evaluation of *V. destructor* Immune-Related Traits of Honeybees	Effects of Immune-Related Traits on the Development of *V. destructor*	References
Hygienic behavior (HB)	Pin-killed brood assay. Freeze-killed brood assay.	Have the potentials of removing diseased brood or *V. destructor*-infested pupae from cells.	Boecking and Spivak [18]; Guzman-Novoa and Morfin [62]; Harbo and Harris [63]; Jack and Ellis [64]; Ellis and Zettel-Nalen [65].
Varroa sensitive hygiene (VSH)	Percentage of uncapping and removal of *V. destructor*-infested broods; can also be tested by pin-killed and freeze-killed brood assays.	Identification and removal of *V. destructor*-infested pupae from cells.	Harbo and Harris [63]; Villa et al. [66]; Panziera et al. [67]; Jack and Ellis [64]; Ellis and Zettel-Nalen [65].
Grooming behavior	Ability of worker bees to remove mites from their bodies; ability of worker bees to bite and injure mites on their bodies; degree of beating wings and legs when mites are placed on their bodies.	Increases the proportion of damaged mites and reduces the tendency of rapid mite reproduction.	Jack and Ellis [64]; Mondet et al. [29]; Ellis and Zettel-Nalen [65].

Table 1. *Cont.*

Honeybee Immune-Related Traits to *V. destructor*	Evaluation of *V. destructor* Immune-Related Traits of Honeybees	Effects of Immune-Related Traits on the Development of *V. destructor*	References
Post-capping brood period/post-capping brood duration	The period taken for brood to cap and the duration from capping to emergence.	A shorter post-capping period is capable of preventing the mites from penetrating into the brood's cells as well as shortening their development due to early emergence.	Rosenkranz et al. [10]; Oddie et al. [68].
Suppressed mite reproduction	Incidence of non-reproduction of mites in capped cells; population development of the mite per colony, which can be evaluated by using powdered sugar, opening broods, and counting the number of mites.	High incidence of non-reproduction of *V. destructor*; capable of interrupting and possibly stopping mite reproduction.	Mondet et al. [29]; Harbo and Harris [69].
Small cell size	Natural cell size with non-foundation-based frame.	Shorter development periods of the bees may lower the reproductive success of *V. destructor* and male absence in susceptible colonies.	Winston [70]; Erickson et al. [71]; Heaf [72]; Olszewski et al. [73]; Oddie et al. [74].

It is believed that some subpopulations of the European and Africanized honeybees are surviving under *V. destructor* infestation in some localities around the world, including Brazil, Arnot forest in the USA, Mexico, Costa Rica, South Africa, Kenya, Tanzania, Uganda, Tunisia, Avignon in France, Gotland in Sweden, and Primorsky in Russia [75].

4. Natural Defense Mechanisms Against *V. destructor* and Integrated Pest Management (IPM) Strategies to Control the Parasite

Developing an effective and environmentally sensitive pest management approach that relies on a combination of common-sense practices is necessary. Regardless of recent knowledge on the biology of *V. destructor* [76,77], beekeepers still face the challenges of developing concrete strategies to effectively manage the mite. Notably, many approaches, spanning from mechanical to cultural to chemical, have been tested to control the mite. Some reported control measures include the following: the physical removal of the mite [8], the immune-related traits of the bees [9,24], and chemical treatments [10,30,31].

4.1. Mechanical and Cultural Approach

According to the United States Environmental Protection Agency (EPA), an IPM strategy that involves a mutual connection between beekeepers and researchers is strongly recommended for the control of *V. destructor* [78]. IPM is a complex pest control approach, with the goal of moving beekeepers towards the use of all proper control measures.

Mechanical and cultural methods are advancing fast because of the resistance of *V. destructor* to soft acaricides (thymol, formic acid, and oxalic acid) [64] and the lethal effect of hard acaricides (amitraz, coumaphos, fluvalinate, and flumethrin) on honeybees [79]. Although mechanical methods (the physical removal of the mites and using essential oils) are less harmful to bees, they have many limitations, including increased labor for beekeepers, sensitivity to fluctuations in ambient temperatures, and minimal differences between lethal doses for mites and honeybees [8]. This has left most beekeepers in doubt and reluctant to adopt and practice this method. Although this method is time-consuming, it is cheap and more reliable. Other mechanical methods include using mite traps, powdered sugar, and screened bottom boards; these are sustainable and less harmful to honeybees. Despite their cost-effectiveness, beekeepers have found them more laborious and less efficient in the control of *V. destructor* [80].

Recently, beekeepers and scientists have adopted cultural methods to control *V. destructor* based on their immune-related behavioral traits. Regardless of the controversies about the reliability of mechanical and cultural methods [64], these methods remain eco-friendly and environmentally sustainable. The controversies among scientists have led to certain questions, e.g., "what method or strategy could be an effective *V. destructor* control measure?". Undoubtedly, the immune-related traits of honeybees can provide resistance against the mite. Therefore, to limit the spread of *V. destructor*, beekeepers should select genetically resistant honeybee colonies without human intervention. In Europe and America, both unmanaged feral colonies and colonies with resistant traits showed lower mortality rates compared to sympatric controlled colonies [75]. Also, evidently, beekeepers have adopted cultural methods associated with social immunity of honeybees to combat the effects of *V. destructor* [29]. For instance, some commercial beekeepers are selecting resistant honeybee colonies against *V. destructor* based on their hygienic behavior (ability to remove dead broods), varroa sensitive hygiene (VSH) (ability to identify and remove varroa-infested broods), grooming behavior, and reduced mite reproduction [3,9,11]. The population dynamics of *V. destructor* and honeybees reveal the importance of time lag from brood to adult bees. According to Messan et al. [81], the large time lag from brood to adult bees could destabilize the population dynamics and push the colony to collapse. In this case, a shorter time lag from brood to adult bees will hinder the development of *V. destructor* and the colony will remain strong and healthy. it is also important to note that the population of *V. destructor* can die out because of seasonal changes [81]. Advancements in our knowledge on using cultural methods has enabled beekeepers to develop more techniques to control the parasite. Oddie et al. [68] communicated that beekeepers can select honeybee colonies surviving *V. destructor* naturally by sorting out those with reduced post capping brood period, while others proposed using a small cell size [73,74]. Recently, after surveying the population of *V. destructor* by sampling fallen mites, it was observed that increased brood population led to decreased *V. destructor* infestation rate [82]. Therefore, it is profitable for beekeepers to adopt the mechanical and cultural methods because it is cost-effective and environmentally friendly. However, the controversies among scientists on the reliability of these methods alone have raised many questions for further research.

4.2. Chemical Approach

Although beekeepers and scientists have developed and tested some chemical substances to control *V. destructor*, they still present some disadvantages. So far, chemical control measures through the use ofthymol, oxalic acid, formic acid, hope beta acids, formamidine, and fluvalinate have been reported to not be sustainable [11]. For example, several studies have reported on the high mortality of honeybees when exposed to hard acaricides (amitraz, coumaphos, fluvalinate, and flumethrin), even at recommended doses [83–85]. However, coumaphos and fluvalinate are more harmful to adult bees [83]. Though bee mortality is also influenced by outdoor temperatures (below 9 °C), significantly higher adult bee mortality could be attributed to treatment methods. For instance, higher adult bee mortality was reported for the trickling method than the vaporizer method [86]. As *V. destructor* mites transmit viruses, including the acute bee paralysis virus (ABPV) and the deformed wing virus (DWV), beekeepers believe that using chemicals could be the most effective control measure. Soft acaricides (thymol, oxalic acid, formic acid) are known to be less toxic to adult bees. After crucial evaluation of soft chemicals, they were found to have low efficacy because of mites' rapid evolution of resistance [10,87]. Again, soft chemical treatment may lethal bees and contaminate the hive products that are consumed by humans [88,89]. Wu et al. [90] reported that fenazaquin, used treat honeybee colonies against *V. destructor*, showed increased larval mortality and deformity rates. They further explained that fenazaquin causes metabolism disorders and physiological dysfunction which disrupt gut microbiota and damage gut tissues [90]. Nonetheless, the failure of formic acid, oxalic acid, amitraz, fluvalinate, and other acaricides in the control of *V. destructor*, especially during summer, does not negate their use and efficacy [64].

Due to the risks associated with synthetic chemical treatments, beekeepers are quickly shifting their attention towards organic chemicals with proven miticide efficacy characteristics [64]. The control of *V. destructor* is challenging because of its resistance to commonly used acaricides [91] and lack of well-recommended techniques. However, efforts are still being made. Integrating multiple tools and techniques of pest management could yield better results than just one strategy. Thus, it is recommended that beekeepers to practice using multiple strategies to mitigate their productivity. Recently, a combination of queen caging and trapping comb techniques associated with an oxalic acid treatment was reported to be an effective mite control strategy [27]. Also, using soft chemicals on honeybee colonies with resistant traits could be an effective control measure for *V. destructor*. The principal control strategies and a recommended technique for controlling *V. destructor* are presented in Figure 3.

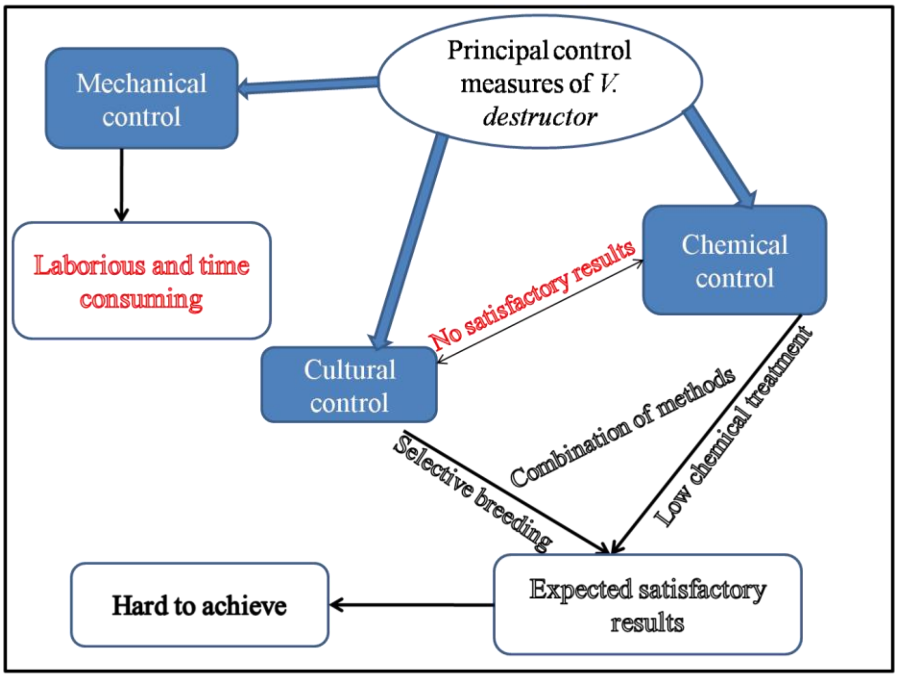

Figure 3. Schematic representation of various approaches and a recommended strategy for controlling *V. destructor*.

5. Behavioral Traits of Honeybees Immune to *V. destructor*

Sociality in honeybees has permitted them to gain social immunity traits against their enemies, including the *V. destructor*. Heritability of *V. destructor* immune-related traits has motivated beekeepers to evaluate and keep colonies with resistant traits. Some behavioral traits of honeybees associated with *V. destructor* immune responses include the following: hygienic behavior, grooming behavior, post-capping brood stage, and suppressed mite reproduction.

5.1. Hygienic Behavior Against *V. destructor*

Many animals display hygienic behavior for a purpose. Honeybees' hygienic behavior is their ability to remove dead or diseased brood from cells, remove unwanted particles from the body, and keep the colony clean. The hygienic behavior of honeybees was discovered in the 1930s when a team of researchers saw that resistant colonies against the American Foulbrood (AFB) virus removed diseased larvae from cells [92]. After this period, the study of the hygienic behavior of honeybees became significantly important. Woodrow and Holst [93] described the hygienic behavior and provided evidence that resistance to AFB is the ability to detect and remove diseased broods before the causative organism reaches the infectious stage of the diseased larvae. The response of hygienic bees to a diseased brood is thought to be common and applicable to all brood diseases of honeybees. Later studies have shown that adult bees from some colonies removed larvae infested with chalk brood from cells within 24 h and this made them resistant to the disease [94–96].

The notion of hygienic response in honeybee colonies may be confusing and sometimes hard to understand or analyze. The two most important methods, freeze-killing and pin-killing, are commonly used in brood assays to determine hygienic behavior of honeybee colonies. Similarly, hygienic behavior has been used to evaluate honeybee colonies for resistance to *V. destructor.* For instance, the removal of *V. destructor*-infested broods and the removal of freeze-killed broods is positively correlated (r = 0.74) [97]. They further suggested the hygienic response to broods infested with *V. destructor* can explain the response of hygienic bees in removing dead and diseased broods. Though many questions are raised on the reliability of the hygienic behavior of honeybee colonies to control the mite, Boecking and Dreschner [97] found higher incidence of diseased brood removal in hygienic bees compared with non-hygienic bees. However, Danka et al. [98] reported a weak correlation. Further investigations predicted the variation could be attributed to specific hygienic behavior rather than just general hygiene. This behavioral specificity has resulted in another form of hygienic behavior known as VSH [64,65,98]. Evolution of the VSH trait in honeybees has promoted the selection of resistant stocks against *V. destructor*. For instance, some honeybee colonies of the Primorsky region in Russia showed high levels of hygienic behaviors which were like those of the VSH stock and these bees were resistant to *V. destructor* [5].

Many years ago, it became evident that the hygienic behavior of honeybees has a genetic origin and is a heritable trait [99,100]. Later, the hereditary nature of resistant traits was evaluated; it was found that the hygienic behavior and the percentage of mites in brood cells had high levels of heritability [101]. In a more recent study, the same researchers documented that the proportion of non-reproducing mites was positively correlated with the expression of VSH [63,69]. As the importance of hygienic behavior and its heritability became known, selective breeding of honeybee colonies against *V. destructor* gained value [5,7,102,103]. The sensitivity of honeybees to this behavior could be improved through field tests with compounds related to larval signaling [104].

5.2. Grooming Behavior of Honeybees' Resilience to V. destructor

While parasites find a host, hosts also develop defensive measures. Grooming is a defensive behavior performed by arthropods to remove either dirt or ectoparasites from their bodies or those of nest mates. Honeybees are not left out. Honeybees practice both autogrooming (removal of ectoparasites by themselves) and allogrooming (removal of ectoparasites by nest mates). In honeybee colonies, Anderson and Trueman [34] described this behavior as being defensive against *V. destructor*. They drew their inspirations from a standpoint that both autogrooming and allogrooming injure mites and may reduce their population and reproductive fitness. However, it is thought that mites could be damaged by hygienic bees during the removal of diseased broods [22]. Grooming effectiveness towards *V. destructor* has been evaluated, and it was found that Africanized bees responded significantly faster and more intensively than Carniolan bees [105]. Later studies have shown that high tolerance to *V. destructor* in Africanized honeybees could be attributed to high grooming effectiveness [18,106]. To destabilize and slow down the effects of the mites in a colony, the intensity of grooming by adult bees could play a key role. Also, the population of mites in a colony could influence the percentage of damaged mites through grooming. Therefore, the grooming behavior is more valuable to beekeepers during high mite infestation [107]. As the number of mites that have fallen to the bottom of a hive is reflective of the intensity of grooming in *A. mellifera*, it is believed that even the presence of dust particles in a colony can elicit grooming. For instance, the percentage of fallen mites in broodless colonies and in small cages with a specific number of mites and bees did not differ, with the effectiveness of removing dust among individual bees being shown in the resistant and control colonies [108]. It is necessary to understand that grooming slows down mites' rapid development, because injured and dead mites were visible after grooming [108].

In the late 1990s, Rath [19] attributed the resistant mechanism of *A. cerana* to several immune-related behaviors, including grooming behaviors. In *A. mellifera*, slow mite reproduction through the grooming behavior [19,109,110] is a reported *V. destructor*-resistant trait. The results of thirteen years of natural selection, used to compare grooming behaviors between resistant and susceptible honeybee colonies, showed that resistant bees successfully removed 10 times more mites through grooming than susceptible bees did [111]. Again, the levels of maturity or experience in honeybees increased their grooming activities compared to young bees with little experience [111]. Though this may sound complex, it is clear that older bees with more experience can identify parasites and groom better than younger bees.

Grooming behavior in honeybees has become an important trait of resistance to *V. destructor*, and scientists have studied the heritability of this trait. The heritability of the grooming behavior was evaluated by assessing the proportion of damaged mites at the bottom of the hive after several generations [26]. Although the heritability of the grooming behavior in *A. mellifera* was estimated to be low, at $h^2 < 0.15$ [26] and at $0.16 < h^2 < 0.42$ [112], Moretto et al. [113] estimated a higher heritability of $h^2 < 0.71 \pm 0.41$. However, it is still to be determined whether the variation in the proportion of damaged mites correlates with the heritable variation in the grooming behavior against *V. destructor* [114].

5.3. Duration of Post-Capping Brood Stage on the Development of *V. destructor*

Aside from the abovementioned *V. destructor* immune-related traits, scientists and beekeepers are still on their way to diversifying the possible routes of eradicating their common enemy. The post-capping brood stage has become a key opportunity for measuring the reproductive success of *V. destructor* because it can only reproduce in brood cells. The exclusive reproduction of *V. destructor* in capped brood cells of their honeybee host has given room to critically understand brood development in relation to mite reproduction.

As reported by Büchler and Drescher [115], fewer mites are capable of reproducing in brood cells of colonies with shorter post-capping brood stage. The post-capping brood duration in honeybees is known for its high heritability [115]. Therefore, it is thought that selecting honeybee colonies with shorter post-capping brood duration will increase the likelihood of slowing down mites' development. This approach seems to be effective because of the rapid inbreeding of *V. destructor* within the colony. Also, Oddie et al. [68] assessed the post-capping brood period in honeybees who survive *V. destructor* by natural selection and found that surviving populations had a shorter post-capping brood period. They further determined that colonies with a shorter post-capping brood period can significantly reduce mites' reproduction and contribute to natural colony survival. Other studies have also outlined that colonies with a shorter post-capping brood period are capable of preventing mites from penetrating the cells and shortening their development because of early emergence [10]. To limit the risk of keeping unselected colonies, it is necessary to test and breed colonies with reduced post-capping brood periods as a strategy to control the spread of *V. destructor* [10].

5.4. Suppressed Mite Reproduction as a Defense Mechanism Against *V. destructor*

The host–parasite relationship between honeybees and the *V. destructor* seems intricate, as honeybees can reduce the reproduction abilities of the mite. Though many reasons could be attributed to the mite's infertility in brood cells, some populations of *A. mellifera* still hinder the reproduction of *V. destructor* in brood cells. For instance, a study conducted on mite fertility revealed significantly lower fertility in Africanized bees compared to European bee strains [46]. Also, no significant difference was observed after comparing the infertility rate of *V. destructor* in two subspecies of *A. mellifera* (*A. m. ligustica*, and *A. m. mellifera*) and the Gotland bees, which are known to be resistant [116]. Evidently, high incidence of non-reproduction in *V. destructor* may interrupt or stop mite reproduction [29]. Under normal colony conditions, reduced mite reproduction may create a favorable atmosphere for worker bees to destroy the remaining populations of the mite by performing hygiene and/or grooming behaviors. Therefore, reduced mite reproduction in honeybee colonies can be considered an intrinsic countering factor against *V. destructor* reproduction [117].

6. Way Forward for Breeding Honeybee Stocks with Resistant Traits Against *V. destructor*

Worldwide distribution of *V. destructor* has been reported in many regions; recently, they have been reported at the port of New Castle in Australia [39]. The continuous spread of the mite is a call for concern.

Reports on the immune-related traits of honeybees, including hygienic behaviors [5,104], VSH behaviors [63,65], grooming behaviors [19,111], the post-capping brood stage [10,68], suppressed mite reproduction [29,69], and small cell size [73,74], have shown promising results and are environmentally friendly. However, selecting and preserving heritable traits of honeybees is hard to achieve. Bearing in mind that this parasite can disrupt the entire food chain and increase the rate of food insecurity by destroying valuable pollinators, efforts must be made to limit their actions. Therefore, four possible ways to fight against *V. destructor* are outlined below.

1. Practicing good colony management: In all apiaries, colony management is a fundamental strategy for a successful beekeeping. Colony management tools and practices are complex because they depend on both individuals and ecology. However, some particular rules of thumb are implemented in most apiaries. Generally, practicing good colony management at different times of the year is critical in controlling some ectoparasites of honeybees. For instance, colony performance can be improved through good management practices that may delay the reproduction of *V. destructor* during spring and summer. It is important to understand the biology of *V. destructor* and evaluate the infestation level during each season of the year to mitigate treatment efficacy and prevent bees from possible chemical exposure. Hence, it is necessary to implement a strategic management plan for *V. destructor* and disseminate it to beekeepers and other scientists. This is because the range of *V. destructor* is not an individual issue; rather, it is a common fight to limit the spread of the parasite. Complementary to these key points, Giacobino et al. [118] highlighted key management practices to prevent high infestation levels of *V. destructor* in honeybee colonies at the beginning of the honey yield season. According to the varroa management plan of the National Bee Unit (NBU) of the United Kingdom [119] and the varroa mite transmission to management (T2M) plan of Australia [39], *V. destructor* cannot be completely eradicated. However, beekeepers must successfully keep productive bees despite the presence of *V. destructor*. The main strategy to accomplish this is for beekeepers to survey and report all possible infestations in a colony in a timely manner and to apply appropriate control measures to keep the population of *V. destructor* below the threshold.
2. Selective breeding for V. destructor tolerance: Earlier, we highlighted the effects of chemical treatment on honeybee colonies. Due to the heritability of the immune-related traits of honeybees and their potential in overcoming the effects of *V. destructor*, it is necessary to evaluate the traits related to *V. destructor*. One possible way to preserve the immune-related traits of honeybees is through selective breeding. The complexity of the mating behavior of honeybees and the difficulties that arise in selecting breeding sites has made it hard for beekeepers to preserve breeding lines over many generations. To accomplish this task, suitable breeding sites for maintaining honeybee genetic resources are being proposed. Recently, Akongte et al. [120] explained the possibilities of breeding and maintaining honeybee colonies in isolated mating stations with diverse characteristics. Therefore, it is recommended for beekeepers to select honeybee colonies with a slow reproduction rate of *V. destructor* and breed through subsequent generations while evaluating their tolerance efficacy.
3. Adoption of associated measures: To successfully fight a parasite, multiple approaches must be combined to increase the efficacy of each approach. For *V. destructor*, selective breeding of resistant lines is fast-developing, and chemical treatments should not

be forgotten. An ideal situation is to associate resistant honeybee colonies with a recommended soft chemical treatment (thymol and oxalic acid); this may weaken *V. destructor* and improve the efficacy of the colony in slowing down mite reproduction. Also, we recommend that, after selecting resistant strains, soft chemical treatment should be applied at the early stage of the honey flow season before rapid mite reproduction. We expect that soft chemical treatment may hinder the fertility of *V. destructor* and create conditions in which the resistant honeybee strains can completely neutralize the rest of the population.

4. Implementation of legislative measures: Another most important way to limit the spread of *V. destructor* is to practice common legislation. Many beekeeping associations should strengthen their capacity and build stronger legislations to fight against *V. destructor*. For instance, existing associations, including COLOSS, National Bee Units in many countries, European Community and UK Legislation, Bee Diseases Insurance, World Organization for Animal Health, International Bee Research Association, and many others, should build farmers' knowledge of the dangers of *V. destructor* and disseminate information on the possible measures available. Also, common control measures should be adopted based on the ecology and season to limit further spread of the parasite. Available resources should be put in place to produce and disseminate soft chemicals and resistant strains to beekeepers under the same geographical region.

7. Conclusions

Despite the many available alternatives for managing the population of *V. destructor* in honeybee colonies, a better solution has not yet been adopted. Recent fundamental and applied studies have produced and proposed control measures using soft chemicals (oxalic acid and fluvalinate) after the dramatic effects of hard chemicals to bees. Even introducing soft chemicals, acaricidal plants, and biological control, *V. destructor* has developed resistance to them. Today, combining mechanical, cultural, and chemical control measures has revealed the importance of adopting multiple approaches. Nowadays, there is a need for researchers to develop an integrated approach against *V. destructor* while laying emphasis on the geographical location, ecology, infestation rate, effects on honeybee health, and management strategies. Therefore, cultural methods based on the genetic selection of honeybees with immune-related traits to *V. destructor* and high heritability could be reliable [121,122]. For instance, using soft chemicals on naturally selected *V. destructor*-resistant traits could yield promising results. Though mechanical methods are laborious, their association with soft chemical treatment on honeybee colonies that have shown traits of resistance to *V. destructor* could be reliable in the control of the parasite. To bolster the naturally occurring immune-related traits against *V. destructor*, intensified research should be conducted with associated soft chemicals that can be applied to reduce the fertility of *V. destructor.* This holistic approach cannot be successful without the intervention of policy makers. Thus, legislative actions should govern a common practice against the enemy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14112094/s1, Table S1: Detailed distribution of *Varroa destructor*; Figure S1: The life cycle of *Varroa destructor*.

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