

Investigation of landscape risk factors for the recent spread of varroa mite (*Varroa destructor*) in European honeybee (*Apis mellifera*) colonies in New South Wales, Australia

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Abstract

In June 2022, an exotic pest of the European honeybee (*Apis mellifera*), the varroa mite (*Varroa destructor*), was detected in surveillance hives at the Port of Newcastle, New South Wales (NSW). Previously, Australia remained the only continent free of

the varroa mite. In September 2023, the National Management Group decided to shift the focus of the response from eradication to management. It is estimated that the establishment of varroa mite in Australia could lead to more than \$70 million in losses each year due to greatly reduced pollination services. Currently, there are no reported studies on the epidemiology of varroa mite in NSW because it is such a recent outbreak, and there is little knowledge of the factors associated with the presence of *V. destructor* in the Australian context. We sourced publicly available varroa mite outbreak reports from June 22 to December 19, 2022, to determine if urbanization, land use, and distance from the incursion site are associated with the detection of varroa mite infestation in European honeybee colonies in NSW. The outcome investigated was epidemic day, relative to the first detected premises (June 22, 2022). The study population was comprised of 107 premises, which were declared varroa-infested. The median epidemic day was day 37 (July 29, 2022), and a bimodal distribution was observed from the epidemic curve, which was reflective of an intermittent source pattern of spread. We found that premises were detected to be infected with varroa mite earlier in urban areas [median epidemic day 25 (July 17, 2022)] compared to rural areas [median epidemic day 37.5 (July 29, 2022)]. Infected premises located in areas without cropping, forests, and irrigation were detected earlier in the outbreak [median epidemic days 23.5 (July 15, 2022), 30 (July 22, 2022), and 15 (July 7, 2022), respectively] compared to areas with cropping, forests, and irrigation [median epidemic days 50 (August 11, 2022), 43 (August 4, 2022), and 47 (August 8, 2022), respectively]. We also found that distance from the incursion site was not significantly correlated with epidemic day. Urbanization and land use are potential factors for the recent spread of varroa mite in European honeybee colonies in NSW. This knowledge is essential to managing the current varroa mite outbreak and preventing future mass varroa mite spread events.

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Introduction

The European honeybee (*Apis mellifera*) is the most common domestic honeybee found in Australia (Department of Primary Industries, 2022). As of 2021, the Australian honeybee industry was comprised of 13,000 registered beekeepers, of which 1300 were commercial apiarists, each with more than 50 hives (Department of Agriculture Fisheries and Forestry, 2021). *A. mellifera* contributes directly to the Australian economy by providing essential pollination services to large-scale agriculture and horticulture, as well as for producing honey. The Australian honey industry is valued at around \$250 million *per annum*, while pollination services from this species are estimated to be valued at \$14 billion *per annum* (Kruszelnicki, 2023). Approximately one-third of Australian food is either partly or entirely dependent on honey-



bee pollination (Clarke & Feuvre, 2021). Australia's commercial beekeeping industry is extremely successful due to the abundant native vegetation that provides bees with large quantities of pollen and nectar. The pollination industry relies on nomadic beekeeper movements across Australia to pollinate these crops, with commercial hives being moved up to 20 times a year to varying locations (Bee Aware, 2023). Additionally, beekeeping as a hobby is becoming more popular in Australia, making up the remaining 11,700 registered beekeepers. Often, these beehives are located in urban areas in close proximity to one another (Perrone & Malfroy, 2014). These industry characteristics make it vulnerable to the impact of disease and introduced pests.

In June 2022, an exotic pest of the European honeybee, the varroa mite (*Varroa destructor*), was detected in New South Wales (NSW), Australia. Previously Australia remained the only continent free of the varroa mite, which has caused significant increases in the cost of bee-related products in other countries where it has been established due to costs associated with the management of the varroa mite (Iwasaki *et al.*, 2015; Department of Agriculture Fisheries and Forestry, 2021). The varroa mite is parasitic and thus requires a honeybee host to survive and reproduce. It weakens and can kill honeybees by feeding on the hemolymph of both pupal and adult honeybees. It also acts as a vector for viruses, of which deformed wing virus (DWV) is the most detrimental to honeybees because it severely weakens colonies and ultimately causes colony breakdown and death. However, there is currently no evidence of DWV in Australia (Kruszelnicki, 2023). There are several ways varroa mite is spread between colonies. Adult varroa mites can live off their host for 5 days; therefore, they can be spread through the movement of beekeeping equipment, including extracted honeycombs as fomites. Another way varroa mite is spread is through close contact between mite-infested drones and worker bees, which drift from hive to hive and even between apiaries. These mites can also be spread during swarming events and absconding colonies (Doug & Madelyn, 2022). The spread of varroa mite can also occur from beehive movement by commercial beekeepers for pollination services. It is estimated that the establishment of varroa mite in Australia could lead to more than \$70 million in losses each year due to greatly reduced pollination services (National Pest & Disease Outbreaks, 2022). Therefore, varroa mite is a serious biosecurity threat to Australia.

V. destructor was first detected in surveillance hives at the Port of Newcastle, NSW, in June 2022. It was quickly detected in other hives, with locations to date ranging from Sydney to the Hunter Valley Region, as well as Coffs Harbour and Narrabri. The source of the outbreak is still under investigation. The NSW Department of Primary Industries (DPI) implemented an emergency response strategy that consisted of movement restrictions on bees, beehives, and bee products within NSW and euthanasia and destruction of all wild and owned European honeybee hives within eradication zones (National Pest & Disease Outbreaks, 2022; Department of Primary Industries, 2023a). As of September 2023, the National Management Group decided to shift the focus of the response from eradication to management of varroa mite (Department of Primary Industries, 2023a). Currently, there are no reported studies on the epidemiology of varroa mite in NSW, as it is such a recent outbreak. There is also little knowledge of the risk factors associated with the presence of *V. destructor* in the Australian context. There have been some risk factor studies on the presence of *V. destructor* in other countries. These studies focused on the management practices of the honeybee hives, climate, and urbanization as risk fac-

tors for high infestation of *V. destructor* in honeybee colonies (Stevenson *et al.*, 2005; Giacobino *et al.*, 2014; Giacobino *et al.*, 2017; Bahrami *et al.*, 2018; Korená Hillyayová *et al.*, 2022). Risk factors for *V. destructor* spread are largely unknown. Given the likely mechanisms of spread, landscape factors might contribute to such spread. The intricate mechanisms governing the interaction between varroa mite and honeybees remain poorly understood. Knowledge of these risk factors might help in the management of varroa mite and limit its spread, as well as provide other information to fill knowledge gaps about the effects of varroa mite worldwide (Chapman *et al.*, 2023). Therefore, this study aimed to determine if landscape factors – urbanization, land use, and distance from the incursion site – are risk factors for the varroa mite detections during the recent varroa mite outbreak in European honeybee colonies in NSW, Australia. We also describe the spatial and temporal features of the early phase of this incursion, including hotspots of detection, which adds to our scant knowledge of the epidemiology of Varroa mite incursions.

Materials and Methods

Data collection and organization

Varroa mite outbreak reports from the first detections in June 2022 up until the end of 2022 were sourced from the NSW DPI Varroa Mite Emergency Response webpage using publicly available data (Department of Primary Industries, 2023a). The data extracted from these outbreak reports included the date of detection by the NSW DPI, the number of infected premises occurring on that day, and the locations of the infected premises.

Infected premises have been defined by the DPI as premises where a mite species of the genus *Varroa* has been detected by either: i) being observed by a person who is a technical expert, or a person who has undergone training by a technical expert in the field of identification of Varroa mite; or ii) diagnostic samples from a site have been received at a department laboratory and confirmed positive by a diagnostician (Department of Primary Industries, 2023b). These reports were collated into an Excel spreadsheet and organized by date of detection. Epidemic day (*i.e.*, the number of days since the first varroa mite detection date, June 22, 2022) was calculated, based on the first detection. The distribution of infected premises by epidemic day was plotted to create an epidemic curve. Descriptive statistics were calculated in Excel. These included the average, median, minimum, and maximum, as well as the interquartile range of the epidemic day. The latitude and longitude of each location were added to the dataset manually using Google Maps. Land use data was obtained from the Department of Planning and Environment website under "NSW Landuse 2017 v1.2". This dataset was updated in June 2020 and is based on aerial imagery and satellite imagery available for NSW. These include, but are not limited to, digital aerial imagery captured by the NSW Department of Customer Service (DCS), high-resolution urban (Conurbation) digital aerial imagery captured on behalf of DCS, SPOT 5, 6 & 7 (Airbus), Planet™, Sentinel 2 (European Space Agency) and LANDSAT (NASA) Satellite Imagery. Mapping also includes commercially available imagery from Nearthmap™ and Google Earth™, along with Google Street View™. Landuse classes assigned are based on activities that have occurred in the last 5-10 years that may be part of a rotational practice.

Statistical analysis

Location data in Excel was imported into ArcGIS v. 10.5 (ESRI Inc., Redlands, CA, USA) and mapped using a shapefile of NSW (Geographic Coordinate System WGS 1984). The Varroa mite incursion was described using a range of spatial and temporal descriptive methods and statistics to produce insights into how this unique incursion evolved and to generate hypotheses about its spread. Such information is lacking in the published literature.

The mean epidemic center weighted by epidemic day and a one standard deviation directional ellipse, also weighted by epidemic day, were calculated (Spatial Analyst, ESRI Inc., Redlands, CA, USA). The spatial distribution of epidemic day was assessed using Moran's autocorrelation statistic (Spatial Analyst, ESRI Inc.). A retrospective space-time analysis was performed using a space-time permutation test for high rates, with a circular scanning window of a maximum of 20% of the population at risk and a temporal window of a maximum of 20% of the study period (Kulldorff M, Information Management Services, Inc. SaTScanTM v 9.6: Software for the spatial and space-time scan statistics. <http://www.satscan.org/>). In this procedure, the locations of infected premises and their dates of detection were scanned for clusters, and the number of observed premises in each cluster was compared to an expectation that the spatial and temporal locations of all premises were independent (Kulldorff *et al.*, 2005). A scanning window of 20% was chosen for each due to the outbreak being a short time frame epidemic, thus allowing us to focus on smaller, discrete, local clusters. Statistical significance was determined via Monte Carlo simulation with 999 replications. Following the descriptive, spatial, and temporal investigation, a series of hypothesis tests were conducted to determine if the infected premises

detected were associated with landscape factors. This knowledge can help inform disease response strategies and the management of the incursion. Buffers (5 km radius, to represent the local landscape) were created around each outbreak location and spatially joined to land use data. Within each buffer, the number of areas of cropping, irrigation, forests, and horticulture were summed. In addition, the land area (sq. m) of each of these variables within each buffer was calculated (ArcGIS v. 10.5, ESRI Inc.). The distance of each outbreak to the nearest rural or urban area was calculated, and each outbreak was classified as being either rural or urban. A Kruskal-Wallis test was performed on epidemic day using the 5 variables extracted using buffers: rural *versus* urban, and the presence of cropping, forests, irrigation, and horticulture using

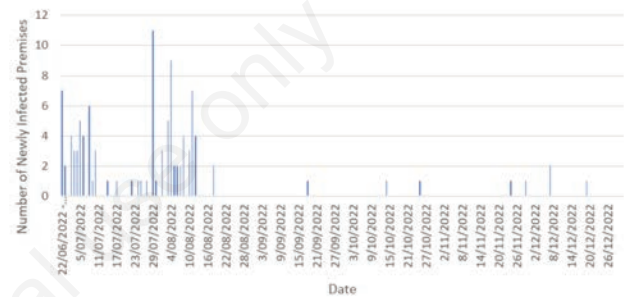


Figure 1. Epidemic curve of varroa mite infected premises from June 22 to December 31, 2022.

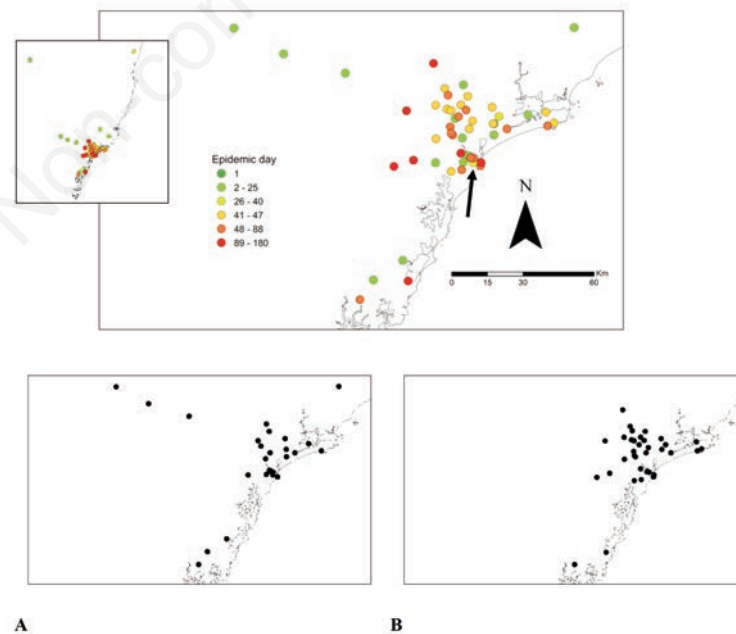


Figure 2. Spatial distribution of varroa mite infected premises in New South Wales, Australia from epidemic day 1 (June 22, 2022) to epidemic day 180 (December 19, 2022). The location of the primary spatiotemporal cluster is indicated by an arrow (13 infected premises detected between July 22 and 29, 2022, 1.82 expected, $p < 0.0001$). Panels A and B illustrate the spatial distribution of infected premises during the first (June 22, to July 29, 2022) and second (July 30, to December 19, 2022) waves of the epidemic.

SPSS v28 (IBM, Armonk, NY, USA). A Spearman's rank-order correlation test was performed on outbreak epidemic day and 5 variables: distance from the incursion site, total areas of cropping, forests, irrigation, and horticulture (SPSS v28, IBM).

Results

Epidemic curve

The epidemic curve analysis showed 2 epidemic waves, the first from June 22 to July 10, 2022, and the second wave from July 29 to August 12, 2022. From this time point until the end of 2022, there were occasional sporadic detections (Figure 1). The epidemic curve reflects an intermittent source pattern of spread. The median epidemic day was day 37 (July 29, 2022), and the interquartile range was from day 14 (July 6, 2022) to day 47 (August 8, 2022) of the outbreak.

Spatial clusters

A primary statistically significant cluster (Figure 2) was detected from July 22 to July 29, 2022 ($p < 0.0001$). The center of the cluster was a single location (32.9167°S, 151.750°E, *i.e.*, Hunter region). There were 13 observed infected premises and 1.82 expected infected premises (observed/expected = 7.13). There were 2 secondary statistically significant clusters detected. The first was detected on June 22 ($p < 0.0001$). The cluster was located at a single location (32.9283°S, 151.7817°E – site of incursion, *i.e.*, Port of Newcastle). There were 7 observed infected premises and 0.46 expected infected premises (observed/expected = 15.29). The second cluster was detected during the period from July 10 to

July 17, 2022 ($p < 0.01$). The center of the cluster was located at 30.3206°S, 149.782°E (Narrabri) with a radius of 294 km. There were 4 observed infected premises with 0.23 expected (observed/expected = 17.12).

Distance from incursion site – Port of Newcastle

A Spearman's rank-order correlation test showed that there was a weak and non-significant correlation between distance from the incursion site at the Port of Newcastle (32.9259°S, 151.7772°E) and epidemic day ($r_{SP} = 0.162$, $p > 0.05$). For the first and second epidemic waves, these correlations were 0.018 ($p > 0.05$) and 0.025 ($p > 0.05$) (Figure 2).

Rural versus urban

A Kruskal-Wallis test showed that there was a statistically significant difference between the median epidemic day of reported infected premises located in rural *versus* urban areas ($H = 6.529$, $p < 0.011$). The median epidemic day for reported infected premises located in urban areas was 25 (July 17, 2022) while for those premises in rural areas, it was 37.5 (July 29, 2022) (Figure 2).

Land types

Cropping

A Kruskal-Wallis test showed that there was a statistically significant difference between the median epidemic day of reported infected premises located in areas with cropping and those without cropping ($H = 48.823$, $p < 0.001$). The median epidemic day for reported infected premises located in areas without cropping was 23.5 (July 15, 2022), while for those with cropping was 50 (August 11, 2022) (Figure 3). A Spearman's rank-order correlation test

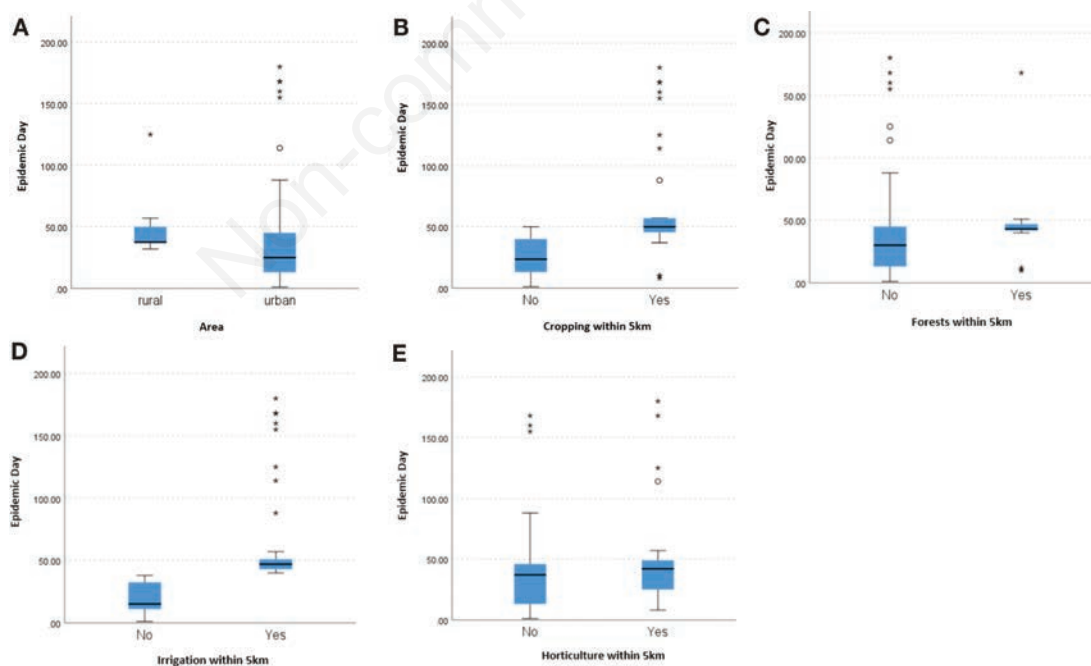


Figure 3. Box and whisker plot of varroa mite outbreak epidemic. Data shown were sampled from the varroa mite outbreak in European honeybee colonies in New South Wales, Australia from June 22 to December 31, 2022. Asterisks indicate extreme outliers (values > 3 times the interquartile range). Circles indicate mild outliers (values between 1.5-3 times the interquartile range). Data were categorized by rural versus urban areas ($H = 6.529$, $p < 0.011$) (A); cropping ($H = 6.529$, $p < 0.011$) (B); forests ($H = 10.322$, $p = 0.001$) (C); irrigation ($H = 79.564$, $p < 0.001$) (D); and horticulture ($H = 3.165$, $p > 0.05$) (E).

showed that there was a strong, positive correlation between the total area of cropping within 5 km of infected premises and epidemic day, which was statistically significant ($r_{sp}=0.668$, $p<0.001$).

Forests

A Kruskal-Wallis test showed that there was a statistically significant difference between the median epidemic day of reported infected premises located in areas with forests and those without forests ($H=10.322$, $p=0.001$). The median epidemic day for infected premises located in areas without forests was 30 (July 22, 2022) while for those with forests, it was 43 (August 4, 2022) (Figure 3B). A Spearman's rank-order correlation test showed that there was a positive correlation between the total area of forests within 5 km of reported infected premises and epidemic day, which was statistically significant ($r_{sp}=0.291$, $p=0.002$).

Irrigation

A Kruskal-Wallis test showed that there was a statistically significant difference between the median epidemic day of reported infected premises located in areas with irrigation and those without irrigation ($H=79.564$, $p<0.001$). The median epidemic day for infected premises located in areas without irrigation was 15 (July 7, 2022), while for those with irrigation, it was 47 (August 8, 2022) (Figure 3C). A Spearman's rank-order correlation test showed that there was a strong, positive correlation between the total area of irrigation within 5 km of reported infected premises and epidemic day, which was statistically significant ($r_{sp}=0.925$, $p<0.001$).

Horticulture

A Kruskal-Wallis test showed that there was no statistically significant difference between the median epidemic day of reported infected premises located in areas with horticulture and those without horticulture ($H=3.165$, $p>0.05$). The median epidemic day for infected premises located in areas without horticulture was 37 (July 29, 2022), while for those with horticulture, it was 42 (August 3, 2022) (Figure 3D). A Spearman's rank-order correlation test showed that there was a weak and non-significant correlation between the total area of horticulture within 5 km of reported infected premises and epidemic day ($r_{sp}=0.115$, $p>0.05$).

Discussion

Australia's European honeybee population is impacted by an ongoing varroa mite outbreak. This has led to financial, ecological, and psychological impacts on beekeepers, agriculture, and horticulture industries nationwide. Therefore, identifying risk factors associated with the outbreak might help to manage the current outbreak and minimize the impacts of varroa mite on the bee industry and pollination-reliant industries. The results of the present study show how land use and urbanization are associated with the current varroa mite outbreak in European honeybee colonies in NSW, Australia.

We found that varroa mite reported infected premises located in urban areas were detected earlier in the outbreak than in rural areas. This could be due to the increasing popularity of urban beekeeping in Australia as a result of increased public awareness of the ecological significance of honeybees (Perrone & Malfroy, 2014). As of 2021, the Australian honeybee industry was comprised of 13,000 registered beekeepers, of which only 1300 were commercial apiarists and the remaining were hobbyists

(Department of Agriculture Fisheries and Forestry, 2021). These urban hives are often located in cities; therefore, the shorter distances might have aided in the spread of varroa mite between premises. Urban beekeeping is also often free of pesticides, which might have facilitated the spread of varroa mite into these naïve hives even further compared to commercial hives, which are often located in rural areas (Perrone & Malfroy, 2014). Urban areas also contain an abundant and consistent source of pollen-bearing plants and nesting areas, compared to rural areas, which attract wild European honeybee colonies (Baum *et al.*, 2014; Wenzel *et al.*, 2020; Bila Dubaić *et al.*, 2021). Wild European honeybee colonies are the result of swarming events whereby managed hives grow in colony number and eventually depart the main hive due to overcrowding (NSW Agriculture, 1999). They were not initially included in the DPI Eradication Program. Therefore, these wild colonies could have been infected with varroa mite, thus aiding in the spread within urban areas at the beginning of the outbreak. The results of the land use analysis found that varroa mite infections occurred later in areas with cropping, forests, and irrigation compared to areas that lack these land types. These findings can also be because of increased urban beekeeping, as mentioned earlier, which provides shorter distances for varroa mite to travel, allowing for more rapid spread locally. Areas with cropping, forests, and irrigation are often spread across larger distances; therefore, the spread of varroa mite into honeybee colonies would have been slower as it would have been dependent on the movement of hives by beekeepers, which was restricted throughout the outbreak (Stevenson *et al.*, 2005; Department of Primary Industries, 2024). Another reason why this might have occurred is that areas with cropping, forests, and irrigation have reduced forage availability and increased use of pesticides (Smart *et al.*, 2016; Ash *et al.*, 2019). The use of pesticides may have slowed the spread of varroa mite in these areas due to some pesticides targeting varroa directly (Johnson *et al.*, 2010). Having reduced forage availability in these areas compared to the diversity offered in urban areas might contribute to honeybee colonies being less abundant in these areas; thus, the spread of varroa did not occur as rapidly. Previous studies have shown that intensive agriculture can contribute to pollinator decline due to nutritional shortages (Dolezal *et al.*, 2019). Cross-correlations between these land use types were generally low (r_{sp} range -0.027 to 0.349), except for the correlation between irrigation and cropping (r_{sp} 0.742, $p<0.001$). The associations identified with epidemic day should be interpreted as the potential influence of the local landscape of Varroa mite detection.

Distance from the incursion site at the Port of Newcastle was shown not to be correlated with epidemic day. This indicated the outbreak was rapid and widespread from the first day of the incursion. This could have been due to long-distance spread events, which are expected to be rapid in Australia because of the migratory nature of the beekeeping industry (Agriculture Victoria, 2023). Activities such as honey gathering, the exchange of equipment, and the trading of hives all play a role in the spread of varroa over larger distances (Stevenson *et al.*, 2005). In Australia, commercial hives are often nomadic in nature, with hives being moved up to 20 times a year to a variety of different locations for pollination or honey production (Bee Aware, 2023). Although movement restrictions were enforced early in the outbreak, the movement of commercial hives before the first detection could have aided in the initial wide and rapid spread of varroa mite.

We found 3 clusters during the outbreak that were of importance. All of these clusters occurred within a narrow time period



during the beginning of the outbreak in June and July 2022. This might have been due to seasonality since the winter period makes the honeybees more vulnerable to parasitic loads. Studies have suggested this is due to the increased energy used to maintain live temperature, which reduces the energy available to maintain immune functions (Ptaszyńska *et al.*, 2018; Liu *et al.*, 2021).

Winter is also when the queen begins laying eggs to replace bees that have died during the winter. Varroa mite require these new brood larvae to reproduce (Agriculture Victoria, 2023). Thus, winter creates an ideal environment for varroa mite infestations. Another reason why this may have occurred could be that the government's initial response to the outbreak, which was to eradicate all hives within a 50 km zone around the Port of Newcastle, was not effective in the early stages; for example, there was insufficient manpower to inspect all hives in the eradication zone within a short time period. However, no other significant clusters were detected after this time period, which might indicate an effective eradication plan that slowed the spread of varroa mite.

Our study findings have to be considered in light of some limitations. There was limited access to specific location data for each premises involved in the outbreak; thus, publicly available data was used instead. Utilizing the publicly available data came with some issues. One of the issues was that there was inconsistency in the reporting of infected premises, with some infected premises assigned to a wider region rather than a town and some premises not assigned to a specific location when multiple infected premises were reported in a day. As the epidemic proceeded, reporting of infected premises became less frequent; therefore, dates of detection may not be accurate towards the end of 2022. Additionally, there is a wider concern about the use of surveillance data during an incursion. We assumed that surveillance intensity was relatively uniform during this short (193 days) study period. The lack of a correlation between distance and the incursion site suggests that this assumption is reasonable. However, it is possible that our study findings might partly reflect the surveillance activities that occurred during the response to this incursion. Separating the effect of risk factors for disease spread *versus* risk factors for surveillance during an outbreak scenario requires access to surveillance intensity data, which was unavailable in this study. In addition, information on the characteristics of the infected premises was unavailable. Specifically, we were not able to differentiate commercial from hobby premises; access to this information might provide greater insights into the evolution of this epidemic. Another issue was that the most recent publicly available NSW land use data was created in 2020. Therefore, the results of this study may not be reflective of current land use if there were major changes to the areas where infected premises were located within the past few years. Another limitation was that there was a lack of previous research on risk factor analysis of varroa mite outbreaks, as most studies focused on management strategies to reduce the varroa mite burden in countries where varroa mite is endemic. This made it difficult to compare studies, and there is a need for further research on varroa mite spread *versus* the presence or infestation intensity of varroa mite.

Better access to specific location data, apiary size, hive densities, and accurate detection dates is needed. This would allow future research into the relationship between local honeybee colony densities and disease prevalence in rural and urban areas, as well as further evidence for the role of land use as a risk factor for varroa mite spread. Further research into how varroa burden is affected by seasonality over a longer time period would extend the

findings of this study and provide further insight into potential drivers of varroa mite infestation and spread. A survey could be conducted of all beekeepers within NSW to determine if management-related factors were associated with varroa mite spread. Previous studies in other countries have shown certain management practices were associated with high levels of mite infestation in honeybee colonies (Giacobino *et al.*, 2014; Giacobino *et al.*, 2017).

Conclusions

In conclusion, this study was able to show that urbanization and land use are associated with the recent spread of varroa mite in European honeybee colonies in NSW. These findings are essential to manage the current outbreak and preventing further spread of varroa mite. This study is the first report on the risk factors associated with the spread of varroa mite in European honeybees in NSW. Further research is necessary to increase resilience and capacity to manage varroa mite within the Australian honeybee industry.

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