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**An investigation of Honey Bee Drone Congregation Area
formation in rural and semi-rural locations in New Zealand**

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Abstract

Male honey bees gather in Drone Congregation Areas (DCAs), and meet with queens to mate in flight. Because they mate on the wing, investigation of these areas using current techniques is not easily facilitated in some areas, which limits research. This study investigates an improved method of studying DCAs in difficult areas; studies the landscape characteristics of DCA locations, and measures pheromone attraction between drones, and honeybee queens and workers, to ascertain any chemical contribution towards DCA formation. Using a camera equipped Unmanned Aerial Vehicle (UAV) holding an artificial honey bee queen with 9-Oxo-2-Decenoic Acid (9ODA), trials were conducted to investigate the different flight behaviours of honey bee drones on their mating flights, and the formation of DCAs. The use of the UAV was found to be successful in carrying out DCA research especially in inaccessible areas. Using this method, drone honey bee mating flight activity was investigated in several distinct areas; hill and valley areas; urban park areas, and flat agricultural areas. The data were analysed using Geographic Information System software ArcGIS 10.4.1. Different patterns of drone activity were found in the different areas suggesting that in hill and valley areas where well defined DCAs exist, landscape features played an important part in their location but that in flat areas, landscape features were not shown to play a part in DCA formation and instead, apiary-dependant DCAs dominated, and away from these apiaries, the abundance of drones flying at random in the flat areas (as opposed to being confined in a hill and valley area) would ensure mating. The results also suggest that contrary to some research, DCAs in hill and valley areas have flexible boundaries that may vary in response to other factors such as queen flight behaviour. In order to find out whether chemical influences could contribute to the formation of DCAs, a four- arm olfactometer test was carried out to investigate drone attractiveness to queens, and drone attractiveness to other drones. Contrary to my expectations, drones and queens were not attracted to other drones but rather to workers, perhaps because only sexually immature drones were available for the tests. The study overall confirms the usefulness of using a UAV in difficult areas; demonstrates a significant difference in the spatial dynamics of drone mating flight in different landscape areas, and concludes that DCA boundaries may be constructs that depend on the mating flight parameters of the queen rather than drones only. Further research, especially on the queen's mating flight parameters is suggested.

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Table of Contents

Abstract	2
Acknowledgements.....	3
Table of Figures	6
1. Introduction.....	7
2. Review of Literature	9
2.1 Drone Congregation Areas.....	9
2.1.1 Multiple mating in honey bees	12
2.2 Drone flight parameters.....	15
2.3 Pheromones and Honey Bee Mating	16
2.3.1 Drone aggregation pheromone.....	18
2.4. The Mating flight of the queen honey bee	19
2.5 Factors that may give rise to Drone Congregation Area location.....	21
2.5.1 Flight altitudes and distance from the apiary	21
2.5.2 Magnetic sense in honey bees	22
2.5.3 The influence of wind.....	23
2.5.4 Optical radiance	24
2.5.5 Physical Landscape Features.....	25
2.5.6 Honey bee pheromone attraction	27
3. Methods of investigating DCAs: Advances and challenges	28
3.1. Summary of the literature.....	28
4. Developing an improved method of investigating certain characteristics of DCAs. (Cramp 2017) 30	
4.1 Study aims	30
4.1.1 Using balloons, kites and artificial queens impregnated with synthetic queen pheromone	30
4.2. Flight trials of the unmanned aerial vehicle (UAV)	30
4.3 Materials and method.....	31
4.4. Results and discussion.....	37
4.5. Conclusions	43
5. Investigating the relationship between landscape and DCA formation in hill areas and a flat, open area using the UAV	44
5.1 Background	44
5.2 Problems associated with DCA research.....	45
5.3 Material and Methods	47
5.4. Analysis of the DCAs.....	51
5.5 Results and discussion.....	52
5.6 Conclusion	57
6. Testing for the abundance of drones in areas in relation to landscape characteristics.	58
6.1 Introduction.	58
6.3 results and discussion	59

6.3.1 Mean times for drones to attend a lure in the areas studied.....	60
6.3.2 Mean times for drone comets to form in response to a lure	61
6.4 Conclusions	62
7. Investigation of drone to-drone, and queen-to-drone attractiveness.....	63
7.1 Aim of the study.....	63
7.2 Background	63
7.3 Materials and methods	64
7.3.1 Test drones.....	65
7.3.2 Test Workers	66
7.3.3 Test Queens.....	66
7.4 Experimental set up	67
7.5 Experimental Process.....	69
7.5.1 Test A. Queen/drone attraction test.....	71
7.5.2 Test A. Results.	74
7.5.3 Test B. Queen/drone only attraction test (worker influence removed).....	74
7.5.4 Test B. Results	75
7.5.5 Test C. Drone/drone attraction test.....	75
7.5.6 Test C. Results	76
7.5.7 Test D. Drone/drone attraction test (workers removed)	76
7.5.8 Test D Results	76
7.6 Discussion.....	77
7.7 Conclusions	79
8. Thesis Summary and Conclusion.....	80
8.1 Conclusions	81
REFERENCES.....	83
Appendix 1. Maps 1 – 45 of Drone Congregation Areas studies.....	90
Appendix 2. Data Tables	119
Data Table 1. Drone Activity in DCAs	119
Data Table 2: Showing the data used to perform regression analysis on four variables	138
Data table 3. Single Lift points to test drone abundance in hill/valley and flat areas.	145
Data Table 4. Test A. Queen/drone attraction tests.....	147
Data Table 5. Test B. Queen/drone only attraction tests (no worker odour source present)	151
Data Table 6. Test C. Drone/drone attraction tests	153
Data Table 7. Test D. Drone/drone attraction tests - no worker influence	160

Table of Figures	Error! Bookmark not defined.
Fig 1 The Phantom Vision 2 camera equipped UAV	32
Fig 2. The UAV showing the extension attachment and the camera.....	32
Fig 3. The artificial queen bee used in the tests.....	33
Fig 4. The queen bee attached to the extension fitted to the UAV	33
Fig 5. The UAV with the queen extension and artificial queen.....	36
Fig 6. The numbered flags show lift points from where the UAV was raised and measurements taken.....	37
Fig 7 showing the drone activity hotspots derived from data taken from the lift sites shown in Fig. 6.	37
Fig 8. showing landscape features superimposed on over the DCA hotspot map at Fig 7.....	38
Fig 9. Showing an aspect overlay on the hotspot data	39
Fig 10. Extract from Data Table 1 showing typical measurements at each site investigated.....	41
Fig 11. An area that was almost impossible to visit on foot or by vehicle.....	42
Fig 12. The areas investigated in this study	47
Fig 13. The area surrounding Apiary 050 which was first investigated	48
Fig 14. Showing UAV flight taking drones up valley sides.....	52
Fig 15. Showing the most of the drone activity.	55
Fig 16. The relationship between DCAs and ground slope	56
Fig 17. Mean times for drones to appear at the lure in 3 distinct areas	60
Fig 18. Distribution of data. Mean times for the formation of comets	61
Fig 19. The drone trapping net after Williams 1987. (Modified).	65
Fig 20. The 4 way olfactometer test equipment showing the air inlets	68
Fig 21. The four way olfactometer.....	68
Fig 22. The VAS four way air pump and air extractor device.....	69
Fig 23. Olfactometer layout.	69
Fig 24. Apparatus layout Test 2. Odour source bowls moved one position anti-clockwise	70
Fig 25. Drone odour source bowl (attendant workers removed).....	71
Fig. 26. Worker odour source bowl.....	71
Fig. 27. Workers in the odour source bowl attend the queen.....	73
Fig. 28. Close up of workers in the bowl attending a queen behind the gauze screen in Test 1. Queen 1.....	73
Fig. 29. Test A. Queen/Drone attraction.....	74
Fig 30. Test B. Worker bee influence removed providing a choice of air or drones.....	75
Fig 31. Drone attraction to workers, drones or air on a four arm olfactometer test	76
Fig 32. Test D. Drone attraction chart with worker influence removed.....	77

An investigation of Honey Bee Drone Congregation Area formation in rural and semi-rural locations in New Zealand

1. Introduction

During the breeding season, the Western honey bee (*Apis mellifera* L) drones mate with virgin honey bee queens in flight. Drones are thought to congregate in certain, well-defined areas called Drone Congregation Areas (DCAs). Several drones will mate with a virgin queen that flies through the area on her mating flight (Bottchner (1971); Gries and Koeniger 1996). However, the factors that determine the presence of DCAs or their boundaries are still comparatively unknown (Scheiner et al 2013). Although not all mating events take place at DCAs, these congregations appear to be important for honey bee reproductive behaviour (Koeniger et al. 2005).

Studying DCAs is important because such areas may have practical uses for genetically controlled mating in bee breeding programs and could be used to delimit conservation areas for subspecies of honey bees (Ruttner 1976); Jandricic and Otis (2003). This aspect now assumes even greater importance in view of the global spread of exotic diseases and the growing resistance to treatments (Aizen and Harder 2009; Neumann and Careck 2010; Oldroyd 2007; Williams et al. 2020).

Finding and determining DCAs however is a time-consuming business which hinders research into these areas, and carrying out research into the parameters of DCAs once found, can be difficult, especially in remote areas. Radar monitoring has been used for this purpose, but is expensive, and the effectiveness of this method can be limited by the local terrain, vegetation, and man-made features (Loper et al. 1987). Landscape analysis methods investigated by Galindo Cardona et al. (2012) have also been used to characterise the landscape features of DCAs and these methods show promising results.

Most research into the flight behaviour of drones in DCAs and DCA boundaries has been conducted using helium balloons or kites holding aloft an artificial queen impregnated with queen pheromone (E)-9-Oxodec-2-enoic acid (9-ODA), dead queens, or tethering live queens to a line (Muerrle et al. 2007; Ruttner 1985). These methods are effective in most cases, but even in ideal conditions, balloons and kites suffer from a lack of directionality. For example, a balloon cannot easily be sent to a particular area over a tree line or river, or over an area of impenetrable bush to locate a DCA or test DCA boundaries, unless the wind is blowing in the right direction. Wind direction therefore can often dictate the days on which investigation of certain inaccessible areas could be carried out. Even

then, intervening tree lines can often prove a barrier because of the possibility of line entanglement. In addition, balloons and kites cannot be used in certain areas inaccessible on foot or by vehicle due to their distance from access points.

Some researchers, for example Butler and Fairy (1964), discard the idea of the existence of areas where drones congregate to mate with queens although a considerable amount of research into DCA formation points to their existence. However, even using the investigation tools and techniques described above, it has been difficult to define them or to find out why they form in certain areas year after year (Loper et al 1987, Winston 1987), and not in other similar areas; or whether they have defined aerial boundaries outside of which drones will not mate with queens. Similarly there is little knowledge on how drone bees, which are new each year and therefore cannot learn from colony experience, are able to find these areas; or how virgin queens, also new, are able to locate them in a single mating flight. There is evidence to suggest that landscape features may play a prominent role in DCA formation and that pheromones could also play a role.

The current study looks at both aspects of DCA formation and consists of three parts. The first part introduces and establishes an improved method of investigating the DCA phenomenon by using a camera-equipped, unmanned aerial vehicle (UAV) to overcome the problems mentioned above in investigating certain aspects of DCAs in inaccessible areas. Part Two analyses the results of the data derived from the UAV based investigation into a series of DCAs. The results provide at a glance maps of drone flight activity levels within each DCA and attempts to locate and map DCA boundaries and look at the role of landscape features in DCA formation. It is known that drones are actively attracted to the virgin queen mandibular pheromone (Wanner et al. 2007), and this attraction could have a possible role in DCA formation. Therefore, the third part involves a four-arm olfactometer test using both virgin queens and drone bees to discover if drones are attracted to other drones as suggested by Brandstaetter et al. (2014), and whether virgin queens are attracted to drones.

2. Review of Literature

2.1 Drone Congregation Areas

The circumstances of honey bee mating are such that observation and research on the subject is not easily undertaken. Because of this high and distant mating behaviour that was rarely if ever observed, it has for centuries been the subject of curiosity and interest amongst scientists and beekeepers. Until comparatively recently, knowledge of how bees mate, and when and where they mate has been limited, causing the subject to be regarded as an unexplained mystery. Indeed, prior to the eighteenth century, some believed that perhaps they did not mate at all. Sir John More (1707) wrote; “There is great contest amongst philosophical bee masters how the bees are generated: some are of the opinion that they never generate, but receive and bring home their seed from flowers; others say that they have amongst ‘em both sexes, yet do not agree which are the males and which are the females.”

Others claimed that honey bee mating occurred in the hive or that the eggs were fertilised in their cells by drone bees. Janscha (1775), however, was able to describe queen orientation flights, subsequent serial mating flights, and the queen’s return to the hive with what was later recognised as, and commonly known as, the mating sign (Woyke and Ruttner 1958). (The mating sign consists of chitinized plates of drone endophallus filled with mucus from the last drone to mate with the queen. Each drone mating with the queen removes the remains of his predecessor). Similarly, Huber (1782), noticed that queens only produced workers following flight and that the return of the queen to the hive with a mating sign was a prerequisite to the queen producing worker offspring. Yet observing what happened during the mating flight, and exactly how and where mating occurred was another matter, and this state of affairs pertained until the mid-20th Century. There had always been much anecdotal evidence that queens in some areas mated better than those in other areas and many beekeepers took their queens to certain areas where drones were supposed to fly in great numbers to have them properly mated (Cooper 1986). Similarly, various authors noted areas where drone bees could be heard flying in abundance and again a large amount of anecdotal evidence was built up around the possible existence of ‘special areas’ where drones congregated to mate with queens.

Drones fly from the hive as young as four days after emergence, but it is more usual for them to fly from age five to seven days onwards. They fly to defecate, orientate themselves and to mate. One of the first references to DCAs was made by Buttel-Reepen (1923) who believed that drones congregated in certain areas, and he cited others who had made similar observations. He concluded that drones usually flew high enough to be out of sight and in sufficient numbers to sound like a

swarm, if heard. After it was realised that a queen mated with several drones on her mating flight (Roberts 1944, Triasko 1951), more notice began to be taken of anecdotal evidence among beekeepers of areas where drone bees could be heard and sometimes seen in large numbers. These areas existed in a number of widely differing climatic and topographic conditions and were often noted year after year in the same location. One such area in Hampshire, UK on Selbourne Common has been seen and heard every year since 1779 and is believed to be the oldest known DCA in existence. Mostly, these drone gatherings were thought to be swarms by beekeepers and because they were not usually seen, they were rarely investigated.

Later observations showed that mating can take place in these special congregation sites (Jean-Prost 1958), and that the queen mandibular gland pheromone 9-oxo-2-decenoic acid (9-ODA) functions as a sex attractant (Gary 1963 a). This knowledge prompted more intensive studies of drones, drone flight behaviour, and congregation areas. Jean-Prost (1958) regarded a congregation area as a definite location where drones regularly assemble; 'independently of the presence of a queen.' Later research showed that virgin queens commence their mating flights significantly later in the day than drones, and therefore DCAs are formed irrespective of their presence (Koeniger and Koeniger 2004).

Gary (1963 a, b) showed that either tethered queens or artificial queens impregnated with synthetic queen pheromone, suspended from lines held aloft by kites or balloons attracted drone bees, and it became evident that virgin queens in flight attracted drone bees to mate with them. This prompted a huge interest in the study of mating behaviour and our knowledge of the subject increased dramatically. It now became possible to investigate this phenomenon in more detail using tethered queens or synthetic queen pheromone-impregnated artificial queens suspended from lines held aloft by kites or balloons. Drone congregations were usually found more than 60 metres from an apiary and drones usually responded to the pheromone from 6 to 30 metres off the ground. The assumption was made that these congregation areas were also mating zones (Zmarlicki and Morse 1963).

Using these new pheromone-based investigation techniques it became clearer to researchers that much of the anecdotal evidence existing in beekeeping circles had substance. Since then, most investigators have used these techniques to unveil honey bee mating behaviour and a great body of scientific literature into these areas has been based on the use of balloons, kites and static lines holding aloft various types of lures.

The research indicates that both in the nest and in the vicinity of the nest, drones are not attracted to virgin queens even when those queens are departing the nest on their nuptial flight (Gary 1963,

Ruttner and Kaisling 1968). Furthermore, it became evident that the drones of *Apis* species do not mate within their colony where they would meet only young sister queens (Koeniger and Koeniger 2004). Instead, drones head away from the nest and congregate in areas known as Drone Congregation Areas (DCAs) (Zmarlicki & Morse 1963, Ruttner 1974, Koeniger et al. 1979). During mating flights, drones fly in the DCA in wide loops until they either mate with a queen and die or return to the colony to feed, ready for another sortie to the DCA if time of day permits (Koeniger and Koeniger 2004).

More recently, Koeniger et al. (2005) found that during an orientation phase, drones chose congregation areas nearer to apiaries, possibly to prolong their stay in the area and thus increase their chances of mating. Generally within *A. mellifera*, several DCAs are found within the flight range of a colony (Zmarlicki and Morse 1963), (Ruttner and Ruttner 1972). Calculations of the relatedness of drone captures in a DCA in Germany revealed that drones within one DCA, originated from 240 different colonies, showing that '*Apis mellifera probably represents one of the most elaborate panmictic systems possible amongst terrestrial animals*' (Baudry et al. 1998). DCAs often have widely differing spatial parameters, varying from 30 – 200 metres and from 10 to 50 metres in height (Ruttner 1966).

Extensive investigations of DCAs in Germany and Austria, using these new techniques led Ruttner and Ruttner (1972) to define DCAs and drone flight behaviour as follows:

- DCAs are specific locations where drones assemble year after year
- DCAs exist irrespective of the presence of a queen
- Drones respond to queens with greater intensity when within a DCA
- Pursuit of queens by drones declines as queens move away from the DCA
- Flight altitude of drones in a DCA varies inversely to wind velocity
- Drones locate DCAs quickly on their first mating flight.

Ruttner and Ruttner (1965) maintained that DCA boundaries were definite and that drones are not attracted to queens outside the area, and Strang (1970) further suggested that DCA boundaries are marked by vertical relief features such as hedges, tree lines or buildings. An early study (Ruttner 1966) observed that drones attracted to a tethered virgin queen left her if she was moved out of a DCA and instead, they re-joined the DCA. However, Tribe (1982) found that drones of *A. mellifera scutellata* would follow a lure for over 2 Km.

Some researchers have questioned the existence of DCAs. Tribe (1982) suggested that DCAs do not exist but that drones are plentiful in an area and will follow and mate with a queen in comet formation, moving upwind to track her; while others (e.g., Butler and Fairy 1964) sustain the idea that drones instead cruise at random and are attracted to queens due to odour/pheromones. Butler and Fairy (1964) suggested that drones find queens quickly because queens have an effective sex pheromone system for attracting them. We know now that this pheromone has a specific receptor site on the drone bee antennae, which makes the pheromone very effective in operation. They state that if virgin queens fly from the hive in a direction perpendicular to the direction of the wind, their area of pheromone dispersion would be maximised enabling drones flying at random in an area to pick up the pheromone more easily in wide, flat, open, agricultural areas.

Even if DCAs exist, the number of drones present in a DCA appears to vary, presumably depending, in part, on the number of available colonies in the area. Koeniger (1990b) observed one queen encountering in excess of 1000 drones at one DCA and Gary (1963) observed drone comets of between 100 and 300 drones. The number of drones at any one time may depend on several factors including the time of day, meteorological factors (temperature, cloud cover, light) and season; factors which can complicate research attempts to replicate conditions in various tests. Over a period of ten years, Ruttner and Ruttner (1972) studied an area near Lunz in Austria and showed that drones from numerous apiaries exhibited a complex distribution among DCAs in the area. Not only were drones visiting several different DCAs but drones from many different apiaries within a 5 Km distance were present in these DCAs.

2.1.1 Multiple mating in honey bees

Flight outside the hive exposes a queen to a myriad of dangers; she could be eaten, suffer a change of weather conditions or even simply get lost. Therefore, presumably, in order to accomplish this multiple mating in as short a time as possible, a virgin honey bee queen will leave her nest and fly through an area where drone honey bees have accumulated prior to her arrival and are thereby available to mate with her in as short a time as possible. Whilst in this area, the drones detect a queen from up to 60m away using a honey bee odorant receptor for the queen pheromone (9-oxo-2-decenoic acid) (Wanner et al 2007). When a queen enters the DCA, drones chase her immediately in a comet like formation, each drone scrambling to attain a lead. (Gary 1963). In his 1963 paper, Gary described the intensive, dynamic flight of drones '*a striking spectacle*', and indeed it is. The formation and disintegration of drone comets around the queen is rapid with hundreds of drones appearing as if from nowhere and disappearing just as quickly only to immediately reappear when a new queen is located. Using high speed film techniques, Gary showed that there were very

coordinated flight movements within a comet, indicating that at this range, the comets are integrated primarily by vision.

Unlike many animals that fiercely compete for females to mate with, honey bee drones may even cooperate in mating. One example of this may be seen in the formation of Drone Congregation Areas, a collective effort that attracts virgin queens. Another is the fact that drones leave a mating sign after copulating with the queen, consisting of chitinised plates of drone endophallus filled with mucus (Koeniger 1990). Some researchers have suggested that the mating sign of the drone was a barrier to following drones who also wanted to mate with that queen. (Woyke 2011). The first drone therefore gained an advantage in that only his sperm would be used by the queen for the next generation. Yet, the fact that the queen mated with many drones anyway tended to contradict this hypothesis. In a two-choice test using models, Koeniger (1990) showed that drones actually preferred queen models marked by the distinctly coloured 'mating sign' compared to unmarked models, and that models carrying copulating drones were more attractive than single models. Thus a copulating drone as well as the mating sign and other coloured markers increased the attractiveness of a queen. The author suggests that the mating sign may indicate a form of cooperation between drones by marking a queen after sperm transfer to facilitate the identification of a queen by the following drones.

The principle hypothesis for multiple mating in honeybees is that of genetic variability (Fuchs and Moritz, 1998). Colonies with greater levels of genetic variability have been shown to be more responsive to environmental variation relevant to foraging (Fewell and Page, 1993). Oldroyd and Fewell (2008) stated that "Multiple mating by social insect queens can enhance colony fitness via an enhanced task allocation system". The essence of the task allocation hypothesis is that in many multiple mating (polyandrous) eusocial insect species such as honey bees, individual workers are genetically biased to perform specific tasks (genetic task specialization). Genetic diversity in these colonies provides a worker force that can perform a range of tasks efficiently and can allocate its workers appropriately across tasks as environmental conditions change. There is an accumulating body of evidence for the hypothesis that more genetically diverse colonies can better respond to changing environmental conditions, and indirect evidence that increased genetic diversity positively affects fitness via task allocation."

It is also evident that the more times the queen mates, the better for the colony (Richard et al. 2007). A mated queen is better accepted by her nest mates on her return to the colony. Worker bees exposed to pheromone from multiply mated queen and to queens who had mated only once showed a preference for the pheromone from the multiply inseminated queens (Richard et al. 2007). There were also differences in the analysis of the queen mandibular pheromone between

the two, as well as differences in brain-expression levels of a behaviourally relevant gene. In other words, insemination quantity alters queen physiology, pheromone profiles, and queen-worker interactions.

The fitness benefits of multiple mating was demonstrated by Mattila and Seeley (2007) who created genetically uniform colonies after artificially inseminating sister queens using sperm from a single drone. These colonies were then compared to colonies headed by queens inseminated by an equal amount of semen from 15 unrelated drones. Artificial swarms from these colonies were then used to assess the fitness effects of polyandry over monandry. They looked particularly at evidence of colony growth following swarming such as colony growth, amount of wax comb built, amount of stored nectar and pollen, forager activity, and amount of brood. Their results showed that the colonies headed by polyandrous queens built significantly more comb in a two-week period after swarming and this lead increased over the year of the trial. This enabled the polyandrous colonies to store increased amounts of food, enabling them to grow more quickly, produce more drones and show a 78% increased amount of forager activity over the monandrous colonies.

Building evidence suggests that more genetically diverse honey bee colonies can better respond to changing environmental conditions (Oldroyd and Fewell 2007,-Mattila and Seeley 2007). Increased genetic diversity results in a more flexible workforce suited to perform a wide range of tasks and better adapted to changing conditions. Natural selection favours queens that avoid inbreeding because inbred queens lose brood viability. The need to avoid inbreeding by mating with related drones begs the question, 'how do queens avoid nest mates?'

Research (Taylor and Rowell 1988, Tribe 1982) has shown that drones are abundant in the immediate vicinity of hives in an apiary and that they are attracted to artificial queens in and around an apiary. Mechanisms that have been suggested to help prevent inbreeding with drones from the natal hive include negative assortative mating in which sibling drones are rejected by queens, or a random system in which the probability of meeting nest mates is minimised (Taylor and Rowell 1988). There is no data to support negative assortative mating, but Taylor and Rowell 1988 developed a distribution pattern that would support a random system, which minimises meeting related drones. The research showed that drone abundance is at its peak over the source apiary and reduces rapidly to zero after 2.4Km. This suggests that queens mating near their apiaries would meet mainly related drones, so they would need to fly sufficiently far away from home to minimize inbreeding. Experiments by Gary (1971), Ruttner and Ruttner (1972) and Taylor and Rowell (1988) showed that even if queens fly a limited distance, e.g., 1.5 to 2Km from the hive, they should encounter a dramatic reduction in the presence of drones from their own source colonies. This is discussed further in section 2.4.

Earlier researchers including Gary and Marston (1971) and Ruttner and Ruttner (1972), and found that queens flew on average 1500 metres to over 2000 metres from the hive, a distance well beyond the peak distribution of related drones. Using these distances Taylor and Rowell (1988) estimate that honey bee populations with densities of 0.39 colonies per Km² with a drone flight of 1 – 1.2Km and a queen flight of 1 - 1.5 Km will be outbred.

2.2 Drone flight parameters

Drone flight can begin as young as 4 days after emergence of the drone but most make their first flight between 6 and 12 days of age and all by 18 days (Currie 1987). Times of drone flight are mainly influenced by time of day, time of year, and meteorological conditions. In earlier research of a DCA near Hay on Wye in 1993 (Cramp 1993), I found that drones flew in sufficient numbers to form DCAs and drone comets between 15.00 to 18.00 hrs. On one day however, rain reduced the flying time to less than 30 minutes and the following day drones formed a DCA and drone comets in response to a lure for an extended period, between 13.00 and 18.30 hrs. The earliest time drones were seen over the 8 day period of investigation was 12.15 hrs and the latest time was 19.00 hrs. The mean daily start time of observed drone flight was 14.17 hrs and the finish time 18.04 hrs. The mean daily formation time of DCAs in which drones would respond to a lure was 14.49 hrs and cease time was 17.40 hrs.

During the mean start and finish time of drone flight, the lowest temperature recorded was 17 °C and the lowest temperature at which DCAs formed was 18.5 °C. It seemed therefore that at this location, drones required a temperature of 17 °C to fly to a DCA and that in order for sufficient drones to be on the wing to form comets, the temperature had to be 18.5 °C or higher.

Although the numbers of drones in the comets were not counted, the size of the drone comets could easily be seen and were larger between 15.00 and 15.30 hrs. I found that drones flying outside of these mean times and heading back to the apiary were not attracted to the lure and that the start of the decline in mean daily temperatures coincided with an increase in the initial height for drones to be attracted to the queen. These observations generally accord with results of other investigations into drone flight parameters in relation to DCA formation and I based my research activities in the New Zealand environment on those times and temperatures.

The time of day that drone flight activity commences has also been correlated with relative humidity, light intensity, the position of the sun and day length (Currie 1987). Despite taking these measurements, in the 1993 study, I was only able to show that a negative correlation existed between temperature and relative humidity but could not draw any conclusion as to what effect this had on the formation of DCAs.

Drone activity may be regulated by a circadian rhythm or an interval timer (Spangler 1972). Daily activity rhythms of individual worker and drone honey bees and complex behaviours such as sun-compass orientation and time learning are clearly regulated by the circadian system in these organisms (Giannoni-Guzmán et al. 2014). Queen flight activity begins approximately 20 hours after sunset (Currie 1987) and it has been suggested that drones and queens may use an interval timer to measure time from sunset before flying again. The period would be different, as drones fly to DCAs well before queens begin their mating flight.

Rowell et al. (1986) found that there were substantial differences in mean departure times of drones from their hives, even among drones within the same hive. Some differences can be ascribed to hive position (Taber 1964), where drones from hives facing towards the sun will fly before hives facing away from the sun. Within-hive differences cannot be explained so easily. The differences did not depend on the age of the drones, although flight duration was longer among older drones.

Drone orientation to DCAs when they leave the hive for the first time to seek a DCA is not well understood. Drones are new born each year and although they do take orientation flights before they are ready to visit DCAs, the first drones can have no hive experience to guide them to the sites. Ruttner (1976) addressed this problem by studying directional preferences for drones on leaving the hive and found a clear preference for places that could be reached by a straight, direct flight from the hive. He noted that even a small elevation in ground between the hive and the DCA reduced the number of drones visiting that DCA from the number expected. He concluded from this that drones use landmarks on the horizon for orientation and that low horizons may attract them. This would suggest that they prefer directions that would take them out of a valley (Ruttner and Ruttner 1977), or towards strong UV radiation on the horizon (Ruttner 1974).

2.3 Pheromones and Honey Bee Mating

The actual mechanics of honey bee mating are well known, having been described by several authors (Woyke and Ruttner 1958, Koeniger, Koeniger and Fabritius 1979, Koeniger and Koeniger 2005). By means of an arm on a rotating mast, a camera triggered from the ground, and a tethered queen, mating has been observed and filmed (Koeniger 1986). However, all the factors necessary to attract drones to a queen, and drones and queens to certain areas, are still not fully known.

The role of olfaction in honey bee mating behaviour was established in the early 1900s by using caged virgin queens to attract drone bees (Salmon 1938), and it became clear that chemical cues were important, evidenced by the successful use of synthetic pheromone 9-oxo-2-decenoic acid (9-ODA) to attract drones, a phenomenon described and tested by Gary (1962). The major component

of the virgin queen mandibular gland secretion is 9-ODA, which acts as a long-range sex attractant (Brockman et al. 2007). More than 45 years after its discovery, 9-ODA remains the only long-distance sex pheromone identified from honey bees (Wanner et al. 2007). Tests by Brockman et al. (2007) of other mandibular gland secretions failed to demonstrate attraction, although some, when added to 9-ODA increased the numbers of drone attracted to a dummy. For the purposes of this study, I used 9-ODA only.

The discovery by Wanner et al. (2007) of a honey bee odorant receptor for the queen substance 9-ODA, progressed our understanding of drone – queen attraction, yet it is clear that other factors are involved in avoiding nest mate pairings. Although Zmarlicki and Morse (1963) were unable to attract drones to queens in the vicinity of an apiary, Botchner (1971) showed that drones could be attracted to queens in the vicinity of an apiary by artificially opening the sting chamber, and that mating could be induced.

Butler and Fairy (1964) and Brandstaetter et al. (2014) demonstrated that drones are attracted to 9-ODA rather than queen mandibular pheromone which more closely resembles the bouquet of mated queens. The latter research was conducted under laboratory conditions and the assay also showed that drones are attracted to groups of drones (but not to workers), which may indicate a role for drone-emitted cues for the formation of DCAs. This is investigated later in this study.

Various authors, including Free (1987), Renner and Baumann (1964), Butler (1971) and Renner and Vierling (1977) believe that the tergite gland secretions aid copulation and act as essential close-range aphrodisiacs, helping the drone to approach the queen and get within visual acuity range. The importance of visual cues in mating behaviour is shown by the fact that drones approach queens from below her flight level, fixing her at a visual angle of between 0 and 40 degrees (Van Praagh et al. 1980). At this angle, they use their largest eye facets; those of the dorso-frontal region of the eye. Drones will also pursue other insects in a DCA and even small stones thrown up into the air (Cramp 1993), although pursuit is short lived probably due to the lack of pheromonal influence. Research on this failed to answer the question of why visual cues are not important outside of a DCA.

Even though it is now easier to investigate DCAs using tethered lures of one sort or another, evidence as to why drones are abundant in certain areas or why they form DCAs in certain areas and not in others has been difficult to find. It has been postulated by Galindo-Cardona et al. (2012) that both behavioural and physical landscape attributes play a part in the location of DCAs. Several authors have studied both of these attributes and these are discussed below.

2.3.1 Drone aggregation pheromone

It is evident that the complex social organisation of honeybees is mediated through pheromones (Trhlin and Rajchard 2011), and there has been much research on this subject, especially in relation to queen-emitted pheromones. Whichever mechanism drones use to fly to a DCA such as visual cues, horizon features or landscape features, drone orientation at the area itself has not been explained. It has been suggested that the mechanism for this may be drone-emitted drone attractant pheromones. Free (1987) suggests that drones may produce an odour that attracts queens and that may attract drones to an area, and keep them in an area once they have arrived. There has been very little research however on the role of drone-emitted pheromones in the mating behaviour to determine whether drones are attracted to other drones. Several authors have suggested that honey bee drones are attracted to consexuales (Brandstaetter et al. 2014, Lensky et al. 1985, Gerig 1971, Free 1987), and this factor may be important in the development of and sustainability of DCAs. Brandstaetter et al. (2014) suggest that other factors in the formation of a DCA such as landscape features do not explain orientation at the DCA itself and that when virgin queens fly out of a DCA, the drones cease pursuit and return to their consexuales in the drone congregation, which acts therefore as a form of drone leash.

Gerig (1971 and 1972) found that extracts of drone heads were more attractive to other drones than extracts of other body parts and suggested that a likely source for a drone pheromone could be the drone mandibular gland. He was able to test this in field trials and most flying drones were attracted to lures containing extracts of mandibular gland rather than extracts of other cephalic tissues. He was able to show that the gland was fully developed at 7 days of age and that after 9 days the gland was no longer active. Although this cessation of activity takes place before sexual maturity (at about 9 -14 days), he found that the product of the gland is stored in the gland lumen for emission during mating flights. The results of Gerig's experiment showed that the mandibular gland pheromone is attractive to flying drones in spite of its small size and atrophied state.

Lensky and Dempster (1985) also suggest that although atrophied, the gland may still contain minor quantities of compounds attractive to other drones. As there does not seem to be a known biological function for this pheromone, it may be important in DCA formation or attracting virgin queens. Less investigation has been made into whether queens are attracted to drones. The drone mandibular gland that is hypothesised by Brandstaetter et al. (2014) to be attractive to other drones may also have an effect on attracting queens. In a new study using a similar walking simulator set up to that of Brandstaetter et al. (2014), Bastin et al. (2017) showed that queens were attracted to drones and not to workers which, if also manifested during the queen's nuptial flight,

could help queens find drones. They also showed that there was no attraction of workers to drones. If virgin queens are attracted to sexually mature drones, it may play a part in directing queens to areas where drones congregate and affect her behaviour within that area.

There are obvious difficulties in testing this in a DCA where drones are flying, however it has been suggested that areas where drones may accumulate such as intersections of flyways (Loper 1992) may result in a DCA forming with more and more drones attracted to the area. In the area investigated in this study, landscape may play a role in directing drone flight, especially in narrow valley areas where hillsides may force drones to fly in wide loops within a confined area. The resulting accumulation of drones in the area may then attract more drones resulting in the formation of a DCA, yet this may not be the case in open, flat areas.

2.4. The Mating flight of the queen honey bee

Most studies into the existence and parameters of DCAs concentrate on drone behaviour, and few investigate the mating flight of the queen. Butler and Fairy (1964) note that very little is known about the mating flight of the queen bee once she leaves the hive.

Queens fly to DCAs after drones and sometimes an hour separates their arrival in the area (Koeniger and Koeniger 2004, Ruttner 1985). This means that DCAs are not dependant on the presence of a queen for their formation and it follows that queen flight is important. Lensky and Demter in 1985 studied the mating behaviour of queens and drones and their mating success throughout the year and found that:

- Worker bees directed virgin queens to leave the hive and take their mating flight
- Cloudiness above 7/8 and wind velocity above 3.9 m/sec. delayed both queen and drone flights
- Duration of queen flights was reduced at 15–20°C and wind velocity of 2.6–3.9 m/sec.
- The peak of flight activity hours of queens and drones overlapped through the year.

This research gives valuable information about the origin of flight and shows an obvious relationship between the environmental conditions and queen flight activity, but it says nothing about the direction, orientation or other important events occurring during flight. What direction does she take on leaving the hive? Does she fly upwind, or towards any landscape feature? Does she open her sting chamber all the time or just when she enters a DCA or area of drone abundance. If so, how does she know she is in one? If she opens her sting chamber only after a certain flying time, would this take her past her own kin peak area? Does she fly upwind to accomplish this?

Currie (1987) speculated that queens could maximise the area exposed to their pheromones by

flying in a direction perpendicular to the direction of the wind. The abundance of drones in an area may also influence the mating flights of the queens, although authors differ on the effects of drone density. Koeniger and Koeniger (2007) found an increase in mean flight duration between areas of abundant drones (10,000 plus) and areas with fewer drones. However, Woyke (1960) observed the opposite effect with an abundance of drones leading to longer flights. The differences may have been due to other factors, such as time of day, temperature and so on. Heidinger et al. (2014) found that there were significant difference in the flight times among different ages of the queens, but no correlation between the number of drone colonies available and the flight duration or frequency, and no difference in the effectiveness of the flights in relation to the amount of sperm received.

Another parameter potentially influencing drone choice of DCA may be distance from the apiary. This was demonstrated by Koeniger et al. (2005) in an experiment using marked drones indicating that drone strategy was to choose nearer DCAs rather than those further away. They found however that there was no direct correlation between distance to the DCA and ratio of drones visiting from each apiary. They concluded that other factors may be at work and suggest the intriguing idea that 'attractiveness' of the DCA may have influenced drone choice. They also suggest that the nearer the DCA to the drone colony, the longer the drone's internal fuel would allow him to stay, therefore increasing his chances of mating with a queen.

The question of how queens avoid drones from their own nest has been debated by several authors. There are two possible ways that queens avoid nest mates (Taylor and Rowell 1988):

- Queens mate close to their colonies but drones from the same apiary fly beyond this range
- Queens fly further than the peak abundance of drones from their own colonies.

There is no data to support the first alternative, and instead, drone abundance tends to drop sharply with distance. Distribution patterns suggest that queens mating near to their own apiaries will meet mainly nest mates.

The second alternative is supported by drone distribution patterns. Earlier studies by Gary and Marston (1971), Ruttner and Ruttner (1972) and Boetcher (1975) indicate that queens fly further than the peak abundance of drones from their own colonies. This indication rests on the assumption that there are unrelated drones/colonies available within the dispersal range and Taylor and Rowell (1988) have shown that honey bee populations with densities of 0.39 colonies per km² with drone flight distances of 1 – 1.2 km and queen flights of 1 – 1.5 km will be outbred. This however begs the question of how the queen passes through the peak abundance of drones from their own colonies, without mating occurring especially when the peak drone abundance is often over or in the near vicinity of the apiary from which the queen flies. It is evident from my

investigations that strong drone comets will form in response to a lure within apiaries, from very low level (1 metre or less) upwards, regardless of wind speed. Further research into this is needed to ascertain if the queen opens her sting chamber and activates the tergite gland only after a certain amount of flying time that would take her past her peak kin abundance of drones. This was already hinted at by Boetcher (1975) when he showed that he was able to get drones to mate with a virgin queen within her apiary by artificially opening her sting chamber.

2.5 Factors that may give rise to Drone Congregation Area location

As discussed above, studies show that there are several factors that may play a part in DCA location. These are summarised below and will be discussed in the following sections:

- Flight altitudes and distance from the apiary
- Magnetic sense in honey bees
- The influence of wind
- Optical radiance
- Physical landscape features
- Honey bee pheromone attraction.

2.5.1 Flight altitudes and distance from the apiary

Flight altitudes and distance from the apiary appear to play a part in the location of DCAs. Experiments by Zmarlicki and Morse (1963) showed that virgin queens were ignored by drones within 30 metres of an apiary, but many drones were attracted at a distance of 60 metres. The same authors noted however that although queens were ignored at a distance of 60 metres from another apiary, when the pheromone lure was raised to a height of 90 – 120 metres, drones were attracted.

Koeniger (1986) showed that drones were attracted to queens when raised 6 – 10 metres off the ground and that the maximum number of drones appeared at 15 – 25 metres. He also pointed out that there is a definite correlation between the height that drones pay attention to queens, and meteorological conditions; in some cases, queens being ignored when offered at different heights within the DCA. This may relate to wind factors which are discussed below. This information has been contradicted by authors reporting that drones will pay attention to queens within a DCA from ground level upwards. (Tribe 1982; Gerig and Gerig 1983). During this research I found drones would pay attention to queens at ground to very low levels regardless of wind conditions.

Flights between DCAs by drones were illustrated by Loper et al. (1987, and Loper (1992) using X-band radar to track drones in flight. They found that drones base their flyways on the most

important physical feature of the area – tree lines, and that where the flyways branched, DCAs would form. The authors suggested that DCAs form in these areas as drones accumulated to reorient themselves for their next flight direction. Their results showed that the maximum flight altitude of drones in the flyways averaged around 21 metres, whereas in the DCA areas, the maximum height averaged 30 to 50 metres. The studies were conducted on a flat area of near desert over a period four years, and indicated that the flyways and DCAs were established in the same locations in each year.

Early studies in Canada with genetically marked queens and drones in an otherwise bee-less area showed that queens were mated more frequently the nearer they were released to a drone apiary, although this wasn't conclusive as some matings occurred across distances up to 10.1 miles. With increasing distances from the drone source, fewer queens mate successfully, suggesting that the nearer the DCA, the better for the queen's success (Koeniger and Koeniger 2005). Queens located at the drone source, and at 3.8 and 6.1 miles distant, began laying eggs at approximately the same time but those located 8.0 miles distant began laying later and those at 10.1 miles later still (Peer 1957).

2.5.2 Magnetic sense in honey bees

It has been known for many years that bees can sense magnetic fields (Gould et al. 1978 and 1980) and the discovery of iron in the abdominal oenocytes of sexually mature drones led Loper (1985) to postulate a magnetic function to drone flight orientation and the establishment of congregation areas, in addition other cues. Honey bees do undergo iron bio-mineralisation, providing the basis for magneto-reception and leading to a proposed model for magnetic reception in honey bees (Hsu et al. 2007). Iron granulates in the abdomen change in size as a reaction to changing magnetic fields, leading to signal transduction for magneto reception and so bees are able to make magnetic maps during orientation flights.

The evolution of magnetic orientation is now well established and was reviewed by Gould in 2008. It is not thought that magnetic sense is the primary means of navigation for bees, but several authors have linked magnetic fields and UV light perception in the orientation of animals (Wiltschko et al. 2011, Winklhofer and Kirschvink 2010, Winklhofer 2010, Yoshi et al. 2009).

Honey bees are known to orient towards the earth's magnetic field (Gould et al. 1978, Gould et al. 1980), and support for this hypothesis comes from observations of free-flying drones forming clusters 30m either side of a 115-kV power line, as well as aggregating in areas where the electromagnetic field fluctuations were the largest. The drones were present only during periods when congregations were observed at sites not associated with the power line. DCAs were also

observed at two other sites where strong magnetic fields were recorded. A hypothesis was advanced that drones respond to the vertical component in magnetic fields by turning and re-orientating, and eventually using other cues in further flight (Loper 1985). Radar was used in these studies. Galindo-Cardona et al. (2012) believe that it may be possible to test for honey bees' ability to tell North from South using magneto-reception and that this ability may be used as a cue in locating and forming DCAs.

2.5.3 The influence of wind

Drone bees are attracted to queen pheromones (primarily 9-ODA) for some distance downwind of the queen (Tribe 1982). This behavioural response causes difficulties for the observer in measuring or defining DCA boundaries due to varying wind velocities and directions. It is also difficult for any investigation into the relative densities of drones at different elevations (Loper et al. 1987). These non-constant factors make it virtually impossible to measure like-for-like on any given day, or to faithfully replicate the research efforts of others. However, there is a certain body of accumulated evidence that may show how wind influences drone flight.

Wind may play an important role in this pheromone driven reproductive system in that it will spread a pheromone over a large area. If a queen flies upwind of an apiary when leaving the nest, she will leave a far longer odour plume than a queen flying with the wind. Tribe (1982) reported that in some areas, drones disappeared from DCAs when the wind dropped and reappeared when wind speeds increased. He added that drones rely on a good circulation of drone pheromones in the air and may fly upwind until they find an area of turbulent air where they wait for a queen. Zmarlicki and Morse (1963) however, found that DCAs were usually protected from strong winds where possible.

Butler and Fairy (1964) and Loper (1992), found that the height that drones operate at is inversely proportionate to wind velocity; the higher the velocity the wind, the lower the drones fly. Loper also found that DCAs could be found in regions where the topography caused air turbulence that more effectively circulated pheromones. This hypothesis is supported by the observations of Cooper (1986), who proposed that whilst mating undoubtedly took place in DCAs, alternative mating sites in the vicinity of the apiary may exist during times of high wind or other inclement weather conditions. He suggests that these areas may also play an important role in the mating of supersedure (new) queens early in the year when few drones are available and major congregation areas have not yet been established.

Atkinson (1993) indicated that apiary vicinity to DCAs may be important even if weather conditions are benign and that nearby DCAs are a normal occurrence. He further hypothesized that there may

be two types of DCA. The apiary-dependent DCAs that forms close to an apiary, and apiary-independent DCAs, which form in the same place each year regardless of apiary distance and to which drones fly to from miles around. Some observers believe that apiary-dependent DCAs only form in response to bad weather conditions, which would deter a queen from flying long distances to find a DCA (Atkinson 1993). This hypothesis has not been fully tested, yet my own observations in Wales in 1993 and during this study, show that DCAs can be found in the vicinity of and within apiaries in both windy and fine weather conditions.

Tribe (1982) hypothesised that wind may play a role in enabling drones to swiftly locate a congregation area, and that if strong winds are evident; more sheltered sites will be chosen. Butler and Fairy (1964) on the other hand, found that the higher the wind speed, the more drones appeared in response to a queen and that drone numbers decreased in periods of calm. These contrasting views may arise because of the different terrains that the researchers were working in.

2.5.4 Optical radiance

Van Praagh and Ruttner (1975) proposed that optical radiance may be one of the characteristics of DCA definition, after observing that measurements of optical radiance in certain DCAs were unique relative to adjacent areas. The idea that bee vision plays a crucial role in 'seeing' a DCA has been postulated in relation to relative humidity. From observations of the apiary-independent DCA on Selbourne Common in Hampshire (Talbot 1993), it has been noticed that drones may congregate above certain tree types rather than others. If this was due to leaf transpiration rates causing differing humidity levels around certain trees, it is hypothesised that bees may be able to detect this difference if the humidity levels affected the plane of polarised light at that point. Atkinson (1993) suggests that this hypothesis can be taken further in that any transpiration thermal may have this effect or even an area of lighter air when it meets heavier air. Both these ideas support Cooper's (1986) hypothesis which stresses the prevalence of thermals in the formation of DCAs. These factors have not been further investigated however.

All of the factors mentioned above that determine the location of DCAs may be influenced by various landscape features such as the presence of hills, valleys, cols, forestry and local geology, I believe the key to understanding why DCAs are formed in some areas and not in others may be found by a comprehensive investigation of those landscape features that are present in DCA locations and by comparing them with adjacent areas where DCAs are not formed (if they can be found).

2.5.5 Physical Landscape Features

Although all of the factors that attract drones to areas where they can mate with queens are not fully known, it has been hypothesised that among other factors, geophysical factors (landscapes) may play a part in drone orientation (Zmarlicki and Morse 1963, Loper 1992). Landscape features may have an indirect effect on DCA formation by determining effect on several factors important to its occurrence such as wind, UV light perception, optical radiance, magnetic sense orientation and the provision of orientation cues such as forests, valleys and hills etc. Geographical features visible to the flying drones can also have a direct effect on drone orientation, influence the location of DCAs, and the flyways used to reach them (Loper 1992).

Ruttner (1966) proposed that physical features on the landscape may play an important role in drone orientation. He noted that drones may head for landmarks on the horizon and that low horizons attract them. They therefore prefer directions that will take them out of a valley for example (Ruttner and Ruttner 1970), or towards mountain cols, i.e. towards strong UV radiation on the horizon-(Ruttner 1974).

The advance of Africanised bees towards the United States in the 1980s gave an impetus to the study of drone flight and mating behaviour. The use of radar gave a new dimension to the study of DCAs and enabled researchers to 'see' drone flight behaviour (Loper et al. 1985, Loper 1992). These studies found DCAs extending up to 30 metres and drone 'flyways' or routes used by drones at or below 15 metres. The flyways appeared to use tree lines as orientation cues and formed parallel to them, and 80 metres away from the strongest tree lines. Where these routes branched, drones accumulated in large numbers, and these points were considered DCAs by the authors. They defined these areas detected by radar as 'distinct perennial sites where drones accumulate, manoeuvre rapidly and fly higher than in the connecting flyways.' They also found that many of the DCAs were located where tree lines branched or ended. At these points, they postulate that drones fly higher to seek out more distant visual cues.

A detailed description of the use of radar in plotting DCAs is given by Loper et al. (1987) and shows the advantages of using this medium for plotting their dimensions. In one case, the radar was able to monitor two congregation areas simultaneously and the authors agreed with Tribe (1982) that the "distinctiveness of DCAs is a function of drone density". At high densities, the number of drones moving among nearby areas and drone flyways can be so great that drones can be attracted to queens almost anywhere and whilst mating normally takes place in DCAs, mating can occur in these flyways (Koeniger et al. 2005). Cramp (1993) noted that drones flying in an area between two DCAs, both of which were formed on what could be termed horizons, flew at a very much lower altitude.

In this area, that could be described as a flyway (Loper 1992), drone density was high and drones were attracted to butterflies and small pebbles thrown into the air. Their initial chase broke off due presumably to the lack of pheromone cues.

More recently, Galindo-Cardona et al. (2012) carried out an analysis of the landscape characteristics of DCAs in Puerto Rico and refer to the “physical DCA hypothesis”, which attributes specific physical (landscape) characteristics of the areas as factors attracting drones and queens to DCAs. An alternative hypothesis is the “behavioural DCA hypothesis” which postulates that DCAs could result from behavioural interactions of flying drones and queens (Loper et al. 1992). It is possible that these two hypotheses are not mutually exclusive and that certain flight paths directed by physical landscape characteristics, may result in particular behavioural interactions.

Galindo-Cardona et al. (2012) also investigated the landscape characteristics of a number of DCAs, discovered by firstly determining the flight of drones from apiary colonies and then using an artificial lure to establish the location and area parameters of the DCAs. The analysis of the DCAs was aided by the use of aerial photographs and GIS software and it was found that a southern aspect may have played an important role in allowing drones flying from different colonies and different apiaries to converge on an area and thus serving as an orientation cue. They hypothesized that the aspect preferred by drones may be a function of their magnetic sense orientation.

Evidence of a magnetic sense in honey bees was provided by Hsu et al. in 2007 and generally in insects by Yoshi et al. in 2009. They also say that this orientation choice could be determined by the distribution of light, wind direction and the skyline as hypothesized by Hempel de Ibarra et al. (2009) in their work on bumblebees. Males of certain species of bumblebee are known to congregate at or near the tops of hills (hill-topping) and this is believed to be a putative form of mate locating behaviour (Goulson, Sangster, and Young 2011). An investigation of two DCAs in the United Kingdom performed by myself in 1993, found that both DCAs were in an area with a Northern aspect and that the horizon may have been important. Galindo-Cardona et al. (2012) however, found that a southerly aspect only played an important role in the location and formation of DCAs in Puerto Rico. They theorised that skyline is believed to be important for drones. and may be similar in this respect to the habit of hill-topping by bumblebees mentioned above.

Thus the physical structure of DCAs varies greatly (Ruttner and Ruttner 1965) from the tight, well bordered DCAs found in valley areas to the more even distribution of drones found in flat areas where queens can attract drones almost anywhere (Butler and Fairy 1964, Tribe 1982, Lahner 1988).

2.5.6 Honey bee pheromone attraction

The possible role of drone emitted pheromones as a contributing factor in DCA formation is discussed in section 2.3.1 above and studies suggest that it could be important in building up drone numbers in certain areas, and maintaining the cohesiveness of DCAs.

3. Methods of investigating DCAs: Advances and challenges

The use of balloons and kites have greatly contributed to our knowledge about DCAs, but their use presents several limitations especially in that they lack directed flight and are unable to access some areas. In areas with a comparatively short flying season, every day during which drones and queens fly is especially valuable and investigation can take years with a only a small gain in knowledge each year (Ruttner 1966). The limitations of using balloons and kites can exacerbate this and can hinder research. Very few scientific papers have acknowledged the main limitation which is the difficulty of investigating potential DCA sites in remote and inaccessible areas. Balloons are effective in most weather conditions in which drones can fly but are effectively restricted to open areas where line entanglement is not a problem (Ruttner 1966).

Where winds are fairly constant kites can be used, but even in ideal conditions, with both methods dependent upon wind direction, neither is directional. Balloons cannot be sent over a tree line or river, or over an area of bush to test DCA boundaries, unless the wind is blowing in the right direction, and even then, line entanglement in high trees and tree lines is a constant problem. As Ruttner (1966) commented; “the balloon method is restricted to open country and to relatively calm days.” Yet most research on DCAs still uses balloons to hold queens aloft.

Weather conditions therefore can dictate the days on which investigation of certain areas can take place. The dependence on weather factors may have unintended consequences for research, producing artefacts, biases, and fragmentary data that may not truly reflect natural conditions.

While also presenting limitations, the use of a camera equipped Unmanned Aerial Vehicle (UAV) to hold aloft an artificial queen (described in Section 4 below) is an advance on current methods in that it is directional, is able to avoid tree lines, and can quickly visit and video inaccessible areas.

3.1. Summary of the literature

Although studying and understanding drone behaviour with respect to DCAs is important for our understanding of conservation generally, the literature shows contradictory information and evidences shortcomings and difficulties in the investigation of this phenomenon.

The many factors that could contribute to DCA formation have been described and again many are often contradictory as are reports of the behaviour of drones within a DCA such as heights and their reaction to different wind speeds. DCAs themselves have been described as varying from 30 to 200 metres wide to those much smaller areas interconnected with flyways. Evidence shows that some have well defined boundaries outside of which mating does not take place, while others are less defined with some reports suggesting that mating can take place almost anywhere.

In other words, although more and more is being learned about the DCA phenomenon, there is still much to discover and this study aims to increase our knowledge by establishing an improved technique for discovering and measuring drone activity, and by investigating two aspects of this subject. It seems that the two major factors that could contribute to the formation of DCAs that stand out in the literature are landscape features and the influence of pheromones. However, with the problems inherent in using balloons and kites to investigate the former in the windy, hilly, bush clad, Wellington area of New Zealand, it was necessary to develop an improved method of investigation.

4. Developing an improved method of investigating certain characteristics of DCAs. (Cramp 2017)

4.1 Study aims

This part of the study aimed to introduce and establish a new and improved method of investigating DCAs that can be used in any weather conditions in which queens and drones fly and that is able to overcome problems of lack of direction and difficult site access.

4.1.1 Using balloons, kites and artificial queens impregnated with synthetic queen pheromone

Finding and determining areas where honey bees mate on the wing is a time-consuming business which hinders research, and with it, the development of better bee breeding programmes and management procedures.

From the literature, it can be seen that most research into the flight behaviour of drones in DCAs and the extent of DCA boundaries has been conducted using helium balloons or kites (Ruttner 1985, Muerrle et al. 2007). My early research in 1993 at Cardiff involved the use of helium-filled balloons and artificial queens impregnated with 9-ODA. It was successful because during that week of investigation there was little or no wind. And this is the main problem with balloons and kites; unfavourable weather conditions disrupting the timing of research. These limitations have been described in section 2.

The use of poles to hold aloft a queen has the advantage of being wind-resistant, but to reach the heights needed to reach some drones congregating above 15 - 20 metres would be impractical and would mitigate against moving the poles easily to cover a large area of ground.

4.2. Flight trials of the unmanned aerial vehicle (UAV)

I spent the first drone-flying season of this research, (December 2014 to March 2015) testing and using balloons and kites with various amounts of success due to both meteorological factors and line entanglement in tree lines. I was then given a grant to purchase a camera-equipped unmanned aerial vehicle (UAV) (Fig. 1). Using the UAV equipped with an inbuilt video camera and carrying a 90 cm wooden probe (Fig. 2) to which an artificial queen impregnated with 9-ODA (Fig. 3) was attached, I was able to investigate all areas of an extensive hill and valley region to find out if there were any DCAs in the vicinity; to determine their extent, their boundaries if any. The UAV could be directed over tree lines, rivers and areas of dense bush, inaccessible on foot, and areas upwind of an apiary. Importantly, investigations were therefore not constrained by the requirement for ideal wind conditions or wind direction for balloon or kite operations. Also, drone activity at a distance is

often not visible even with binoculars. The UAV was able to video activity (or lack of it), stream the information back and at the same time record it for later analysis. (Civil Aviation Authority (CAA) rules however prohibit flying the UAV out of sight of the operator).

The UAV camera recorded the development of, and disappearance of drone comets, and showed when drone activity commenced and ended. The video picture was streamed down to the operator and also recorded for later download.

This system enabled me to rapidly investigate a large area of difficult terrain, and visually and automatically record:

- the time of arrival of drones to the lure;
- the height of drone activity around the lure;
- areas where drone activity drops off;
- areas not accessible on foot such as over rivers, swamp areas, tree lines or areas of dense bush; and
- areas upwind, which would not always be possible to investigate using kites or balloons.

4.3 Materials and method

In January 2015, trials were started using a camera equipped (still and video) UAV which could fly in any conditions that drones can fly in and could also hold aloft a queen lure and fly at the same speed as a queen bee.

The artificial queen (Fig. 3) was made from a small 3 x 0.5 cm piece of dowel covered in a soft cloth and was attached to the probe by a stiff wire, bent to bring it into line with the aircraft camera (Fig 4). It was attached to the end of a 90 cm light wood, cylindrical probe extending out to the front of the machine. The extension was designed to limit the downdraft effect of the four rotors and to aid in avoiding the destruction of a downwind odour plume. The queen was impregnated with 0.075 mg of 9-ODA in solution with 0.5 ml acetone and was made of black, soft material wrapped around 8mm wooden dowel 3cm long.

The whole attachment weighed about 90 grams in total (the UAV can carry over a kilogramme of extra weight). The extension was designed to keep drone bees and drone comets away from the downdraft of the rotors, although downwash turbulence sometimes appeared to initially confuse the bees as to the nearby source of the pheromone. For the purposes of my investigations, this was not a factor.



Fig 1. The Phantom Vision 2 camera equipped UAV

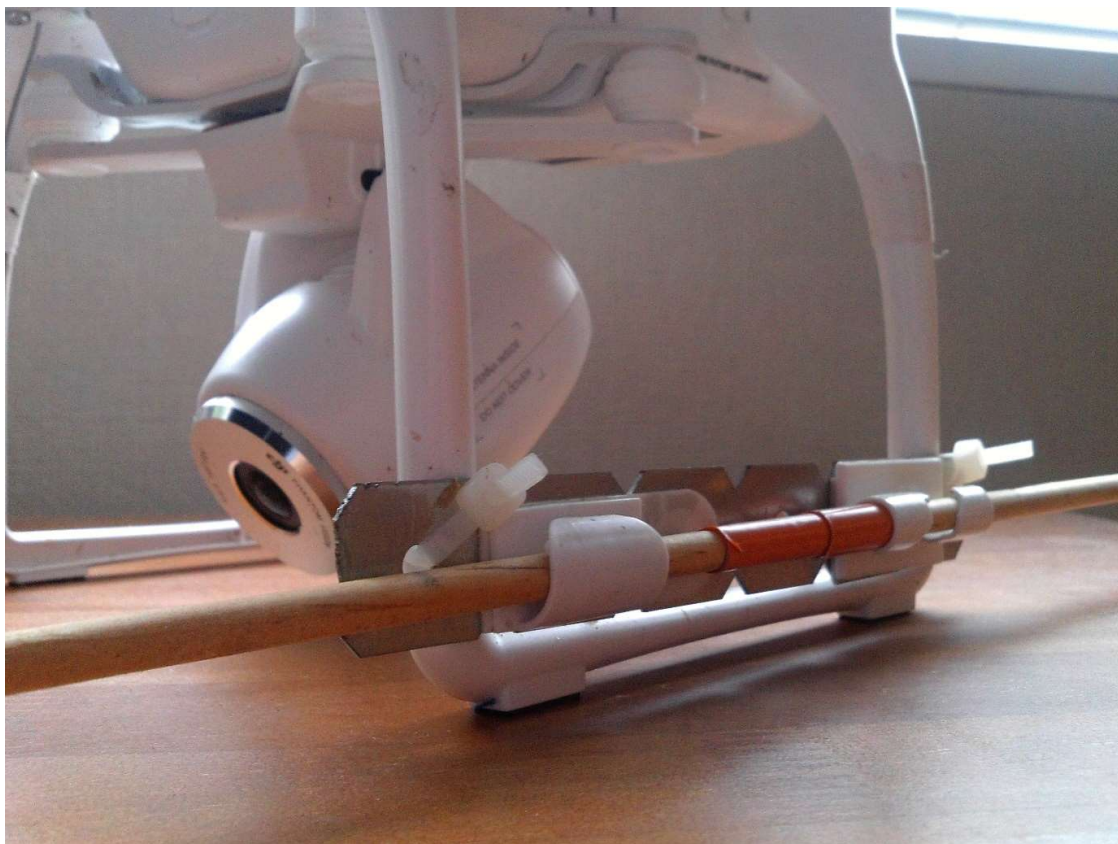


Fig 2. The UAV showing the extension attachment and the camera



Fig 3. The artificial queen bee used in the tests



Fig 4. An artificial queen bee attached to the extension fitted to the UAV

The UAV can fly up to 15 metres per second (54km/hr), in any direction, and it can fly in winds up to 27 Km/hr, enabling it to fly any conditions in which drone bees fly. It is not affected by strong gusty winds due to its GPS positioning system which prevents it moving in space unless directed. The rechargeable battery lasts for around 25 minutes and using two batteries enabled around an hour of investigation.

The UAV was evaluated from January 2015 to March 2015 in the Wainuiomata area, in order to determine the best way to investigate areas abundant in drones and the following protocols were developed:

In order to locate an area abundant in drones:

- Areas in the vicinity of apiaries were tested for drone activity by raising the lure.
- The lure was raised and lowered vertically in various positions along a line across an area. In these tests there was no use of horizontal flight.
- 1mg of 9-ODA in 0.5ml acetone was applied to the queen.

The following scale was developed during the vertical lift trials to classify the time taken for drones to form a comet:

- Immediate to 5 seconds - 1
- 6 - 10 seconds - 2
- 11 - 19 seconds - 3
- 20 - 30 seconds - 4

At each lift, the approximate size of the comet was classified as follows:

- Intense drone activity of hundred or more drones - 1
- Large – 30 – 100 drones - 2
- Medium - Up to 30 drones - 3
- Small - few drones but forming small comets - 4

An estimated height class was assigned to each drone comet (informed by the UAV control panel) as follows:

- Ground to 5 m – 1
- 6m to 15m - 2
- 16m - 25 m -3
- Above 25 m - 4

Occasionally, there was some drone interest but no comet formation and these outcomes were scored as follows:

* Drones in small numbers visited the lure but no comets formed.

** Drones in larger numbers visited the lure but no comets formed. Possibly flying through.

Thus each lift position was given a unique lift number followed by the three scores of drone interest. Wind speed/direction and temperature in degrees Centigrade were also recorded giving a line of figures thus: 068/322/3.2/045/22.4 is translated as:

GPS 068/comet formed at 11 - 20 seconds with up to 100 drones at 6 – 15m. Wind speed 3.2 Km per hour. Wind direction 045 degrees, temperature 22.4C.

The lure was kept aloft for a maximum of 30 seconds. If a comet formed within 30 seconds then the site was recorded as a DCA as it is evident that the drones were there in that area or very nearby (Fig. 5).

Throughout the tests, wind speed, wind direction and cloud cover readings were taken at each UAV lift. This was done not to see if there were any relationships between them and drone flight in the areas but to ensure that conditions for taking measurements in DCAs remained broadly the same for each test and that each test day was conducive to drone flight.

In order to simulate a queen's flight in an area with drones and determine any boundaries:

- The lure was flown horizontally upwind through an area that was abundant in drones until a diminution or absence of drone activity was noticed. The drone was then flown downwind, again until a diminution or cessation of drone activity occurs. This was to try and ascertain DCA boundaries. Tree lines, rivers and areas of impenetrable bush could be flown over.
- In areas where no drone activity was noted, the UAV was kept aloft for several minutes and maintained in that area to ascertain whether drones could be attracted to the lure over time and form comets.
- The areas were investigated no more than every other day when weather conditions allowed to avoid drone habituation.
- Temperature, wind speed and direction and cloud cover in oktas (an 8-point scale) were measured. The lure was moved in upwind steps even when crossing rivers etc.
- DCAs were designated in and around points when drone activity was immediate (within 30 seconds) and where comets formed in response to the artificial queen



Fig 5. The UAV with the queen extension and artificial queen rising to see how high the drones would follow. The usual comet shape formation of the following drones is less apparent probably due to the turbulence caused by the rotors changing the usual shape of the pheromone trail.

4.4. Results and discussion

In this study I established a new method of investigating DCAs, (Cramp 2017). Maps 1 – 41. (Supplementary material) show the areas that were investigated by the UAV and the individual, numbered markers indicate drone strength, time to reach that strength and heights where the drones appeared as illustrated in Fig. 6.

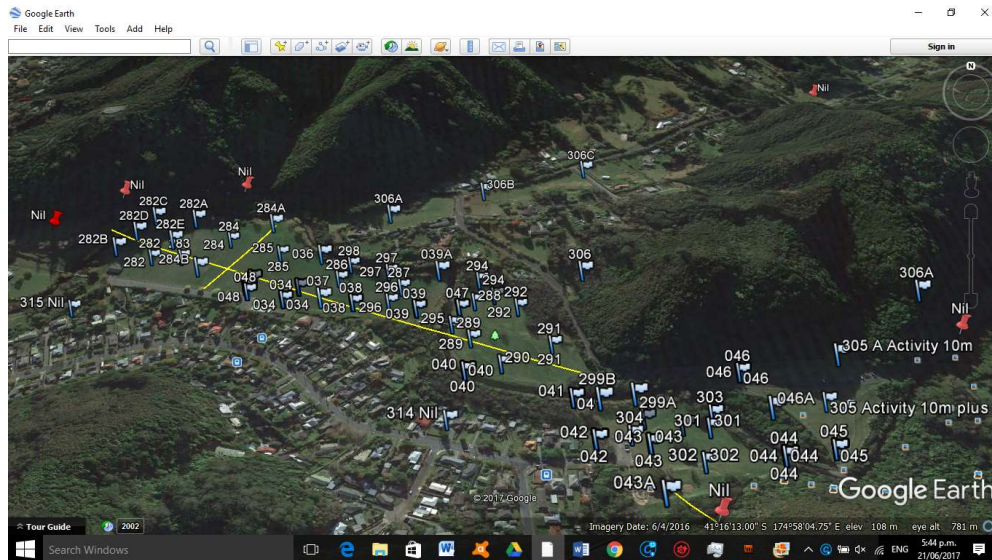


Fig 6. The numbered flags show lift points from where the UAV was raised and measurements taken. The information shown in Fig. 6 indicates a series of numbered points. The blue numbered flags show locational data and each point where the UAV was raised and where measurements described in section 4.3 were taken. Each flag represents a point at which the UAV was raised. Using the data measured at each of these points, hotspots of drone activity within the DCA could be located and marked (Fig. 7).

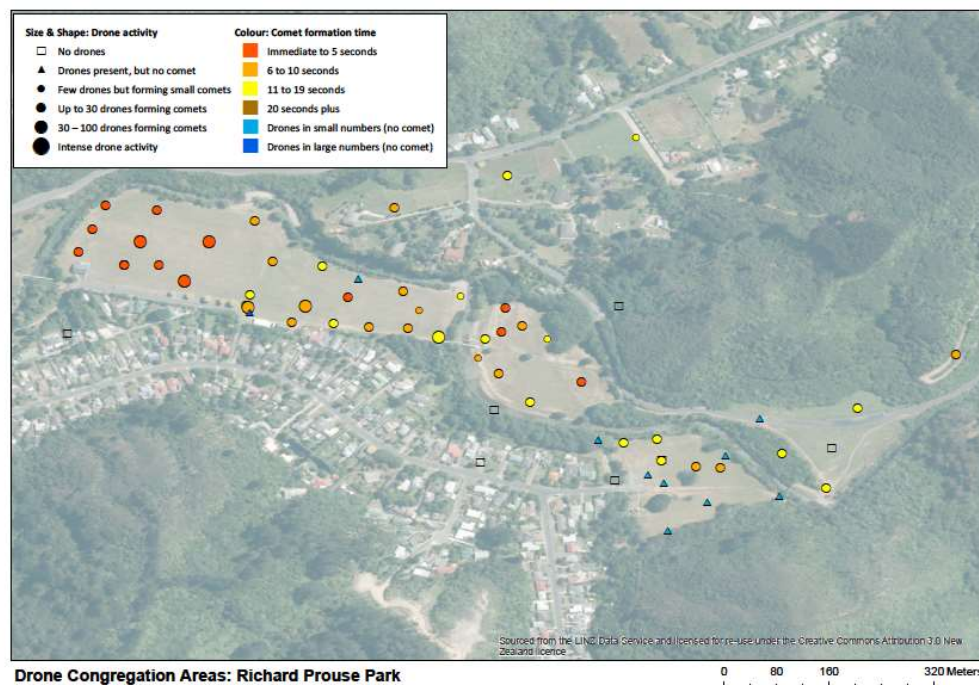


Fig 7. Showing the drone activity hotspots derived from data taken from the lift sites shown in Fig. 6.

The DCA hotspot maps could then be superimposed with landscape information and aspect information and further analysed to ascertain the possible effects of landscape features on the DCA. The landscape map and the aspect map overlays of the hotspot map (Fig. 7) are shown at Figs. 8 and 9. Equivalent maps of each of the areas tested are shown in the Supplementary material.

Drone Congregation Area: Richard Prouse Park

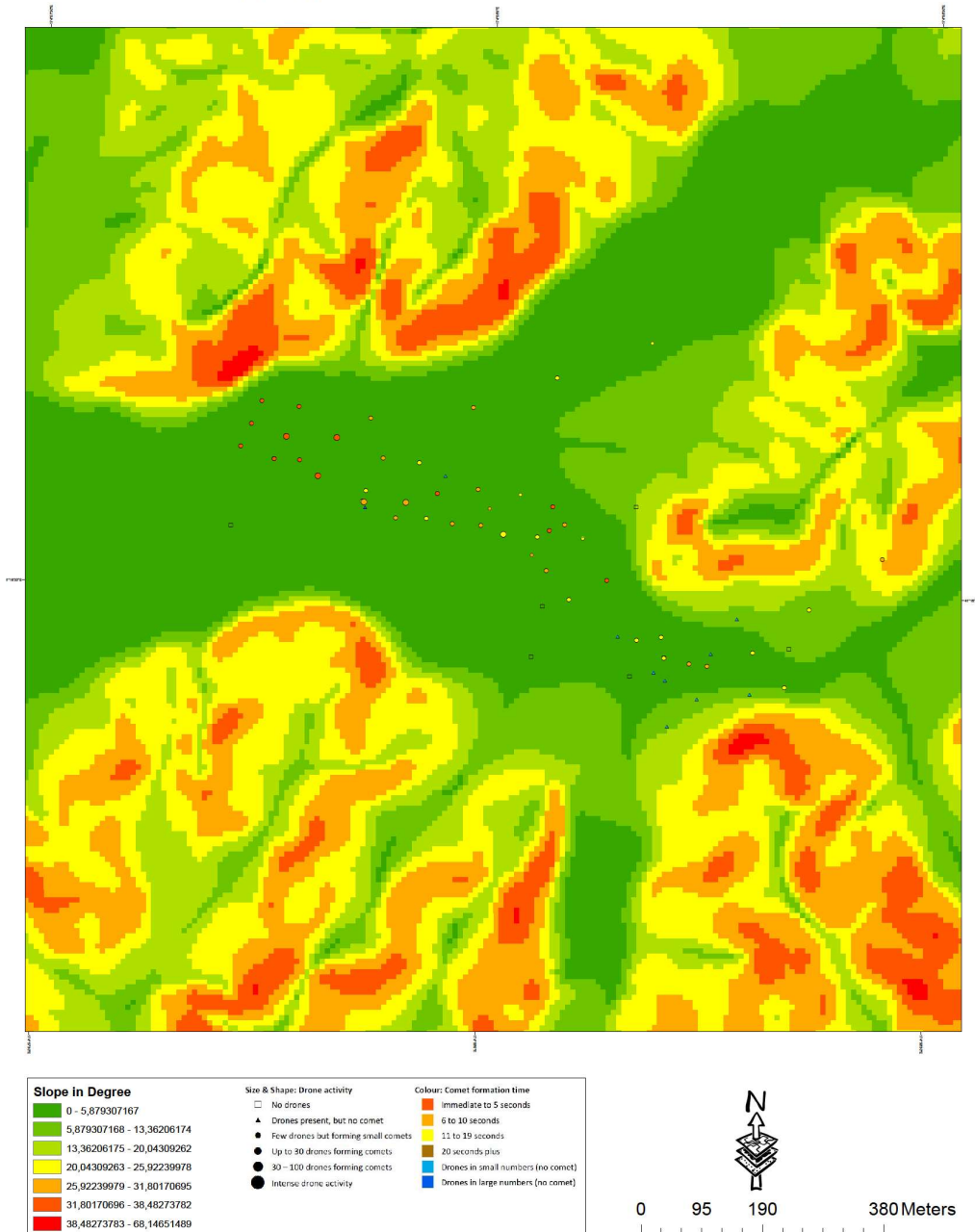


Fig 8. Showing landscape features superimposed on over the DCA hotspot map at Fig 7

Drone Congregation Area: Richard Prouse Park

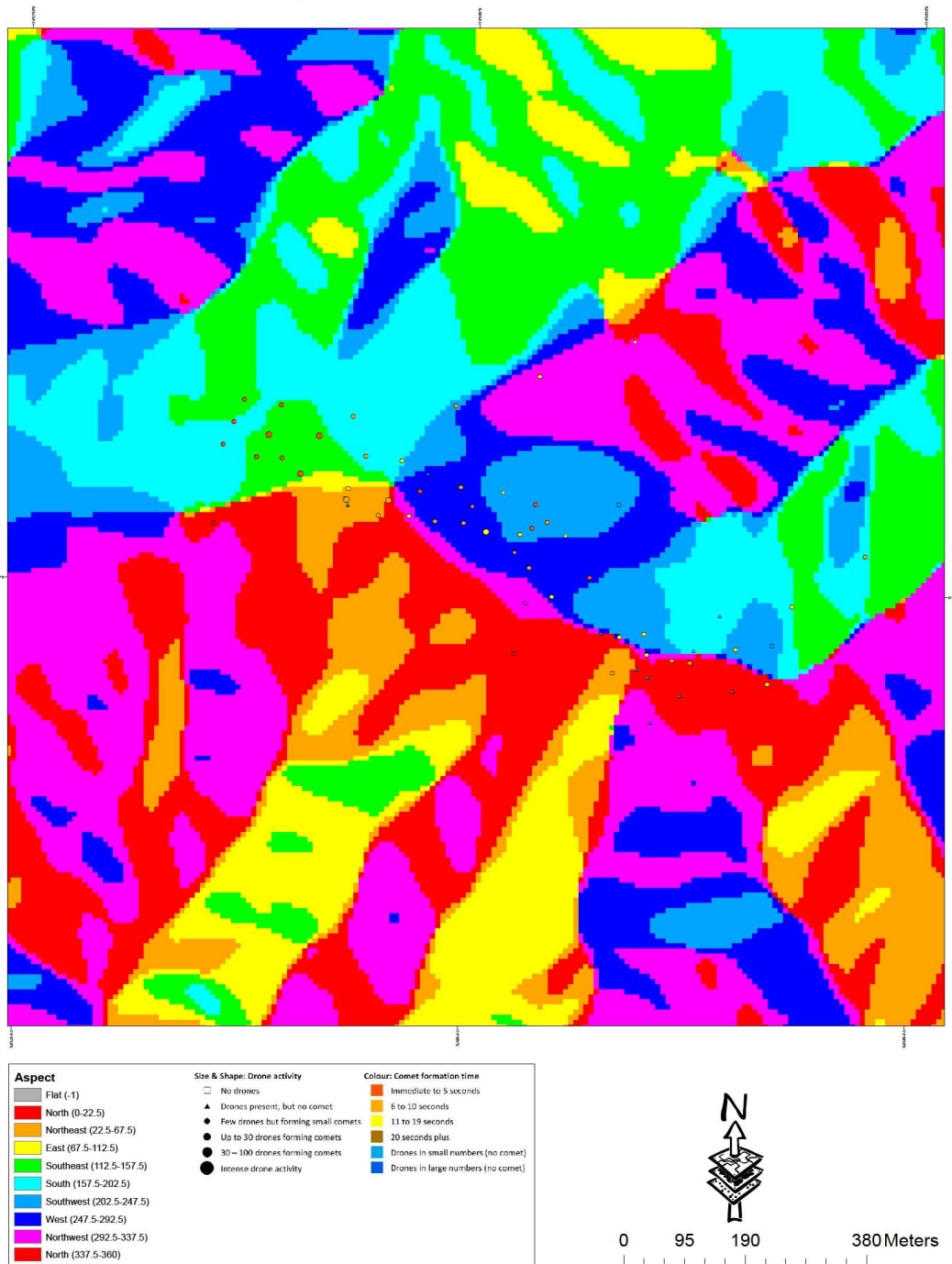


Fig 9. Showing an aspect overlay on the hotspot data

This area was not untypical of the areas studied. By using the UAV, a Phantom Vision II, equipped with an inbuilt, moveable still and video camera (Figs. 1 & 2), success was achieved in being able to investigate DCAs in an extensive hill and valley area, and in a flat, open area and determine drone numbers, heights of drone flight when forming comets and DCA boundaries, or absence of, detectable boundaries. The UAV was able to be flown over tree lines, rivers and areas of bush that were impassable or inaccessible on foot, regardless of wind direction, and it streamed live video

back and recorded this activity for later analysis. The rising sides of the valley, covered in dense bush could also be easily and quickly investigated. This was important as it was able to show that drones would follow a lure beyond identified boundaries where a single lift of the UAV showed no drone activity. The inbuilt camera recorded the time of appearance of drones, the development of, and disappearance of drone comets, and showed when drone activity began and dropped off in both vertical and horizontal dimensions.

DCAs in this study were accurately and rapidly investigated in any conditions in which drone bees flew, over even the most difficult of terrain. This enabled me to rapidly investigate areas not accessible on foot or by vehicle such as river areas, swamps, over tree lines or areas of dense bush; and areas upwind of the operating site. This was not always possible using kites or balloons.

The use of the UAV also enabled me to discover that what were thought to be several DCAs in the area (discovered by using balloons and kites), were in fact one large DCA with variations of activity and comet formation. I was not able to discover this using balloons and kites due to the control problems identified earlier.

The main problem when using a UAV to try and determine the aerial boundaries of an area abundant in drones as accepted in much of the literature is that drones may follow the lure, for example over a natural boundary such as a tree line and if the lure is moving upwind, more and more drones flying downwind of the lure may be attracted. This however is how a queen flies so it can be assumed that they would also do this if the lure was a queen. The literature suggests however that they don't; Ruttner (1966), Ruttner and Ruttner (1965) for example, suggests that drones will only mate within the boundaries of a DCA and are not attracted to queens outside that area if the queen flies past an invisible boundary.

Using the UAV in the Wainuiomata area, I was unable to find such a boundary. In fact, in places during this investigation, drones followed the lure over trees, rivers and up hillsides suggesting that if a queen is available they would mate with her anywhere. This was tested by developing protocols around the use of a lure in determining where drones are attracted to queens and these are outlined in Section 4.3, materials and methods.

Another potential problem that could negate useful research into drone flight behaviour when approaching queens is that downwash from the UAV interferes with the normal dispersion of the pheromone and so it was not always possible to see the normal arrow shaped comets of drones forming in response to the queen (Fig. 5). A far more dispersed pattern of approaching drones is evident and so using a UAV would not be successful if drone behaviour near a queen was being investigated. This study however was interested in the presence or absence of drones, drone

numbers, heights above ground, and whether drones would follow the UAV queen past obvious landscape boundaries or boundaries determined by testing. The abnormal dispersion of the pheromone was not a factor in achieving results from these tests.

Sound waves/noise and any electro-magnetic fields generated by the UAV did not appear to affect or deter the drones from attempting to approach the queen. Although the drone comet pattern was more dispersed, drone numbers were similar to those appearing when using balloons and kites.

A final problem associated with using a UAV is time in the air. Each (very expensive) smart battery lasts for approximately 25 minutes and then takes about an hour and a half to fully recharge. This period in the air is further reduced by the UAV computer that requires sufficient fuel in reserve to enable the UAV to regain its starting point in case of low battery. Even with two batteries, time operating the UAV and carrying out research is limited and the machine must be used with the utmost care to ensure that the designated area is covered. Obviously, purchasing several more batteries would overcome this limitation, at a cost.

Using the data indicated by these markers (Data Table 1), I used ArcGis software to produce maps of the DCAs and surrounding areas showing levels and intensity of drone activity within the DCAs. For example the short extract from the data table below at Fig. 10 provides an example of measurements taken in the Richard Prowse Park area of Wainuiomata (shown in Figs. 6 to 9).

The table shows three UAV lifts in the park on 12/12/2015 at 14.40, 14.45 and 14.52. The positions on the map are GPS blue flags 036, 037 and 038. The figures in column 5 (333) indicates that drone comets formed in 11-19 seconds from lift off, up to 30 drones were present at 16 – 25 metres. The figure of 233 shows the same except that the drones appeared within 10 seconds. Column 6 gives wind speed; column 7 gives wind direction; column 8 gives temperature and column 9 gives cloud cover. The meteorological data were measured to ensure that overall, they aligned with drone flight and queen flight parameters and that the measurements were not being taken in conditions where drones would not be expected to fly, or where their numbers would be unusually reduced.

Location	Date	Time	GPS	Drones	W/S	W/D	Temp	Cloud
RPP	12/12/2015	14.40	036	333	0-1	45	24	5/8
RPP	12/12/2015	14.45	037	333	0-1	45	24	5/8
RPP	12/12/2015	14.52	038	233	0-1	45	23	5/8

Fig 10. Extract from Data Table 1 showing typical measurements at each site investigated

(The full data tables for all the areas tested are shown in the supplementary material).

Vegetation types and other landmarks and relief features were also mapped using ArcGis, enabling

analysis of the DCAs in relation to landscape features. The results of this analysis are shown and discussed in section 5 of this paper.

The inbuilt camera recorded the time of appearance of drones, the development of, and disappearance of drone comets, and showed when drone activity began and dropped off in both vertical and horizontal dimensions. Fig. 11 shows an area cut off from access by bush and a river that could easily be investigated by the UAV.

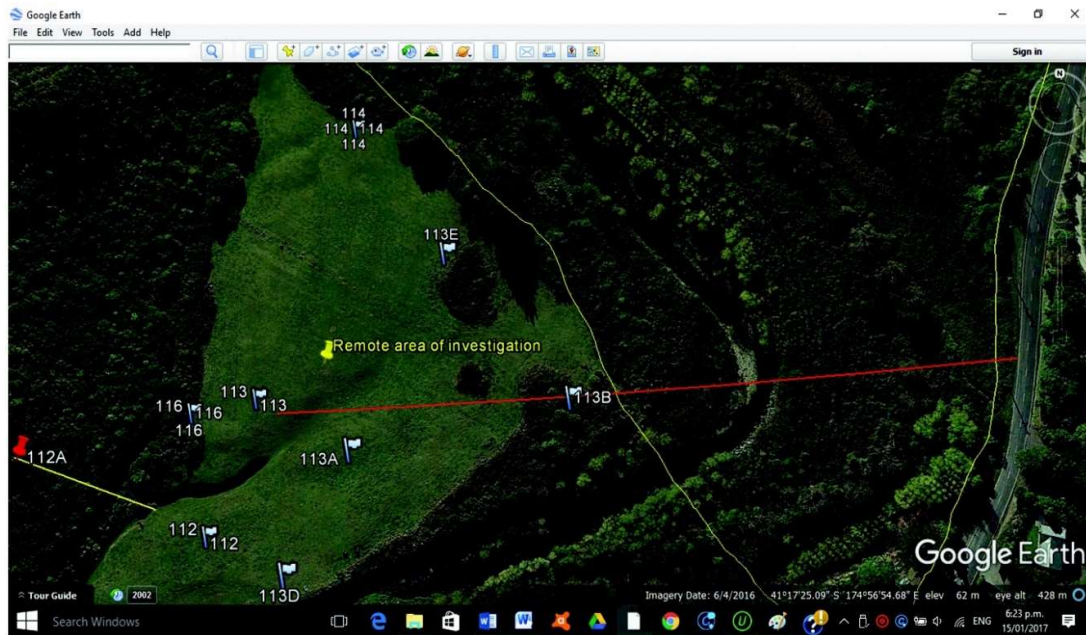


Fig 11. An area that was almost impossible to visit on foot or by vehicle was able to be inspected remotely by the UAV.

The main advantages of using a UAV for this investigation were found to be as follows:

- The UAV is multi directional.
- It is not dependant on wind direction.
- It can cross over areas of impenetrable bush, rivers and high tree lines without risk of entanglement and provide video footage of events while doing so.
- It can visit areas not possible on foot or by vehicle.
- It is able to provide information as to height, speed and location to the operation at all times.
 - It can stream back to the operator live video information and record this for later analysis. This in turn means that no field glasses are required to view drone activity around the lure if at a distance.
- It is able to replicate the speed of flight of a queen through an apiary.
- Drone bees do not appear to be concerned about the motor noise or disturbance of

the rotor downwash but their flight behaviour does change.

The main disadvantages of using a UAV for this investigation were found to be as follows:

- Even with two batteries, the UAV has an operating time of less than an hour. (Two times 25 minutes).
- The rotor downwash rapidly disperses the synthetic queen pheromone. This was not a factor in this research but may be in research aimed at observing drone flight behaviour around a virgin queen.
- Although the UAV is GPS stabilised, pilot error can cause crashes which in turn can cause a major disruption to the research programme, and occasionally it can fly out of control on its own. This latter problem necessitated placing a GPS locator device on the UAV which downloads the position of the UAV when lost, to the operator's mobile phone app.
- Civil Aviation Authority (CAA) NZ rules dictate the circumstances in which UAVs can be used which can be limiting. (CAA NZ 2017).

Despite these disadvantages, the results found in this evaluation confirmed that this method is useful in investigating DCAs in conditions that would be difficult if not impossible using balloons and kites. Spreading the investigation over four seasons ensured that the DCAs found were formed in the same areas each year. In view of these findings, this research relies for the data, on the use of a UAV.

The data produced by the UAV soundings in each of the areas are shown at Data Table 1. The locations marked on these data tables are shown on Maps 1 - 45 at Appendix 1 and form the basis of the next part of the study.

4.5. Conclusions

Even a brief analysis of the results of this study shows that studying DCAs can be difficult due to the nature of the phenomenon when bees fly on the wing to mate. Using a UAV has aided this investigation, the results of which have been published in the Journal of Apicultural Research and are described below. Further investigation into the use of UAVs for DCA research is recommended.

5. Investigating the relationship between landscape and DCA formation in hill areas and a flat, open area using the UAV

5.1 Background

The study by Galindo-Cardona (2012) where he investigated the relationship between landscape and DCA formation was the first experiment of its kind and so the researchers were unable to compare their results with surveys undertaken in other areas such as those in the southern hemisphere areas. By comparing any findings between both hemispheres we could raise our understanding of the reasons for the global decline of bees and latitude-dependent factors.

A landscape analysis of DCAs in the New Zealand environment, may allow the development of a predictive model to aid in future DCA research and breeding programmes by highlighting the importance of different cues for DCA formation in rural and semi-rural locations in temperate southern latitudes. A comparison of the findings of this research with those in other areas could be undertaken in future and could help in determining a potential hierarchy of landscape and other characteristics important for DCA formation. Future research may also be able to highlight differences between the Northern and southern hemispheres. This analysis is the first of its kind in the southern hemisphere.

The review of literature on this subject showed that landscape may play an important part in determining the location of DCAs (Galindo Cardona et al. 2012, Ruttner 1965, Koeniger et al 1979). Landscape variation might explain why DCAs occur in some areas and not in other seemingly identical areas. In view of the relative importance of landscape in previous studies, I carried out investigations of four main valley areas, two urban park areas, and a flat agricultural area in the Wellington and Kapiti Coast regions using a UAV to investigate the existence of and parameters of these DCAS and relating the findings to the landscape of the area. Overall, this study aimed to shed further light on the existence of defined areas where queens and drones mate on the wing.

To this end, this research carried out a survey of several sites of rural and semi-rural DCAs in New Zealand to seek an answer to the question: *“Do landscape features determine the spatial form of honey bee mating areas?”*

To answer the question, the research tested two alternate hypotheses as follows:

- Drone honey bees fly away from the apiary to special areas (Drone Congregation Areas) with defined and fixed boundaries dependent on landscape features, to mate with virgin honey bee queens that fly through the area (Zmarlicki and Morse 1963, Ruttner and Ruttner 1965);
- DCAs do not exist and drone honey bees fly at random from the apiary and their sheer

abundance ensures mating with queens (Butler and Fairy 1964).

These hypotheses were tested by:

- Investigating drone honey bee activity based around known apiaries as well as away from apiaries;
- Measuring the intensity of drone activity in response to an artificial lure in hill and valley areas and flat, more open areas;
- Using a defined protocol to assess whether the areas were DCAs or not;
- Investigating the spatial parameters (if any) of these DCAs if found by measuring drone activity within them and building a picture of drone activity within the areas;
- Relating these parameters to landscape and other features using GIS mapping techniques;
- Testing assumed DCA boundaries to discover whether drones would follow a queen past those boundaries;
- Testing the abundance of drones in areas not considered DCAs in this study;
- Comparing the DCA areas to similar areas where no DCAs formed;
- Investigating whether drones are attracted to an artificial queen in areas not designated DCAs if the queen remained in the area for an extended time; and
- Measuring each area studied for magnetic anomalies.

Because there are several theories about the existence of DCAs and with some researchers such as Butler and Fairy (1964) believing that DCAs did not exist, it was decided to carry out two tests. Firstly to use a queen lure to locate and measure areas with an abundance of drones and plot their coordinates; and when found, attempt to discover if these areas had boundaries and whether these boundaries were rigid. These areas would then be correlated with the local landscape characteristics by imposing the data onto a map using GIS software. The two types of areas could then be analysed and compared. Secondly, areas with no recorded drone activity but with similar landscape characteristics were investigated to see if drones could be attracted to the queen over time.

5.2 Problems associated with DCA research

In any field work studies, environmental factors cannot be guaranteed to remain constant. There are several problems associated with examining DCAs and these mainly revolve around the number of continually changing variables pertaining at any one moment. Many research papers give the impression that each examination of a DCA is a repeat of the previous one and that annual examinations of DCAs, for example, are examining the same phenomenon as the year before. At a general level this is the case, but the detail cannot be the same and the bees' behavioural reaction

to the many variables pertaining on different days or years may be quite different. For example, if DCA 'X' is tested at a certain time on a certain day in a certain month, this test can never again be repeated exactly. There are simply too many changing variables. Even a test of DCA 'X' the next day will be different.

Drone honey bees will not fly (or fly in reduced numbers) if there is rain; if the sky is 8/8 overcast, or if the temperature is below 18 degrees (if they do fly below this, they don't form drone comets). They will also not fly on mating flights before around mid-day or after around 18.30. These factors reduce the number of days during which research can be carried out. Other climatic variables may also be significant.

In research in the UK, I was not able to find any significance effects of light levels, or relative humidity on in the DCA under investigation (Cramp 1993), but there is little research of whether these affect the frequency or duration of drone mating flights or queen mating flights. Because of the range of variables, including unknown variables, it is not possible to investigate DCAs in exactly the same conditions. Therefore, to minimise the impact of climatic factors it is necessary to ensure that a basic set of variables are maintained within certain boundaries.

Another significant problem in investigating DCAs is that the number of apiaries in the area around the DCAs is usually unknown. Especially in New Zealand, hives are moved at regular intervals to follow crops both for honey production and to pollinate crops. Thus the dynamics of a DCA may change throughout the year and from year to year. Thus when a known DCA is checked while the temperature, time of day, cloud cover and all other factors are conducive to DCA formation, there may be a very different result if the same DCA is checked a week or two later under the same climatic conditions if several hundred hives have been moved into or out of the area. Feral colonies that would in the past have played a part are now largely absent since the arrival of the mite *Varroa destructor*. Feral colonies derived from swarms will set themselves up and be active for a while, but will soon die out because of *Varroa*, again changing the local bee numbers.

The problem of the existence or not of unknown apiaries is common in most areas of the country. Some apiaries will be unknown because the owner wants it that way to prevent theft and observation, and because they may be unregistered therefore avoiding hive levies (registration is compulsory in New Zealand). These hives will generally not be in view of any road and will be actively hidden from the sight of thieves, the authorities, and bee researchers. Also, the number of small apiaries of one or two hives in gardens and even on rooftops is rapidly growing and these are an unknown factor when trying to gauge the number of hives in the area. Because of these factors, it is difficult to carry out studies of DCAs' relationship with all apiaries in the area, but some

difficulties could be overcome by locating known apiaries, noting their presence over the years of investigation and noting the large-scale movement of commercial hives into and out of the areas being studied.

Finally, the impregnation of an artificial queen with a given amount and strength of queen pheromone (90DA) may not mirror a natural scenario over time. Does the applied pheromone have the same effect after an hour or two of use for example? I have found no research on this variable and have not measured any change in this study.

5.3 Material and Methods

Apiaries in the Wellington and Kapiti Coast areas were selected for investigation (Figure 12). A mix of local DCAs serving these apiaries was looked for within a 2 km radius of each apiary. DCAs were declared if drone activity occurred according to the test protocols established for the use of a UAV for this research and outlined in Section 4.3. (Speed of arrival of drones and formation of drone comets). Areas near to these DCAs that showed nil drone activity sufficiently quickly for DCA to be declared were also re-tested at a later date and the queen held aloft for an extended time.

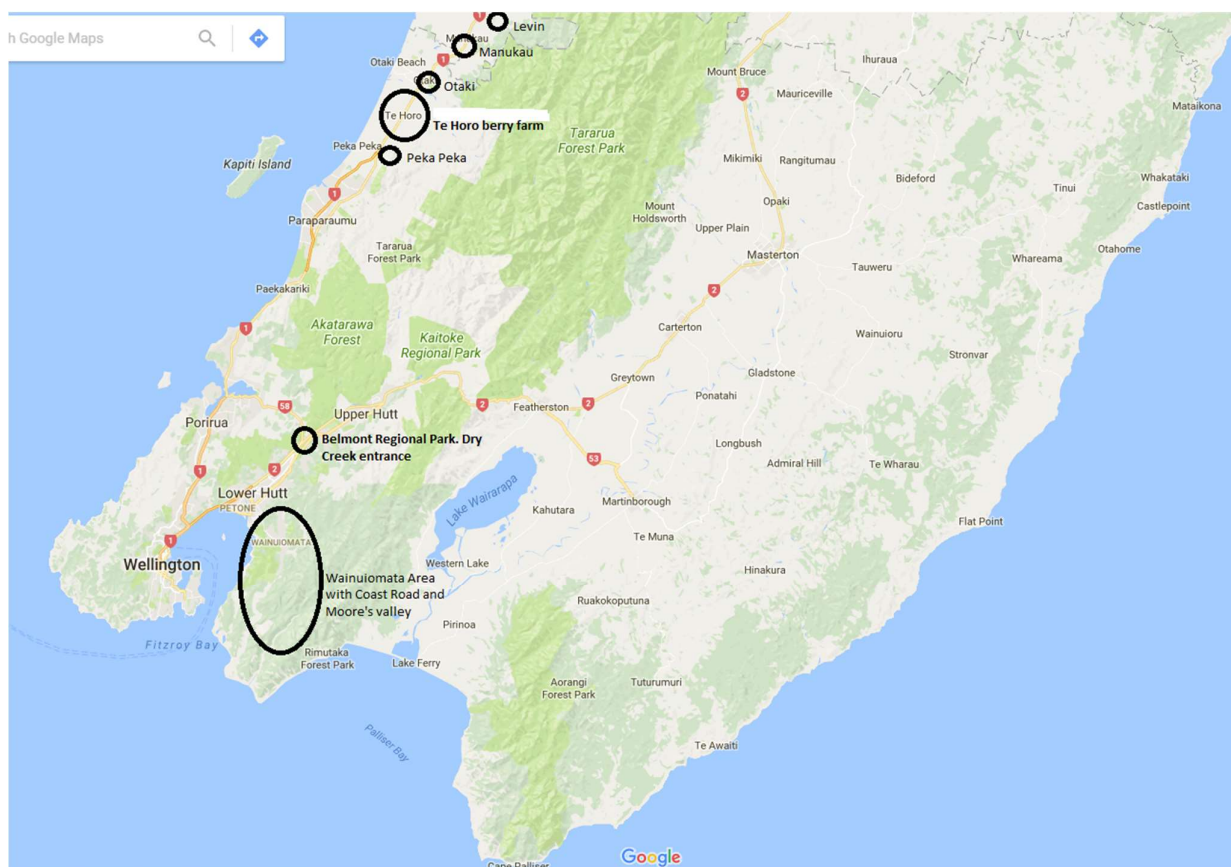


Fig 12. The areas investigated in this study

During season one (2014 to early 2015), balloons were used in the Coast Road area 2km South of Wainuiomata in an area where several small apiaries exist. Commencing in the Wainuiomata apiary

in Coast Road near to the town of Wainuiomata (Fig. 13), a large helium balloon was used to raise to raise an artificial lure (a small, queen-sized object made of a piece of dowel rod covered with soft material and containing approximately 0.1mg 9-ODA dissolved in 1 ml of acetone). Plettner et al (1997) state that 0.074mg of 9-ODA equals the amount of 9-ODA in a virgin queens' glands, although Butler and Fairy 1964 suggest that 0.1 mg of 9-ODA is as attractive to drones as the quantity of pheromone released by a virgin queen). In this study, I used 0.1 mg per artificial queen so that as far as possible I was able to ensure that the results explored the natural situation.

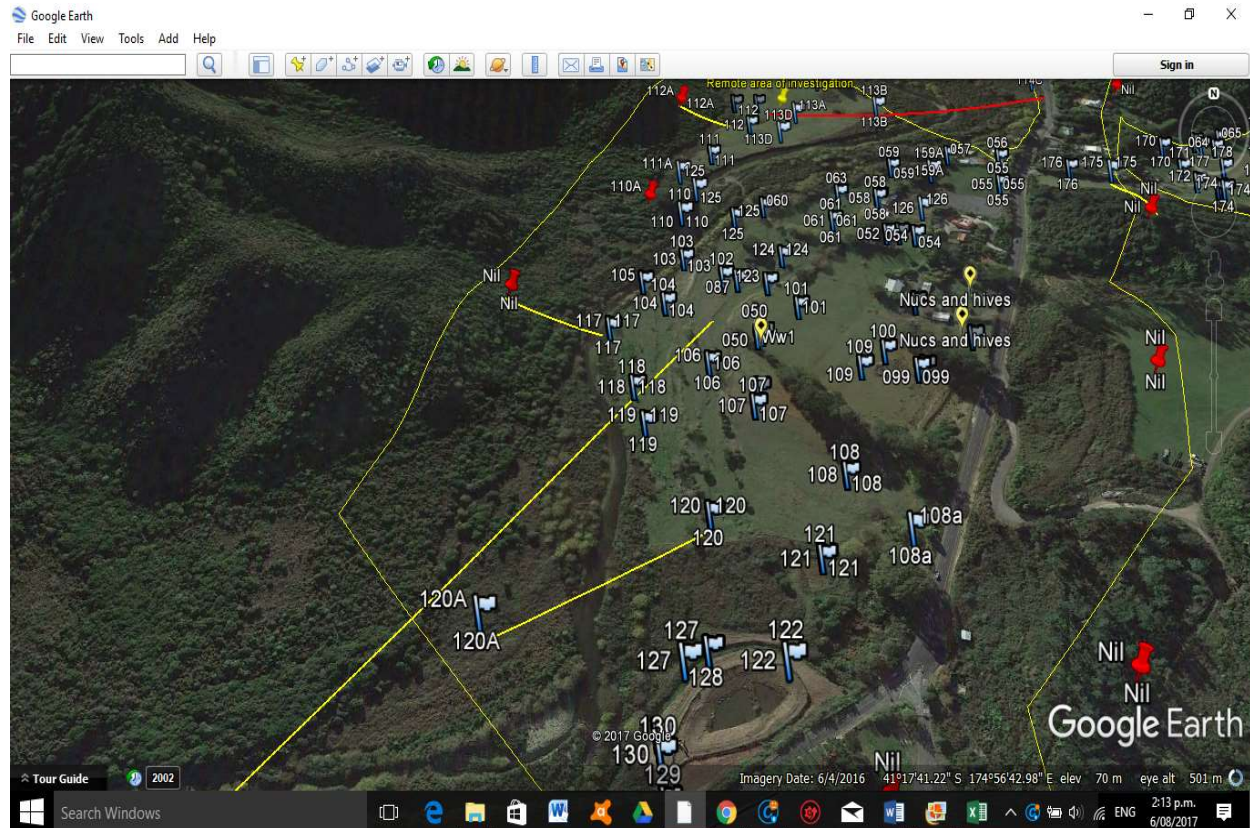


Fig 13. Google Earth map of the area surrounding Apiary 050 which was first investigated

The blue flags represent lift points described in Fig. 6. Some of the locational label numbers are repeated but for example all numbers 121 represent the same point. The yellow symbols represent groups of bee hives. The red and yellow lines represent distance measurements used for my own purposes. The basics requirements for test commencement were: temperatures above 18.5° C; cloud cover less than approximately 7 to 8/8; no rain, and testing only between 1300 hrs and 18.30 hrs. In other words, the field research was only carried out when these variables were conducive to drone mating flight.

Starting at the centre of the apiary near to the hives and initially using balloons, the queen was raised every 50 metres. At each raising position, the presence or absence of drones was observed and if present, the time taken to form drone comets was measured. The approximate height at which drones formed a comet was noted as well as the approximate number of drones. The lure was then lowered and moved in a set direction for 50 metres. This distance was approximate

because on many occasions, landscape features and trees/bushes got in the way. The area immediately surrounding the apiary and the area of Wainuiomata (Wainui) Camp (Fig. 13 and maps 7 to 10), were mapped out in this manner. Problems arose however with access to certain areas and the use of the UAV helped to overcome access problems and enables quick mapping of other areas.

Ruttner (1966) commented that *“the areas (DCAs) exhibited an astonishing power of attraction to drones”* and that if queens (a lure) departed the area, drones did not follow. Other researchers including Ruttner and Ruttner (1965) and Koeniger and Koeniger (2005), Galindo-Cardona et al. (2012) also report this phenomenon. In order to test apparent DCA boundaries in hill and valley areas, the UAV was carried up a hillside through dense bush in some of the more accessible areas and flown. Areas where no drones appeared were marked. Adjacent areas on the flat ground where drone hotspots did appear were marked. The line between these areas was assumed to be a boundary where activity occurred on one side and no activity occurred on the other side. The UAV was then flown from active areas where drones were attending the lure to the nil activity areas.

The lure was not used in areas on consecutive days in order to avoid habituating drones to the area by using 9-ODA, although unfavourable weather conditions also played a part in delaying subsequent visits to the area. This method of locating DCAs was used in the Coast Road area of Wainuiomata (Maps 17 and 18), and the Wainuiomata camp area (Maps 7 to 10). These areas were subsequently investigated by the UAV. All other areas were located and examined by the UAV only.

The camera equipped Phantom Vision 2 UAV was set up in accordance with the tests described earlier referring to the testing of the UAV for this type of investigation and was flown in the same way as described according to the protocol developed.

5.3.1 Three distinct types of area were tested defined by landscape features as follows:

- Urban Park areas
- Hill and valley areas
- Flat, agricultural areas.

Using the UAV in urban areas was not allowed so only urban park areas were tested. The code of conduct for UAV users in urban areas include not operating over parked vehicles or park roads, not operating within 20 metres of, or flying over park buildings and overhead wires and not flying over adjoining private properties.

Areas where drone activity was tested by the UAV are shown in Fig. 12 and on maps 1 to 45 in Appendix 1, and their locations are summarised below:

Location and GPS marker	Latitude	Longitude	Type of area	Map No
Wainuiomata GPS marker 050	47°17'41.07S	174°56'44.25E	Narrow valley	Map 6
Ngatura Park Wainuiomata marker 140	41°17'13.77S	174°56'56.43	Urban park	Map 8
Golf Course Wainuiomata GPS marker 354	47°17'41.07S	174°56'44.25E	Wide valley	Map 18
Lower Coast Road GPS marker 366	41°20'51.41S	174°54'47.99E	Wide valley	Map 22
Wainuiomata Camp GPS marker 066B	47°17'34.20S	174°57'11.03E	Narrow valley	Map 12
Richard Prowse Park marker 293	41°16'26.11S	174°58'04.67E	Urban park	Map 26
Lower Moore's Valley Road marker 316	41°15'26.97S	174°59'00.94E	Wide valley	Map 30
Upper Moore's Valley Road marker 191	41°16'26.11S	174°59'00.18E	Wide valley	Map 32
Belmont Dry Creek marker 267	41°09'33.73S	174°58'06.18E	Narrow valley	Map 38
Te Horo berry farm GPS marker 271	40°47'49.98S	175°07'23.48E	Flat area	Map 42
Peka Peka marker 400	40°49'36.58S	175°05'54.68	Flat area	Map 47
Otaki marker 360	40°44'51.16	175°09'55.50	Flat area	Map 48
Manukau marker 390	40°44'05.31	175°11'14.85	Flat area	Map 49
Levin marker 370	40°39'29.91	175°19'22.97	Flat area	Map 50

Each area (except the last four above) was tested over three seasons to ensure the stability of the DCAs or the absence of drone activity. The DCAs appeared to be consistent each year apart from variation in the intensity of activity in each area, a phenomenon that is explained in section 6.2. The last four areas mentioned above were areas where DCAs did not exist but were tested for the eventual presence of drones.

These same areas are shown with drone with drone activity displayed as coloured graphic symbols, showing where drone activity was more active and where it was less active. A further series of maps show the areas overlaid with geographic/landscape information displaying aspect and slope information.

Magnetic anomalies are caused by magnetic deposits or rock formations that cause the field to be different within a local area. Because honey bees undergo iron bio-mineralisation, providing the basis for magneto-reception leading to a proposed model for magnetic reception in honey bees (Hsu et al. 2007), each area covered by balloons or the UAV and measured for drone activity was also tested for magnetic anomaly variation using a TriField Natural EM meter. The meter was carried across each area on foot and any anomaly looked for. No anomalies were found in the areas where drone activity was recorded in this study.

5.4. Analysis of the DCAs

The capture data from the UAV lift points marked in detail on the area maps (example shown at Fig. 13), was noted in an excel file and the KML points provided were imported into ArcGIS 10.4.1. The points that were not located in the area of interest were removed and the point data were assessed against the table data provided. The data that didn't match were not used. The X123 field was split into the three separate fields referring to height of drone comet formation, drone numbers in the comet and time of comet formation. The maps were then prepared on Google Earth background of the areas tested and the key variables symbolised. This produced the series of DCA drone activity maps shown in Appendix 1. Hotspots of intense drone activity are shown and the 'layout' of each DCA is graphically represented using the symbols provided as a key to each of the maps. Each area tested was overlaid with raster layers showing slope and aspect.

The relationship between landscape features and the degree of intensity of drone activity and the formation of drone comets within the DCA areas was analysed by comparing the numbers of drones and the speed of reaction to the lure in the hotspots shown on the drone activity maps, with four independent landscape variables, decided upon by an analysis of past research.

This research indicates that the following landscape features play an important part in determining the location of DCAs: slope, aspect, ground cover and shelter from wind. This last variable was classified here as 0 – no shelter around the spot and 1, nearby shelter in the form of trees. The landscape cover overlays were found to be too generalised and so data from the area maps was used to determine ground cover for analysis.

Landscape cover was categorised as: pasture/grass (1); bush (2); margin – bush/pasture (3); low forest (4); forest (5); urban industrial (6); park/grass (7), and trackways (8). Slope was measured in degrees, and aspect was measured in radians (Galindo-Cardona 2012) to avoid statistical complication with numbers (for example, 360 degrees is very near to 1 degree). The raster layers used in ArcGis for aspect and slope are shown in colours in the maps for each area in the Appendix and show on the maps as a range of measurements for each point. The mean of each range was used in the calculations to determine the effect of each variable and to determine the predominant variable.

The dependant variable (drone intensity) was measured in drone numbers present as follows:

- 6 = 100+ drones;
- 5 = 30 – 100m drones;
- 4 = 20 – 30 drones;
- 3 = 4 – 20 drones
- 2 = 1 to 2 drones

1 = no drones.

Regression analysis of four variables was used to determine the degree of effect of each variable. The areas in this study were tested over three seasons to ensure that those areas found during the first season were not casual aggregations, but were a constant feature.

5.5 Results and discussion

The drone activity maps shown in the Maps at Appendix 1 show that drones within DCAs are not distributed evenly over the DCAs but were concentrated in certain areas, with fewer drones in some areas of the DCA and no drones in other areas. These findings accord with the findings of Ruttner (1966) who carried out research in Austria and Germany, and are a strong indication that Drone Congregation Areas exist as defined areas in which drones wait for queens to appear and can then be mated. Although these areas appear to be extensive in hill and valley areas, their boundaries were not rigidly fixed as suggested in many studies.

In most cases, drones would follow the lure past previously established nil points to nil points on hill sides where a launch at that point had not previously attracted drones. These lure movements are shown on each map as short yellow lines moving up valley sides to a red nil marker such as those shown below (Fig.14). Another example was reported on 21 Jan 2016 the drones at points 264 and 272 in the Belmont park area (Map 33) where the UAV took drones up the hill side to an area where drones were not present on a launch at that point.

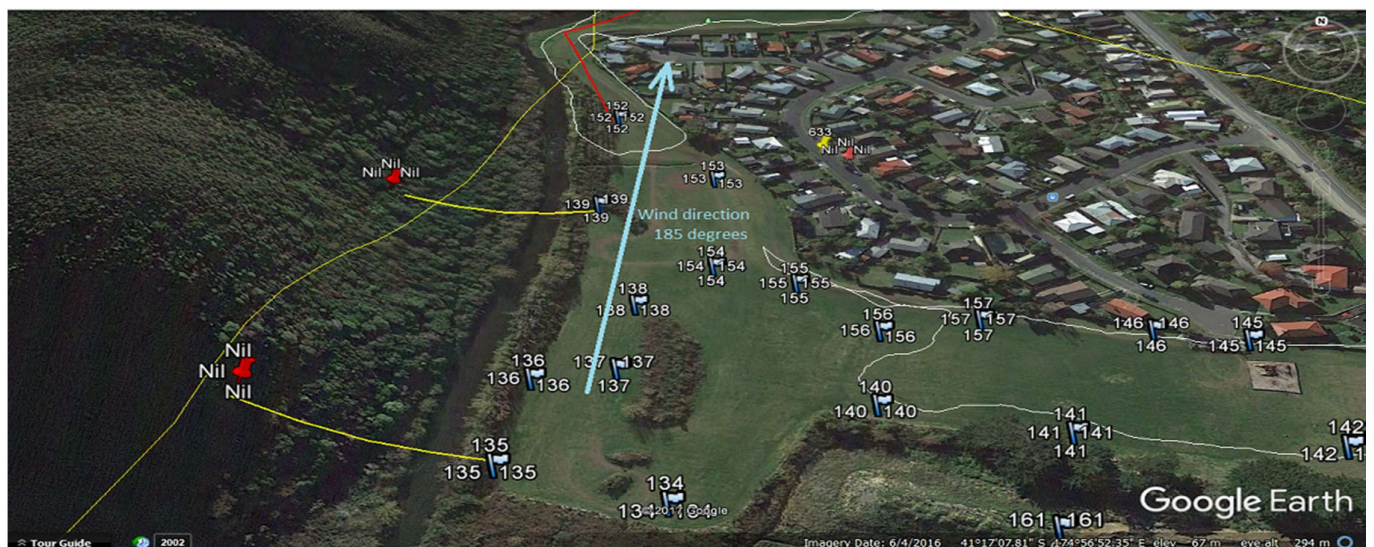


Fig 14. Showing UAV flight taking drones up valley sides (yellow lines) from a DCA until no further pursuit occurred. The light blue line indicates wind direction.

These flights indicate that drones will fly past zones thought to be DCA boundaries in pursuit of a lure. They only lost interest when the lure was flown further up valley sides even if those valley sides were on the edge of the DCA. Maps 1 and 3 illustrate this and the yellow flight lines marked on

the map show the drone activity hotspots from which the UAV was flown up the valley sides until drone pursuit ceased.

The height of Drone activity in the DCA itself around the hotspots in the DCA would often exceed the height that drones would follow a lure up valley sides, indicating a relationship between height and a landscape feature. The hotspots on the map were around 75 – 80m above sea level (asl) and the lure was flown to approximately the 100m asl point in this area. Activity within the park DCA would often occur up to 30m plus indicating that height alone was not a factor in drone decision not to pursue a lure. This feature occurred in all of the other hill and valley areas investigated when the UAV was flown up valley sides.

This suggests that DCA boundaries may exist in response to certain variables pertaining at the time but that they can be flown past in pursuit of a queen. This further suggests that more needs to be discovered about queen mating flights. It is likely that a queen would depart her apiary and fly upwind or at an angle to the wind direction as suggested by Currie (1987) so as to leave a large downwind pheromone plume. Taylor and Rowell (1988) found that queen leaving the nest flew in an Easterly direction when on an East facing slope with a prominent east – west line of sight. Queens flying from a hive on a North facing slope with a prominent east west line of sight also flew east which was also downhill.

Gary (1971) points out that when leaving the hive on a mating flight, queens usually flew at low level, suggesting that a downhill slope, or a prominent line of sight may induce them to fly in certain directions. If her requirement is to maximise the pheromone plume while taking into account landscape features (slopes and clear lines of sight), then it is unlikely that she would fly towards a hillside and in the study by Galindo-Cardona et al (2012), no DCAs occur in areas with more than a 19° slope. (In this study, no DCAs occurred in areas with more than a 21.73° slope).

Therefore in the examples shown in Fig. 14, with the wind blowing up the valley in a north easterly direction, it is suggested that a queen flying up the side of the valley would present an unlikely scenario, but if it happened, drones would probably follow, as they did a lure. This in turn suggests that the boundaries of DCAs are constructs that depend on the normal behaviour of the queen during her mating flight, which may in turn depend on the predominant wind direction and modified by landscape. In other words, it may be that there are no constant boundaries for drones in DCAs, except those caused by queen flight behaviour, and these boundaries would depend on wind direction. Wind direction varied during the tests over three years but was predominantly up or down the valley.

If however the UAV followed a flat route, drones would follow until the UAV was almost out of sight. This suggests that in flat areas such as Kapiti or in areas such as broad valleys where the UAV is flown up or down the valley and not up the valley sides, no boundaries are apparent that a drone in pursuit of a queen will not pass.

Finding DCAs in hill and valley areas and urban park areas was easier than in the flat area of Kapiti where much searching discovered only one major DCA at Te Horo (Maps 37 to 41). The drone distribution maps show a clear representation of drone activity within DCAs. The key to each map shows that 'hot spots' of intense drone activity in Hill and Valley DCAs and those in the urban parks appear to be more spread out over the areas than the DCA in the Te Horo berry farm area of Kapiti.

In areas of low drone activity in which Comets did not form, where drones were seen, their direction of flight was noted. In these circumstances, some drones would approach the queen but would break off and continue on their way almost immediately. Other drones seen in these circumstances did not approach the queen but carried on their way suggesting that drones were moving between areas of activity, or from areas of less activity to areas of greater activity. Drones were frequently seen moving between the Coast Road (apiary 050) and the Wainuiomata Camp area (Maps 7 to 10).

The maps show clearly that drone activity tends to be more intense in some areas of a DCA than in others but whether the data allows establishing definite DCA boundaries caused only by landscape features is difficult to assess. For example, in the Moores Valley North DCA, (Maps 27 and 28), the nil points moved outwards considerably following the arrival in the next paddock of a large number of commercial hives prior to the third season of testing. The DCA existed strongly before the arrival of the new hives but altered in size following the arrival of a new apiary. This known variable suggests that DCA size depends to some extent on the amount of drones available rather than purely on landscape features.

Regression analysis of the relationship between drone activity intensity and slope, aspect, cover and shelter found that there was no correlation other than a weak correlation with shelter for drone activity intensity within DCAs (Data Table 2, Appendix 2). Galindo-Cardona et al. (2012) found a significant relationship between DCAs and a southerly aspect. By measuring the most intense drone activity areas in this study, it was found there was no significant relationship with aspect or any other measured variable. This last variable seemed important when examining the drone activity map of the Te Horo berry farm shown at Fig. 15 below where it is evident that the most intense activity occurred between tree line wind breaks and rarely exceeded the height of those breaks.

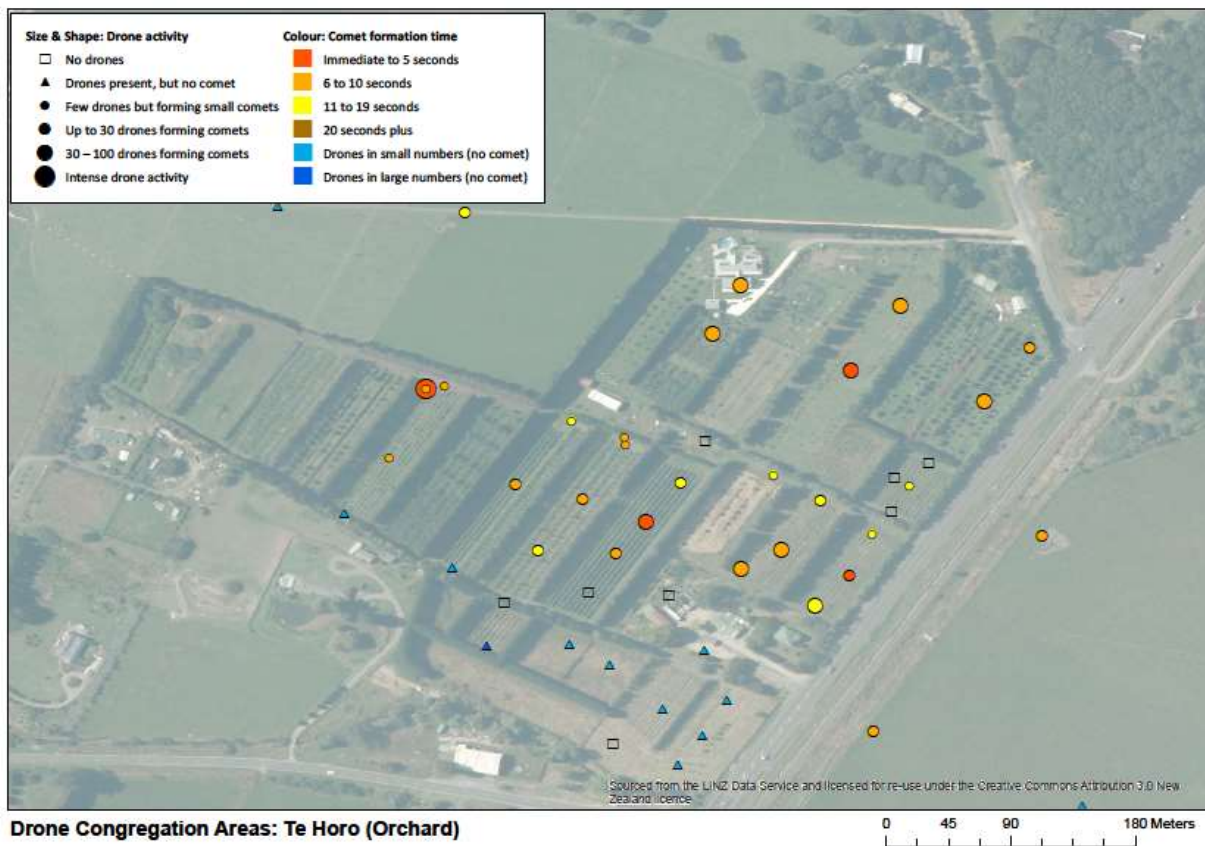


Fig 15. Showing the most of the drone activity and the more intense drone activity within the tree lines (wind breaks).

Although the areas of high drone activity within DCAS showed no significant relationship with the variables measured, the DCAs as an entity did show a relationship with slope (see Fig. 16) with 75 percent of the area of the DCAs having an average slope of 2.76°. This analysis shows that throughout all the hill and valley and urban park areas studied, high drone activity predominated in flatter areas. As ground slope increased, drone activity decreased. The relationship was shown by regression analysis R square 0.729359 (adjusted - 0.594039), P =0.036626. A relationship with aspect was not shown.

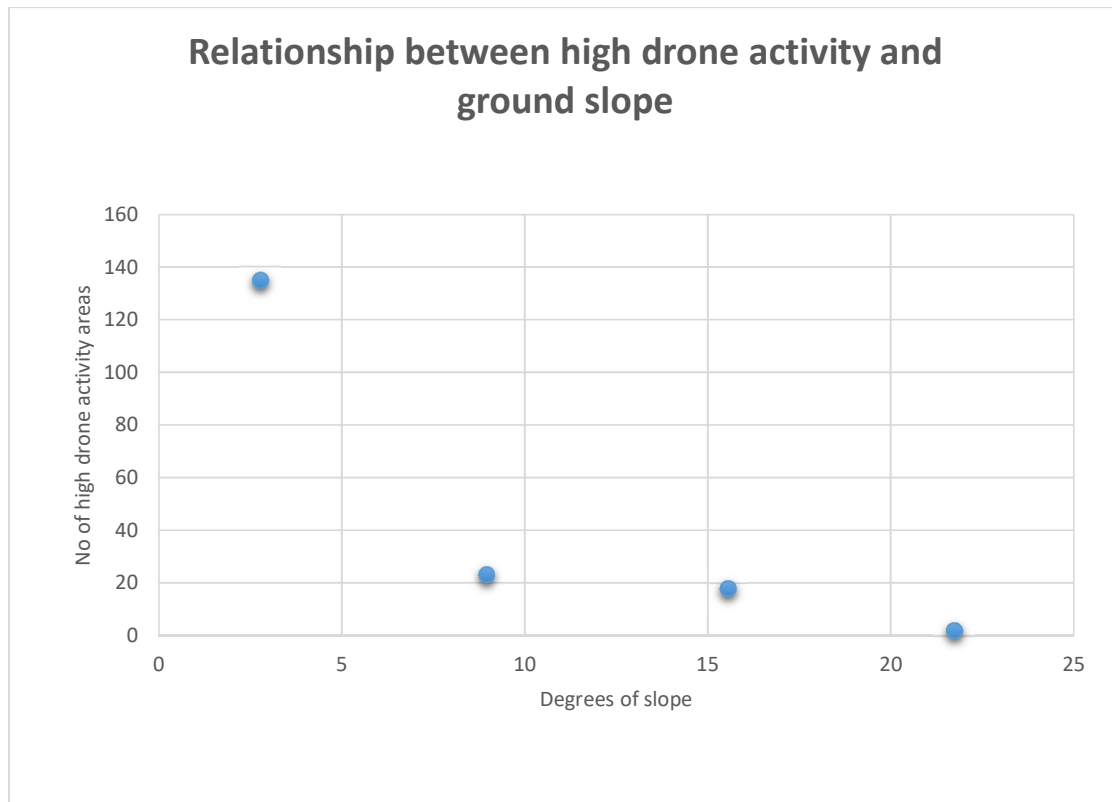


Fig 16. The relationship between areas of high drone activity and ground slope

This does not mean however that this slope played a determining part in DCA formation. The area of the Wainuiomata Golf Course (Maps 13 to 16) and Coast Road (Maps 17 to 20) have similar slope and aspect characteristics and are in the same valley yet only in the northernmost part of the golf course is there any lower density drone comet activity and the drone activity in these latter areas are more aligned itself with that of the flatter Kapiti area. The differences in the areas are that the valley is wider at these points and further from the Wainuiomata DCA with drone comet activity just beginning in the golf course nearest to the Wainuiomata DCA (Map 13).

The other factor that may contribute to DCA formation being proximity to an apiary in the case of Wainuiomata, Belmont and Te Horo, and despite the regression statistics, the lack of any comet activity over ground with a slope of more than 20 degrees.

Thus, the DCAs and DCA areas of intense drone activity were found in narrow valley areas (Belmont and Wainuiomata Camp), broader valley areas (Moores Valley and Wainuiomata), urban park areas (Ngatura and Richard Prowse parks), and DCAs were also found in the flat areas (Te Horo) in the vicinity of apiaries. DCAs were not found however in similar areas with the same slope and aspect characteristics but wider valley areas such as Wainuiomata Golf Course and Coast Road South, and the flat areas in the Kapiti region not in the vicinity of apiaries.

5.6 Conclusion

The UAV was successful in investigating the DCAs in the manner intended and enabled large areas to be studied in a shorter time than using other methods and was able to demonstrate drone activity is not evenly distributed in a DCA, determine boundaries even in inaccessible areas and show that queen flight parameters may play a part in determining DCA boundaries in certain cases.

As noted above, in any field work, there may be factors unknown to the researcher that cause changes in bee reactions. For example, in January 2016 in the Belmont study area (Map 33) the eastern area nearest the main road showed nil drone activity on 16 January, yet on 20 January showed much activity in similar conditions (i.e. time of day, temperature, cloud cover and so on). It is difficult to know the causes for these variations in activity but it could be because of the movement of hives into or out of the area, or some climatic variable (which are important factors). However, unknown factors influencing results overall can be mitigated by ensuring that the tests were carried out according to the protocols established in this study relating to drone and queen flight parameters.

6. Testing for the abundance of drones in areas in relation to landscape characteristics.

6.1 Introduction.

It has long been hypothesized that among other factors, geophysical factors (landscapes) may play an indirect and a direct influence in drone orientation (Zmarlicki and Morse 1963, Loper 1992, Ruttner 1966). Landscape features may have an effect on wind, UV light perception, optical radiance, magnetic sense orientation and the provision of orientation cues such as forests, valleys and hills etc. Geographical features visible to the flying drones can also have a direct effect on drone orientation, influence the location of DCAs, and the flyways used to reach them (Loper 1992).

In their extensive research in 1964 in the UK, Butler and Fairy found that drones behaved very differently on mating flights from those studied by Ruttner and Ruttner (1965 and 1972), and Koeniger and Koeniger (2004) in Germany. A possible reason for these discrepancies could be that researchers were carrying out their studies in areas with very different landscape features. Ruttner and Ruttner and Koeniger and Koeniger and other researchers carried out their research into DCAs in predominantly hilly areas in Austria and Germany and found that drones remained in DCAs with defined boundaries. In contrast, Butler and Fairy's research was carried out in flat areas of the English Fens and they found no DCAs but suggested that drones flew in abundance in general and found queens for mating due to well-developed pheromone attraction abilities.

They also suggested that although drones might gather in areas where they had previously smelled queens, aggregations should not be interpreted as DCAs according to Jean Prost's (year) definition of DCAs which mandates that drones congregate in areas before a queen is present. Ruttner and Ruttner (1966) point out that '*the physiographical structure of Apis mellifera DCAs seem to vary greatly*' and Tribe (1982) agreed with Butler and Fairy (1964) that drones are distributed more uniformly in plains and less structured areas and could be attracted to queens wherever they were placed.

Another key difference between studies deals with the formation of drone congregations before or after queen appearance. Ruttner and Ruttner and other researchers in Europe and other countries theorise that drones congregate in well-defined areas before the arrival of a queen, whereas Butler and Fairy theorised that if drone aggregations occur, it is because a queen has already flown through that area, or that they congregate in response to a queen's presence.

This test therefore aimed to answer the question, 'do drone honey bees behave differently in their quest for a virgin queen in areas with different landscape features?' Because of the difference in theories I expected to find a significant difference in behaviour with drones in the hill and valley

areas remaining in defined DCAs joined by flyways between areas of intense drone activity, (Map 7), and drones in the flat areas of Kapiti, behaving more in alignment with Butler and Fairy's research (1964). The collected data will also provide information related to the need for the queen's presence to form drone congregations.

6.2 Methods

In order to test drone congregation in areas with different landscape features, the UAV was flown at 25 points in each area where no drone activity had previously been recorded under the protocol established in this study for signifying a DCA (25 points for Urban Park areas, 25 points for Hill/Valley areas around Wainuiomata, Belmont and Moores Valley, and 25 points for Flat areas around Peka Peka, Otaki, Manakau and Levin). These sites were previously marked as 'Nil' on the maps presented earlier and designated as red nil pins (Maps 41 to 45).

The test looked at whether drones would visit the queen after a period of time and if they would form comets after a longer time. In order to designate an area a DCA, the UAV measured drone activity occurring within 30 seconds. In these tests the UAV was held aloft for a maximum of four minutes and the time taken for drones to appear at the lure was recorded as was the time taken for sufficient drones to appear to form comets, however small. The numbers of drones, heights and immediacy were not recorded. The anticipated problem of limited flying time due to UAV battery limitations occurred in the hill and valley areas necessitating testing over several days.

I expected to find drones outside of DCAs in flat areas would find queens over a larger area faster than drones outside DCAs in hill and valley areas. I used the chi square test as an ideal measure to confirm this as it measures how well the observed distribution of data fits with the distribution I would expect if the variables are independent. The data used are shown at Data Table 3.

6.3 results and discussion

At some of the nil markers across all areas, drones did not appear even if the UAV and queen were kept aloft for 4 minutes. For example, nil activity markers in Richard Prowse Park (Map 21) remained at nil when the UAV remained aloft for 4 minutes. It is difficult to know whether this was due to other factors including meteorological factors at the time of the tests that may have affected the result, but the tests were carried out using the basic criteria needed for drone flight and DCA comet formation described earlier.

At other points between the areas mapped for this study, and in some areas near to the active drone areas where the UAV registered no drone activity within the protocol time limit, it was found that if the UAV and queen were kept aloft, drones would often gradually appear in small but increasing numbers after an amount of time

If the UAV was maintained in this mode for a further time, small comets would often eventually form after sufficient drones had been attracted to the location. The data showed that the response in attending the lure in these tests varied but was much more frequent in the flat Kapiti areas. Comets formed 10/25 times in the two urban park areas, 11/25 times in hill and valley areas and 20/25 times in the flat Kapiti areas (Maps 42 to 45). These differences among areas was statistically significant ($X^2=9.792$ $P=0.007$).

These areas were not considered DCAs for the purposes of this study, (DCAs were designated when drones react within 30 seconds or faster indicating that they were at that location before the arrival of a queen). The markers displaying these locations are numbered in the 500 and 600 series on the maps and these are used as observational points which may further show that Butler and Fairy (1964) were correct in their suggestion that drones fly at random in sufficient numbers to locate and find a queen in flight and appear in an area in response to the presence of a queen.

6.3.1 Mean times for drones to attend a lure in the areas studied

The mean times for Drones to appear at the lure are shown below (Fig. 17).

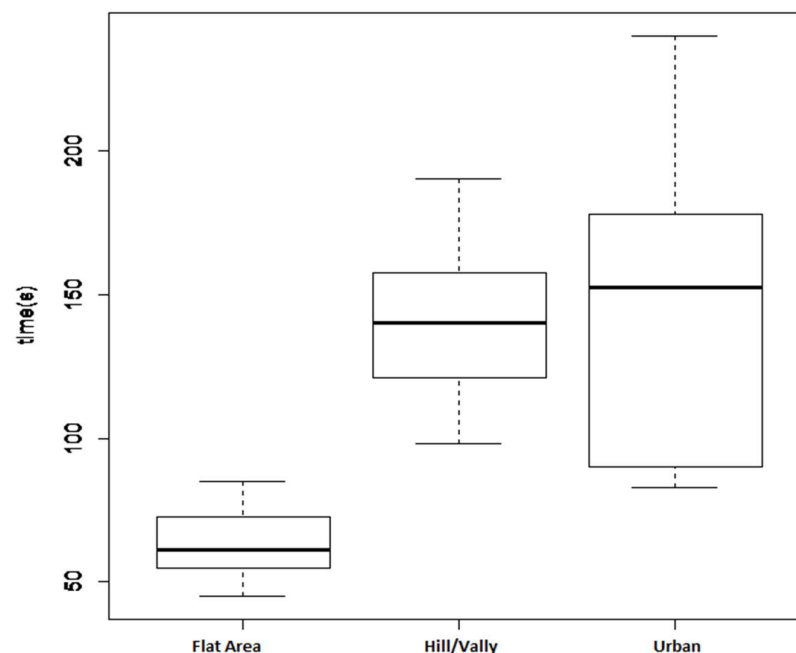


Fig 17. Mean times for drones to appear at the lure in 3 distinct areas

Urban areas: 145.1 seconds ($s=50.77$)

Hill/Valley areas: 140.46 seconds ($s=8.67$)

Flat areas: 62.8 seconds ($s=12.80$)

HSD (.05)=24.6; HSD (.01)=31.28

Urban vs Hill/Valley not significant difference

Urban vs Flat $P < .01$

Hill/Valley vs Flat $P < .01$

There was a significant difference in the mean times of drone response, between urban park and flat areas and Hill/Valley after a chi square test and a Tukey HSD test corroborated this.

6.3.2 Mean times for drone comets to form in response to a lure

The mean times for the formation of comets in these areas is shown in Fig. 18 below.

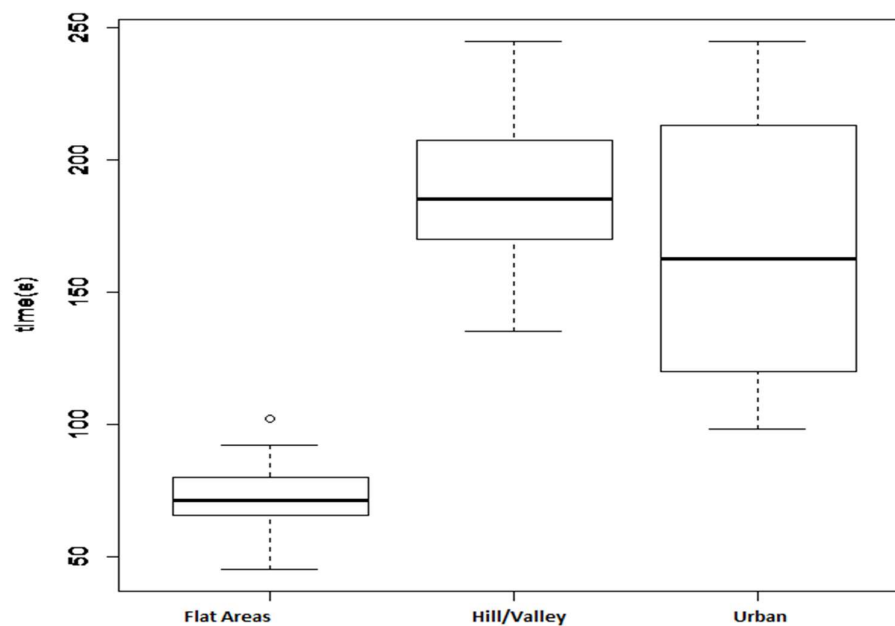


Fig 18. Distribution of data. Mean times for the formation of comets

Urban areas: 167.7 seconds ($s=55.73$)

Hill/Valley areas: 191.41 seconds ($s=233.45$)

Flat areas: 72.95 seconds ($s=13.81$)

Tukey HSD Test:

HSD (.05)=31.2; HSD(.01)=39.68

Urban vs H/V not significant difference

Urban vs Flat $P < .01$

H/V vs Flat $P < .01$

There was again a significant difference in the mean times of comet formation, between urban park and flat areas and Hill/Valley after a chi square test, and Tukey HSD test corroborated this. .

These results support those of previous research especially the studies of Butler and Fairy (1964), Koeniger (1986) and Ruttner (1976) who found differences in the way drones behaved according to landscape characteristics. This indicates that in the flat zone of the Kapiti Coast region, those areas not categorised as DCAs according to the protocol established for this study, drones fly in sufficient numbers in those areas tested to find and visit a virgin queen and to form comets in response to the presence of a virgin queen. This accords with the findings of Butler and Fairy (1964) who found this scenario to be operating in the flat Fenland district of the UK.

The greatest difference in both response to the lure and response time to form drone comets was found to be between the urban park zones of the Wainuiomata area ($s=50.44$ seconds) and the Flat areas of the Kapiti region ($s=12.81$ seconds), and it may be theorised that drones tend to remain in the more confined park areas in order to avoid built-up areas where the presence of buildings and other man-made structures might cause air disturbances that may affect the drone response to the queen pheromone.

This study also suggests that large drone congregations or drone intensity hotspots in flat areas tend to be apiary-dependant but that away from apiaries, drones fly in sufficient numbers to form comets for queen mating, whereas drone activity hotspots are found in hill and valley areas where no apiaries are known to exist.

6.4 Conclusions

The two hypotheses tested were as follows:

- Drone honey bees fly away from the apiary to special areas (Drone Congregation Areas) with defined and fixed boundaries dependent on landscape features, to mate with virgin honey bee queens that fly through the area; and
- DCAs do not exist and, instead, drone honey bees fly at random from the apiary and their abundance ensures matings with queens.

The results show that these two apparently contradictory statements could be amended to read:

- In hill and valley areas, drone honey bees fly away from the apiary to special areas (Drone Congregation Areas) to mate with virgin honey bee queens that fly through the area. The boundaries are not fixed in space and depend on both landscape features and the flight parameters of a virgin queen; and
- In flat areas, DCAs exist in the area around apiaries but at increasing distance from the apiary, drone honey bees fly at random and in sufficient abundance to ensure matings with queens.

7. Investigation of drone-to-drone, and queen-to-drone attractiveness

7.1 Aim of the study

In Part Three of this study, a third hypothesis was tested as follows:

- The formation of DCAs is aided by drone pheromones and consequent behavioural interactions of flying queens and drones.

This part of the study sought to answer the question, ‘are honey bee queens attracted to drone pheromones?’ and to help confirm the research study of Brandstaetter et al (2014) that suggested that drones are attracted to each other possibly due to an aggregation pheromone emitted by sexually mature drones, and Bastin et al. (2017) that suggested that queens are attracted to sexually mature drones rather than workers. Butler and Fairy (1964) stated that no evidence existed that queen honey bees are attracted to drones, and that such an attraction would be unlikely given the strong olfactory attraction that drones have for queens, but until recently (Bastin et al. 2017), little research on this subject has been carried out. Bastin et al. (2017) showed that virgin queens in a laboratory simulator are attracted to drones, and there has been speculation that if virgin queens were attracted to drone pheromones, this factor could play a part in attracting queens to DCAs.

7.2 Background

Chemical communication in the honey bee is very complex with 15 exocrine glands known to produce various pheromone chemicals (Torto et al. 2013). We know *a priori* that drone honey bees are attracted to a virgin queen’s mandibular gland pheromone and that many researchers since Gary (1962) have confirmed this. In 2007, Wanner et al. discovered a drone honey bee odorant receptor for 9-oxo-2-decenoic acid, a major component of the mandibular gland or ‘queen substance’.

Since then, researchers have tested for a drone emitted pheromone that may be attractive to other drones and also queens. Lensky et al. (1985) and Brandstaetter et al. (2014) have shown that there is an attraction between drones which may be a possible contribution to the formation of DCAs. Loper et al. (1992) reported that based on radar studies, drones congregate at intersections and branching points of flyways and reorient themselves. Brandstaetter et al. (2014) suggest that this loitering time while they re-orientate may be sufficient for the odour of drones to build up in the area while they fly in their characteristic orientation loops, thus attracting more drones. They also suggest that this may be an explanation for the clear-cut boundaries of congregation areas found by other researchers such as Ruttner and Ruttner (1965), Ruttner (1985), and Loper et al. (1992). They go on to ask whether queens are also attracted to olfactory cues emitted from groups of drones.

The first experiment in this test described below sought to confirm the work of Brandstaetter et al. (2014) on the question of drone/drone attraction. Part two of the test sought to answer the question of whether queens are attracted to these drone-emitted olfactory cues.

7.3 Materials and methods

Control of the experimental variables was attempted by ensuring that the tests took place during the hours, temperatures and other climatic variables normally associated with queen and drone nuptial flights. The tests were carried out in a glass conservatory in order to provide the bees with the usual multimodal sensory information they receive during mating flights apart of course from the fact that they are not flying. For the purposes of this series of tests, the following equipment was used and the following animals caught and tested:

- Drone trapping net (modified from Williams 1987) with an artificial queen impregnated with 0.1mg of synthetic 9-ODA (Fig. 19)
- A room open to the sun and clouds with the same ambient temperature as outside
- Four arm olfactometer described below (Figs. 20 and 21)
- VAS portable control air pump and extractor (Fig. 22)
- Mobile phone video camera fixed over the test olfactometer to record some of the tests.
- Helium balloons and control line to hold the net aloft
- Drone bees for use as pheromone/ odour emitters
- Drone bees as experimental subjects
- Worker bees as pheromone/odour emitters
- Virgin queen bees as test subjects
- Holding containers with comb, water and honey
- Kestrel pocket meteorology station

All test bees were captured and held in accordance with Standard Methods for Maintaining Adult *Apis mellifera* in cages *in vitro* laboratory conditions, outlined in Coloss Bee Book Volume 1 (Williams et al 2012).

The drones and workers were caught from the comb by brushing the bees into containers. Worker bees from brood combs were used for these tests and so would likely mostly be nurse bees. Because of the absence of flying drones, drones were also caught directly from the comb. The capture colonies were checked free of American Foul Brood disease and other diseases, and had been treated for varroa. The virgin queens were between four and seven days old when tested. Following the tests, the drones and workers were released and the queens either returned to the queen breeder or used by the author for making up nucleus hives.

7.3.1 Test drones

Several attempts at catching drones were made in late March, early April for the purpose of pheromone attraction tests in an insect olfactometer. Drones are often more numerous in the late summer/early autumn period than during the summer or spring and are usually very active in DCAs at this time. The drone trapping net similar to that used by Williams (1987) (Fig 19) was suspended from helium balloons in known and very active DCAs at Coast Road Wainuiomata DCA, Wainuiomata camp DCA, Moores Valley DCA and Belmont Park DCA, over several days conducive to drone flight. Inside the trap, an artificial queen was suspended, made with soft material impregnated with 0.25 mg more than previous used in the DCA testing to ensure greater attractiveness) in solution with 0.5 ml acetone.

Drones flying in a DCA are likely to be sexually mature and this is a simple way of ensuring that drones used for testing were of the right age for the tests. It was intended to capture drones from the hive at peak drone flying time to ensure that these latter group of drones would be unlikely to be sexually mature. In this way, a comparison of virgin queen attractiveness to sexually mature and sexually immature drones caught in the hive could be tested.

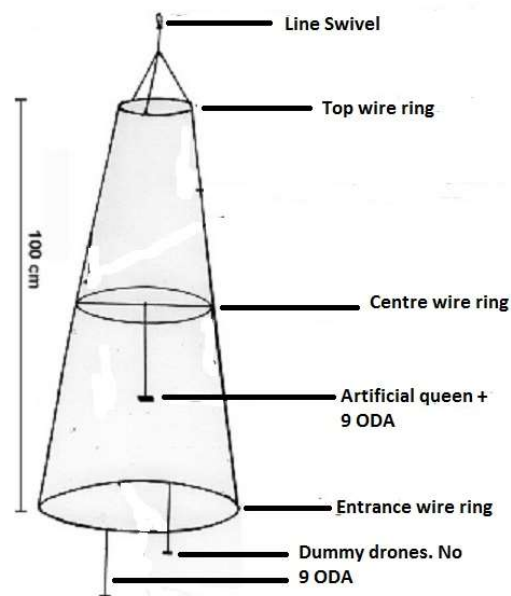


Fig 19. The drone trapping net after Williams (1987). (Modified).

Unfortunately, the period of several weeks leading up to the olfactometer tests, was abnormally cold and wet and not conducive to drone production in the colonies and beekeepers reported that drones were being evicted from the colonies far earlier than is usually the case. Drone numbers in a hive are regulated by the workers and they are evicted from the hive in times of scarcity of nectar. The process is gradual but if the scarcity continues, most drones, especially the older ones will be

evicted. The abnormally bad weather associated with the summer and early autumn of 2017 caused an earlier than usual eviction.

Consequently, when the drone trap was used in several known DCAs that were usually very active each year, there was nil drone response. An inspection of hives in the area showed very few drones present, although as the weather was improving there was some young drone brood in the hives. Beekeepers in several parts of the Wellington region advised me that they had some drones emerging in colonies and although there was still a failure to capture drones in flight in DCAs, hive drones of indeterminate age were captured and used in a series of modified tests.

Captured drones for experimental use that same day were transferred to an odour-free, phthalate- and BPA- free plastic box with a few workers in a ratio of two drones to one worker and a piece of wax comb providing honey and water before being placed in the test apparatus. Drones, especially young drones, tire easily when confined without workers in the odour-source bowls and so new drones captured were used for testing immediately and then released.

Drones were used as both pheromone lures and as experimental subjects. These two groups were taken from separate hives. 10 drones used as pheromone/odour emitters were placed in the experimental set up prior to each experiment. Unlike workers, drone bees are usually accepted in any hive and drone 'drift' is an accepted phenomenon in apiaries. It was therefore considered unlikely that there would be any antagonism between drone subjects and workers in the worker odour-source bowls.

7.3.2 Test Workers

Test workers used as pheromone/odour emitters were caught in hives in an apiary in Moore's Valley and placed in an odour-free, phthalate- and BPA-free plastic box with a piece of honeycomb with honey and water. Ten workers were used per experiment as per Brandstaetter et al. (2014). Test worker numbers were not restricted in any way.

7.3.3 Test Queens

With few drones available for successful matings, few queen breeders were producing virgin queens at this time for sale. Test queens were bred in batches of four especially for this study by a Wellington-based queen breeder in Days Bay and supplied as required in queen cages accompanied by attendant workers; each cage having a candy plug for food. Queens kept overnight were supplied with water drops and kept at temperatures above 26 degrees centigrade. The virgin queens were between 5 and 6 days old during the experiment and were either returned to the queen breeder after testing or used to form new colonies for overwintering by the author.

For the attraction assay tests, drones, queens and workers were not caught in the same hives so as to avoid hive odour recognition by the test animals which might have skewed the results in favour of attraction to nest mates.

7.4 Experimental set up

In order to test drones' odour preference, a four-arm olfactometer was used with four currents of air, one from each arm (Fig. 20). Before reaching the central part of the olfactometer where the subject queen or drone was situated, the air from each of the four arms passed through a glass globes containing either 10 workers (two worker compartments), 10 drones or an empty globe which passed filtered air only. The arms faced North, South, East and West as shown in Fig. 21. The set-up was placed on a table in a glass conservatory so as to ensure natural light conditions as far as possible. The temperature of the conservatory was kept the same as the outside temperature.

Continuous air flow through the odour sources was provided by two portable VAS Volatile Assay System controlled push-pull airflow, hydrocarbon filtered air pumps (Fig. 22). So as to avoid the saturation of odours, the air was extracted via a central vent in the olfactometer which was also the entry point of the test bee (drone or queen).

On entry to the olfactometer device, the drone or queen was free to move around to familiarise themselves with the apparatus for two minutes before the air currents were switched on (Brandstaetter et al. 2014). Their start quadrant was wherever the subject bee was when the airflow was switched on. A video camera (mobile phone) suspended above the olfactometer recorded some of the tests as soon as the air pumps were switched on.

A mesh insert on each arm of the apparatus leading to the odour sources prevented the test bees from joining the odour source bees, and also prevented the odour source bees from entering the olfactometer. Following each test, the olfactometer was cleaned with a standard, odour-free wet wipe.



Fig 20. The 4 way olfactometer test equipment showing the air inlets

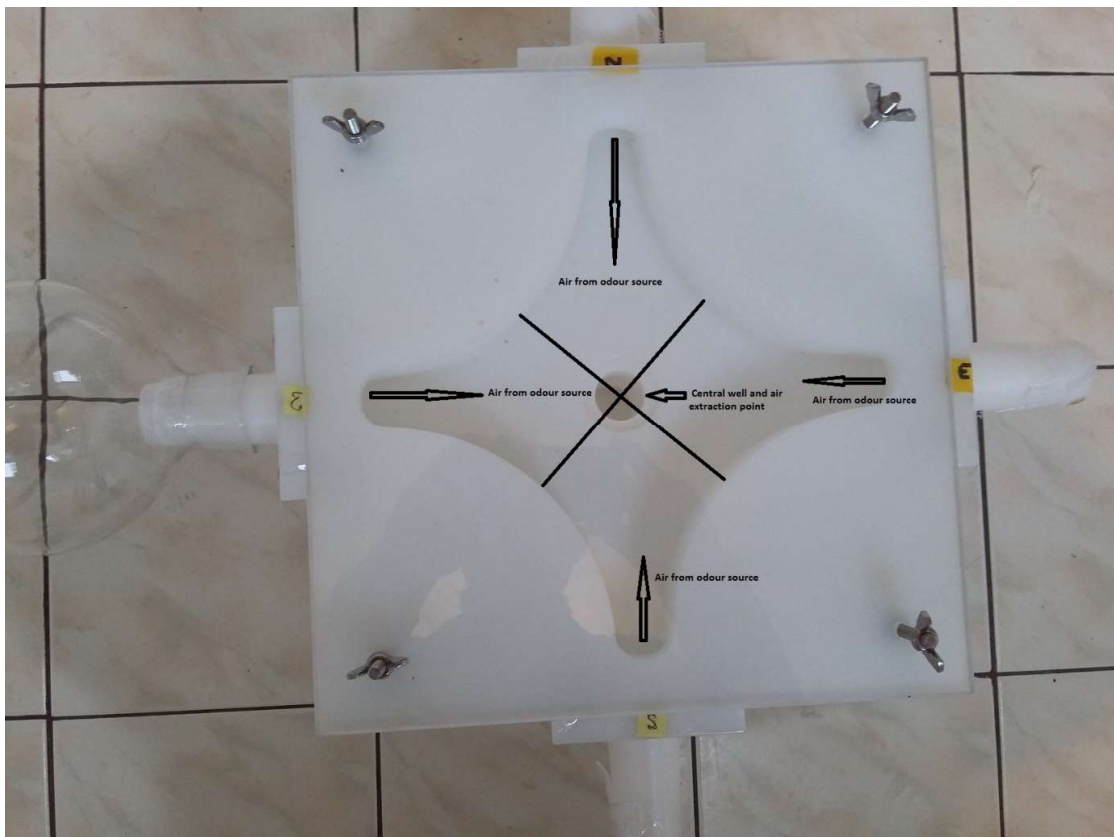


Fig 21. The four-way olfactometer. Detail showing the four quadrants and odour intake and extraction points



Fig 22. The VAS four-way air pump and air extractor device

7.5 Experimental Process

Three glass bowls attached to the olfactometer were filled with 10 bees each. Two bowls were filled with worker bees (W1 and W2) and one with drone bees (D). The fourth bowl was left empty and only filtered air passed through this bowl (A). For the first test in each series of four, the olfactometer and odour sources were placed as shown in Fig. 23 below.

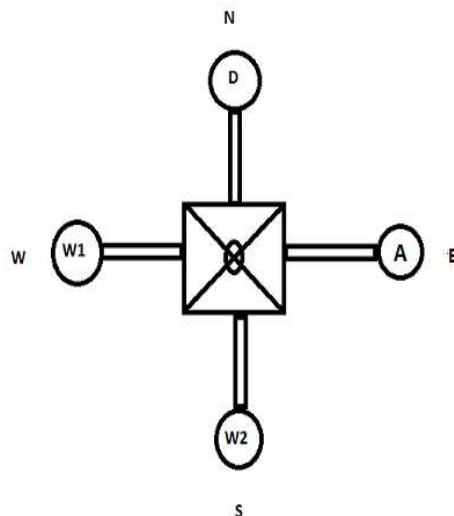


Fig 23. Olfactometer layout.

Key:

D. 10 Drones were placed in the North position.

W1. 10 Workers were placed in the West position.

W2. 10 Workers were placed in the South position.

A. No bees were placed in the East position. Only filtered air passed through this portal.

Workers W1 and W2 were taken from the same hive. Test queens were taken from separate hives as were test drones.

Following test 1, the apparatus was cleaned and the glass odour bowls were moved to the next position anticlockwise as shown in Fig 24 below.

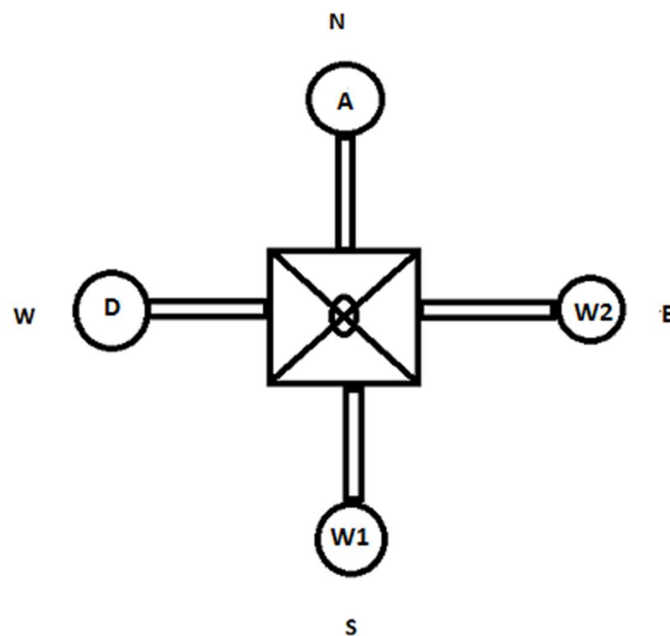


Fig 24. Apparatus layout Test 2. Odour source bowls moved one position anti-clockwise

The glass bowls were moved one position anti-clockwise after each test for a total of four tests.

Thus each subject animal was tested four times. Illustrations of the odour bowls with attendant bees are shown in Figs 25 and 26 below.



Fig 25. Drone odour source bowl (attendant workers removed)



Fig 26. Worker odour source bowl

7.5.1 Test A. Queen/drone attraction test

The aim of this test was to find out if virgin queens are attracted to drone pheromones or would

preferentially be attracted to workers.

A queen test consisted of setting up the odour sources as shown above and introducing a virgin queen to the olfactometer through the central well entrance to the olfactometer (which doubles as the air extraction point). A small ladder was available for the queen to climb up onto the floor of the olfactometer. Once in the olfactometer, she was left for two minutes to acclimatise to the apparatus before the air flow was started. Each of the four input air flows was set to 0.25 Litres per minute and the extraction pump to 1 L/min so that all the air entering the olfactometer was extracted.

The queen was carefully observed at this point. She was timed in each quadrant and finally the time when she disappeared from sight into one of the tunnels leading to an odour source was noted. At this point, the queen was left for one minute in the tunnel and was observed at the mesh entrance to the odour bowl to ensure that the position was a permanent choice. During this time, the queen was noted being attended to by workers through the mesh separating her from them (Figs. 27 and 28). Workers can be seen clinging to the net preventing them from entering the olfactometer chamber with a queen on the other side of the net.

Following the test, the queen was recovered, placed in a queen cage, the olfactometer cleaned, and the queen replaced in the olfactometer well. The odour sources were moved one position anti clock wise and the test repeated with the same queen. Four tests were carried out on each queen with the odour sources moved each time.

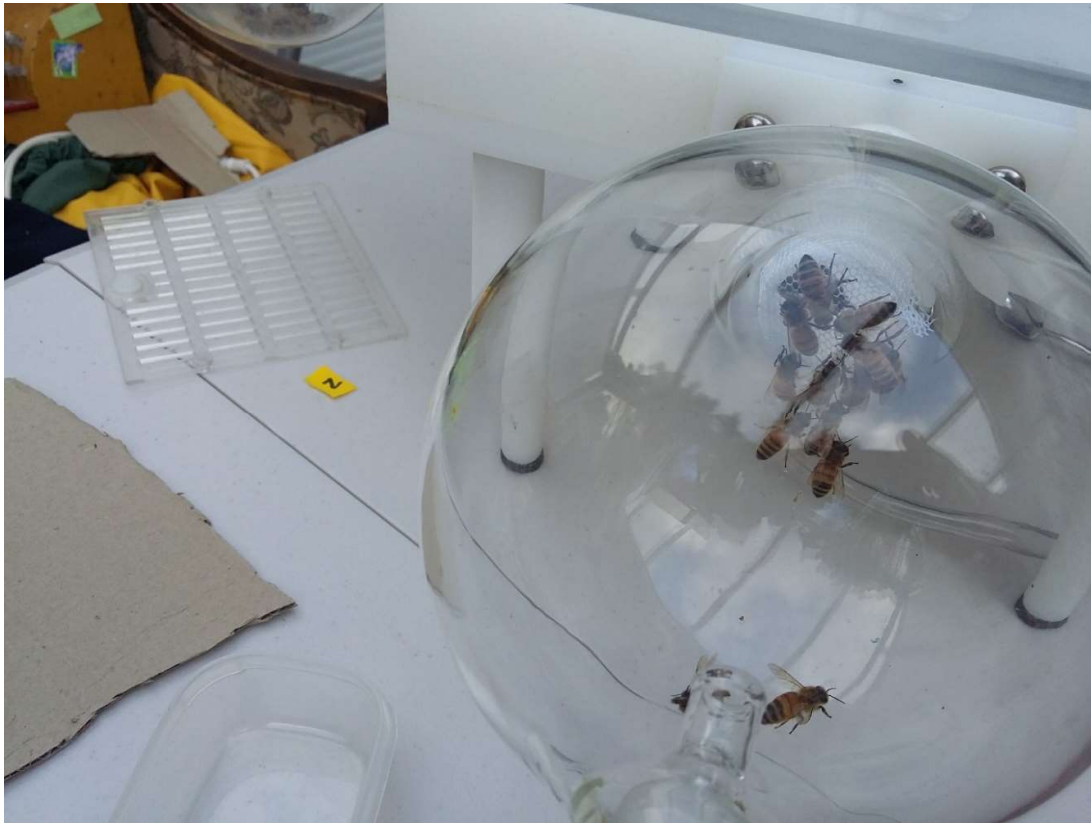


Fig. 27. Workers in the odour source bowl attend the queen behind her gauze screen during Test: Queen 4. Test 4.

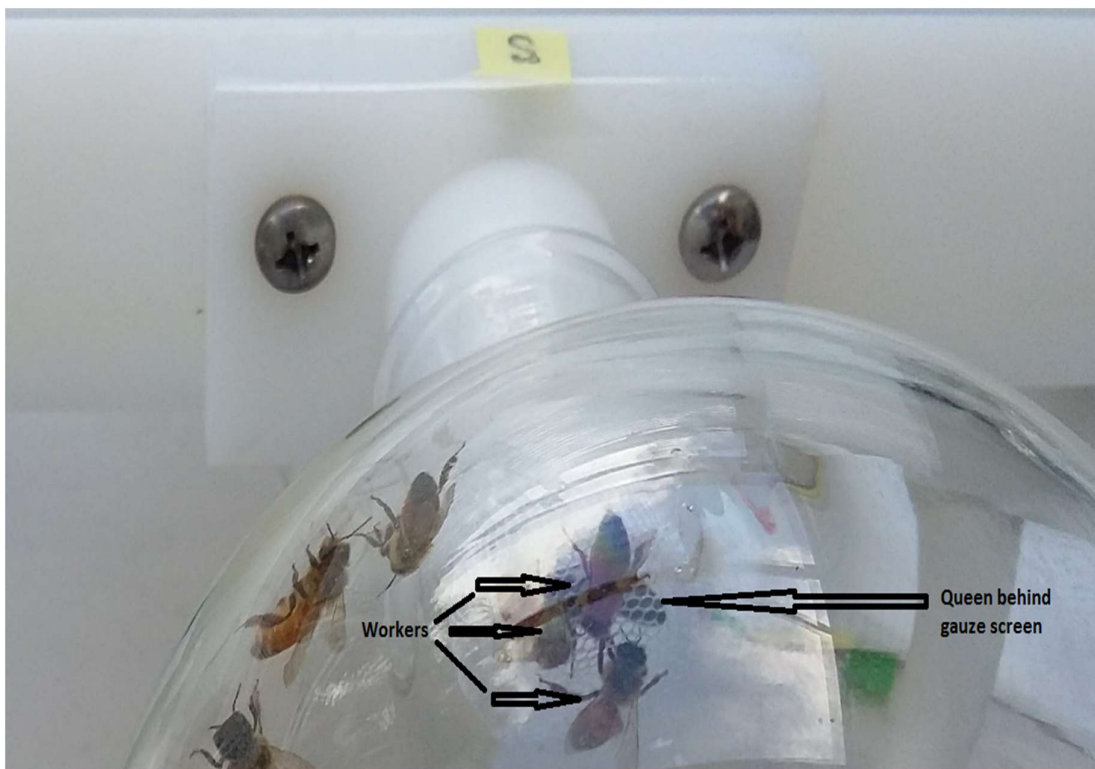


Fig. 28. Close up of workers in the bowl attending a queen behind the gauze screen in Test 1. Queen 1.

Once the odour sources had been moved, the queen was recovered, the olfactometer floor cleaned and then the queen was allowed to return, and the test was repeated with the odour sources in the

different positions. A third and fourth test followed in the same way for each test animal. The queen was then recovered and placed back in her cage with attendant workers. The apparatus was then cleaned and the bowls refilled with fresh worker bees or drones and a new queen introduced. Ten queens were used and each queen was tested four times, providing data from 40 tests. The data from these tests is shown at Data table 4.

7.5.2 Test A. Results.

It was evident from simple inspection of the data that the virgin queens were attracted overwhelmingly to workers, thus showing the opposite results to those of Bastin et al. (2017). Out of 40 tests, the queens went to the worker odour sources 100% of the time (Fig. 29). Some went to the worker odour sources faster than others and on occasion some lingered in the drone quadrants but always finally went to workers.

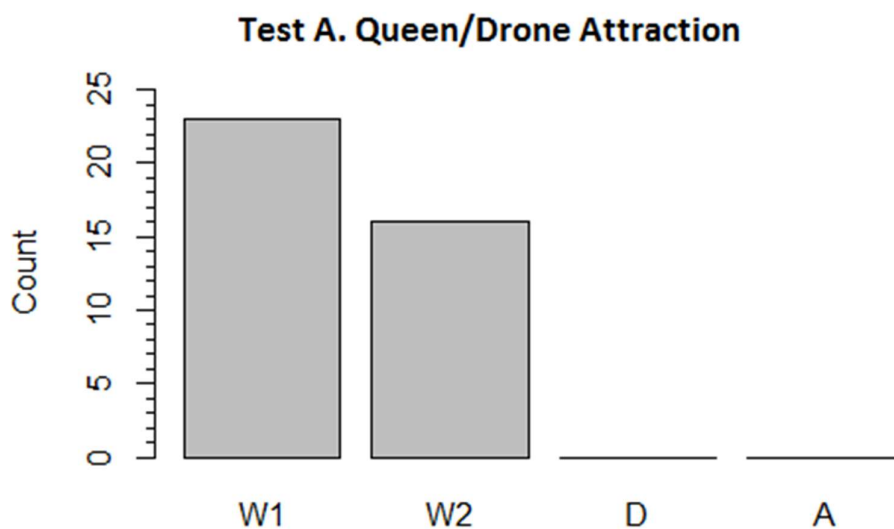


Fig. 29. Test A. Queen/Drone attraction. W1 = Workers 1. W2 = Workers 2. D = Drones. A = Air

Figure 33 shows that when provided with a choice of workers (W1 and W2), drones (D) or air (A), the virgin queens in the test chose workers 100% of the time ($\chi^2=0.02$, $P<0.0001$). There was no significant difference between Worker 1 and Worker 2 odour sources ($P= 0.337$). If a queen went to W1 or W2 during her first test, it was not always faithful to that group. Six queens visited the same worker group every time. Four queens visited more than one worker group during their four tests. $\chi^2= 0.4$, $P= <0.527$.

7.5.3 Test B. Queen/drone only attraction test (worker influence removed)

The aim of this part of the study was to observe the queen's choice of odour if the worker odour

sources were removed and there was only a choice between one drone odour source and three sources using only filtered air. The process was the same as in the queen drone attraction test above. The drone source started in the East position and moved anticlockwise between each test. Each queen was tested four times using the same protocols as above.

7.5.4 Test B. Results

With the worker odour sources removed, the queens went predominantly to the drone source (Fig. 30). Out of the 20 tests, queens went to drones 18 times rather than air, and one test was invalid as the drone fell in the well, was damaged on rescue and did not continue with the test. The test results are shown at Data Table 5.

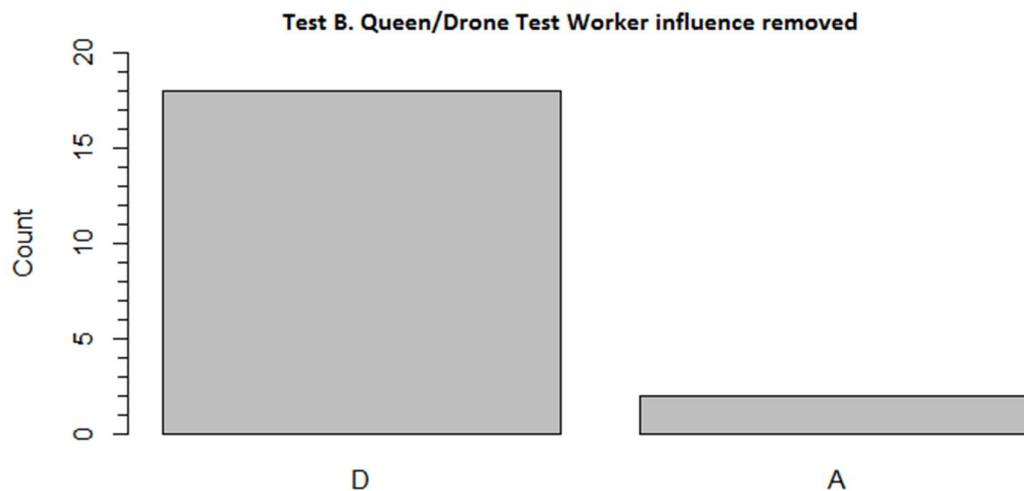


Fig 30. Test B. Worker bee influence removed providing a choice of air or drones.

D = Drone, A = Air Chi squared (X^2) equals 12.800 with 1 degree of freedom showing that the difference is statistically significant ($P= 0.0003$).

7.5.5 Test C. Drone/drone attraction test

The aim of this part of the study was to revisit the drone/drone laboratory attraction assay carried out by Brandstaetter et al. (2014), which tested innate odour preferences of drones under experimental conditions. Again, a four-way olfactometer was used and a 4-way orientation test was designed, in which drones were presented with 3 biologically relevant odorants and plain, filtered air as one of the sources. The process was identical to that of queen testing with drone subjects entering the olfactometer from the central well, being given time to acclimatise to the situation for two minutes and then starting the air pumping through the three odour bowls and the filtered air bowl and with the olfactometer extraction pump switched on. The results from these drone tests are shown in Data Table 6.

7.5.6 Test C. Results

On simple inspection the drones in this test were attracted mainly to workers. Out of 80 tests, 71 resulted in drones going to worker odour sources with 14 going to drone sources (drones went to the worker odour sources 90% of the visits – Fig. 31). Again this is a significant result using a chi Square test ($X^2=49.64$, $P= <0.001$). Some went to the worker odour sources faster than others and on occasion some lingered in the drone quadrants but always finally went to workers. No drones went to the air sources. There was not a significant difference between drones going to the two different worker groups ($X^2=3.6$, $p=0.058$).

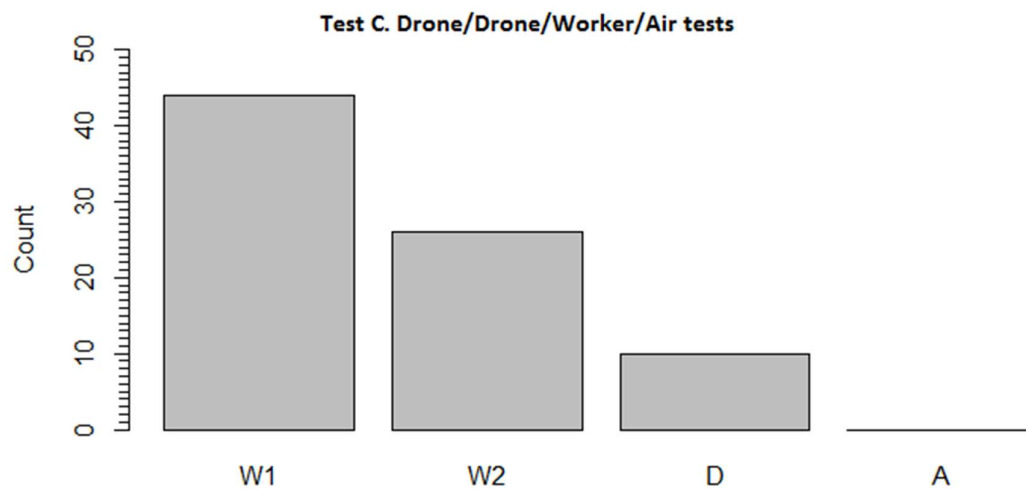


Fig 31. Drone attraction to workers, drones or air on a four arm olfactometer test.

W1 = Workers 1. W2 = Workers 2. D = Drones. A = Air

7.5.7 Test D. Drone/drone attraction test (workers removed)

The aim of this part of the study was to observe the drone's choice of odour if the worker odour sources were removed and there was only a choice between one drone odour source and three sources using only filtered air. The process was the same as in the queen drone attraction test above. For this test, the worker influence was removed and the results are shown at Data Table 7.

7.5.8 Test D Results

Out of 40 tests, of those drones that completed the test, they went to the drone quadrant and remained there 82.35% of the time (Fig. 32). 3 drones were not able to complete the test, 3 drones finished in the air quadrants and 34 finished in the drone quadrant. The result showed that in this study, drones significantly prefer drones over air ($X^2= 25.83$; $p<0.001$).

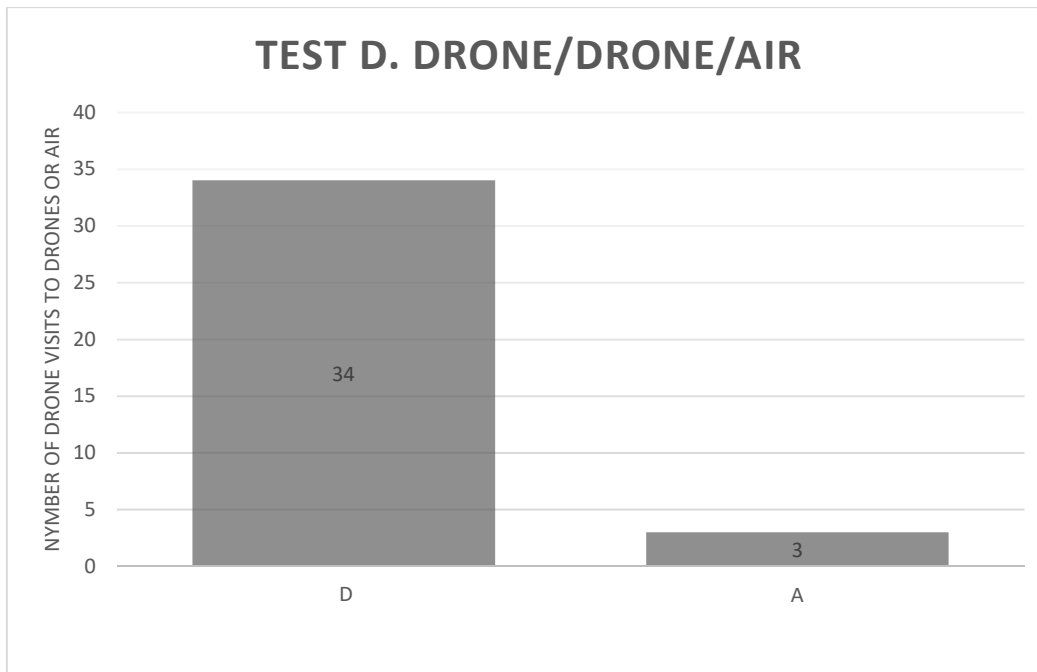


Fig 32. Test D. Drone attraction chart with worker influence removed. D=Drone and A=Air.

7.6 Discussion

It was evident from simple inspection that 7 day old virgin queens went to workers rather than drones when there was a choice and to drones when worker influence was removed. It is also evident that if queens went to a group of workers in one of the bowls during the queen's first test, she would invariably return to that group of workers during tests 2, 3 and 4. The queens were not nest mates of either the drones or the workers and so the influence of hive odour was discounted in the experiment.

When in the hive, queens, virgin or mated, are fed and attended to by workers and perhaps this attraction factor motivated the queens to choose a worker quadrant when workers were present.

Queen bees do not fly from the hive except for mating flights (virgin queens), or in swarms (mated queens). Whilst in the hive, queens are either mated, or they are virgins that have not left the hive on mating flights and in both cases drone presence is not a significant factor in their behaviour. However, on a mating flight when worker influence is removed, there may be an attraction to sexually mature drone pheromone, something that this experiment was not able to determine due to inability to determine drone age and carry on the experiment from there. Bastin et al. (2017) have recently shown that queens are attracted to drones in a laboratory setting. Using a walking simulator similar to that used by Brandstaetter et al. (2014), and using sexually mature drones, they concluded that:

- The experiments may reveal an attraction of queens towards drones;

- no attraction of queens towards a bouquet of living workers; and
- no attraction of workers towards drones; and

These findings are contrary to those in this study which found that there was no attraction of queens to the bouquet of living drones and there was an attraction to the bouquet of living workers. This difference in results is likely due to the age of the drones used in the experiments. Bastin et al. (2017) used sexually mature drones, this study necessarily used drones that were most likely not sexually mature.

During the drone/drone attraction tests, again it was evident from simple inspection that in the olfactometer, drones went to workers rather than other drones when there was a choice, and that they went to drone quadrants rather than filtered air quadrants when the worker influence was removed. Unfortunately this result again highlighted the main flaw in the experiment and that is the lack of age knowledge of the test drones and odour source drones. For the first few days of their lives drones are fed exclusively by workers. This is followed by a period in which they are fed by workers to a lesser extent, and when they also feed themselves from honey cells. They rarely fly before the sixth day (Witherell 1971). For the first week of their lives therefore drones depend entirely on workers for sustenance and it can be conjectured that this factor may have influenced the drone attraction tests if the age of the drones was 7 days or less.

Because of the method of capture (from the comb) it was not possible to determine their age with any accuracy and because of the fact that drones were expelled from the hive early in the season, the drones in the tests are likely to have been new ones. It may also be likely that while any older drones were still being expelled from the hive, new, young drones would still be fed by workers- (Free 1957). The behaviour of workers toward a drone appears to depend upon his age. At the same time as some of the older drones are often attacked by workers the younger drones present are still being fed by workers.

This 'unknown age' factor was a major drawback to the successful outcome of the intended tests which required knowledge of the age of the drone and high numbers of test animals. As mentioned earlier, the drone mandibular gland pheromone builds up in the gland before the drone is sexually active and then the gland atrophies with only trace amounts of the pheromone remaining. The original experimental design intended to find out if there was a difference in attraction, both drone/drone, and queen/drone, between young, non-sexually mature drones, and older, sexually mature drones. In the event, drones taken from the hives were used for the tests and it is likely that the majority of these would have been young, non-sexually mature drones that were still being fed by workers and would likely still be dependent on workers.

As it is likely that the drones in this experiment were young and not yet sexually mature, the attraction to workers (who feed them) it could be conjectured that this could have been a significant factor in their choice of quadrant. Experiments such as that performed by Brandstaetter et al. (2014) used drones of known age, (in bad weather age marked drones were caught inside hives and during good weather, drones exiting the hives on nuptial flights were caught). The results of their laboratory tests suggested that there could be a drone/drone attraction which in turn could assist in the cohesion of DCAs.

In this study however, when the influence of worker odour was removed, a likely scenario during a mating flight, drones went to the drone quadrant in the olfactometer rather than air quadrants. If the odour source drones were young, it can be conjectured that this preference is unlikely to be due to any pheromone attraction.

It was disappointing that the intended experiments were unable to be fully realised. The lack of drones generally and the lack of drones of a known age and the consequent paucity of queens produced by queen breeders was a major drawback that prevented sufficient data being generated. The intention was to carry out Test A using non-sexually mature drones as odour sources and then repeat the experiment with drones captured in flight from DCAs which would likely be sexually mature. Had there been a significant result showing an attraction to drones by queens, or drones and drones in test C, I would have further investigated this by exploring the chemical makeup of the drone bee mandibular gland pheromone in the laboratory for young drones and mature drones and testing the components against live drones and virgin queens.

7.7 Conclusions

It was evident that workers were the main destination for both drones and queens in preference to drones or air and both results were probably due to the dependence on workers in the hive by young virgin queens and young, sexually immature drones. The same virgin queens may have reacted differently to drones outside of the hive on a mating flight in the absence of worker influence. It is recommended that future research is continued and carried out earlier in late spring/early summer to ensure that a short drone season doesn't affect the research. It is further recommended that if drone/drone and queen/drone attraction is shown, further tests be carried out to determine the source of the attraction in the drone mandibular gland pheromone.

8. Thesis Summary and Conclusion

The study of DCAs in the Wellington and Kapiti coast areas was divided into three parts:

Part One sought to develop an improved method of investigating various aspects of DCAs, particularly areas that are difficult or impossible to access by using a camera-equipped, unmanned vehicle (UAV), and developing a set of operating protocols that together ensured good coverage of a large area of ground. The results were successful and showed beyond doubt that areas that were difficult to access could be surveyed regardless of wind direction, high tree lines or other impediments that could prevent survey by balloons and kites. Despite various disadvantages, this method will enable researchers to carry out studies of DCAs on any days that are conducive to drone and queen flight rather than waiting for the right wind direction or other conditions to improve.

Part Two used the UAV to investigate a series of DCAs in the Wellington and Kapiti areas of New Zealand. Three types of area were investigated: Urban park areas; hill and valley areas, and a flat agricultural area. Using the UAV according to protocols developed in part One of this study, the UAV provided data which enabled a description of each DCA, an 'at a glance' map of the intensity of drone activity within each area and was able to relate the DCA locations and activity levels to various land cover and land formation features, such as land cover, slope, aspect of the areas and whether wind shelter areas contributed to areas where drones were most active in a DCA.

No correlation was found between DCA concentrations and aspect, slope or land cover type. A weak correlation was found between drone intensity and shelter. Part two also looked at drone response in areas that were not designated DCAs in accordance with the protocols outlined in this study in order to find out at the abundance of drones in the three types of area (urban park, hill and valley and flat, agricultural areas) and compare the responses in each area and relate them to landscape features. There was a significant difference between the hill/valley and urban park areas when compared to the flat areas, meaning that drone mating flight behaviour is different in each area.

Part of the research above theorises that the supposed boundaries of DCAs especially in hill and valley areas may be constructs that depend on the direction of the queen's flight as well as landscape and perhaps other factors. Another question about DCA boundaries of DCAs is drone numbers. In this study, what appeared to be DCA boundaries moved when more bees were moved into the immediate vicinity of the DCA. I believe that the DCA puzzle will not be complete until extensive research is carried out on the mating flight of the queen.

Part Three tested for queen/drone and drone/drone pheromone attraction and answer the question 'do drone pheromones have a role to play in the formation and maintenance of DCAs?' The experiments clearly showed that if given a choice in a laboratory olfactometer test, queens and drones preferred workers to drones. Only when workers were removed and the only choices were drones or air, were drones mainly chosen. Due to unforeseen meteorological circumstances, the tests were flawed in that the age of the drones was not known and the tests likely showed only a reaction to young drones rather than sexually mature ones, which are present in DCAs. The tests did however indicate that young drones did not attract other young drones or queens. It would be useful if these tests could be repeated with drones of known age to establish if there are differences between sexually mature and immature drones.

8.1 Conclusions

This study has shown some relevant results in terms of methods of exploring DCAs with a UAV; provided visual information about the make-up drone activity within DCAs in different landscape zones, and has been able to demonstrate differences in drone mating flight activity in different landscape zones.

The landscape features measured did not correlate with the intensity of drone activity within DCAs except for shelter. There was a correlation however between ground slope and DCA formation, although contrary to the study by Galindo-Cardona et al. (2012), there was no correlation with aspect. With other nearby areas sharing the same characteristics it appears that other factors come into play and these could be proximity to apiaries and valley width. It seems therefore that the areas studied with intense drone activity were either apiary-dependant DCAs, with drone activity diminishing with distance from the apiary, or some other factor such as drone reorientation points as described by Loper (1987) in his radar studies, which lead to a build-up of drones in a confined valley area. This could explain the DCAs at Belmont, Wainuiomata camp and both DCAs in Moores Valley, where DCAs existed with no apiaries in the immediate vicinity (the apiary at North Moores Valley appeared only during the third season although the DCA existed before this). This feature also aligns itself with suggestions that a drone-emitted aggregation pheromone might be at play; the pheromone building up in the narrow valley confines.

The observation of Butler and Fairy (1964), that DCAs do not exist but that drones fly at random in sufficient abundance in an area were partly shown as possible in this study where attracting drones outside of DCAs in flat landscape is far easier than in hill and valley areas. The Te Horo DCA also shows however that apiary dependant DCAs also exist in these flat areas and gradually fade out to the Butler and Fairy model with distance.

The observations of other researchers that DCAs are fixed in space, with rigid boundaries is also open to question from the results of this study, which suggest that DCA boundaries are not fixed but can be affected on a day to day basis by other factors including landscape, wind direction, queen mating flight behaviour and numbers of drones in an area. This in turn suggests that in hill and valley areas, the boundaries of DCAs may be constructs that partly depend on the normal behaviour of the queen during her mating flight, which may in turn depend on the predominant wind direction modified by landscape. In other words, it could be said that DCAs exist as shown in the DCA maps found as a result of this research, but that there are no constant boundaries for drones in DCAs that drones would not cross if a queen crossed them. But there may be areas or landscape features towards which queens would not fly, thereby defining the boundary.

This study has also shown that queens and other drones are not attracted to young drones but was unable to show if this was the case for sexually mature drones.

Further areas of research in two aspects of DCA formation has also been recommended following from this research:

- A further investigation into whether virgin queens are attracted to sexually mature drones to confirm the recent research carried out by Bastin et al. (2017);
- If the attraction is confirmed, an investigation into the compound responsible; and importantly,
- An investigation into virgin queen nuptial flight behaviour without which, we are looking at only one side of the equation.

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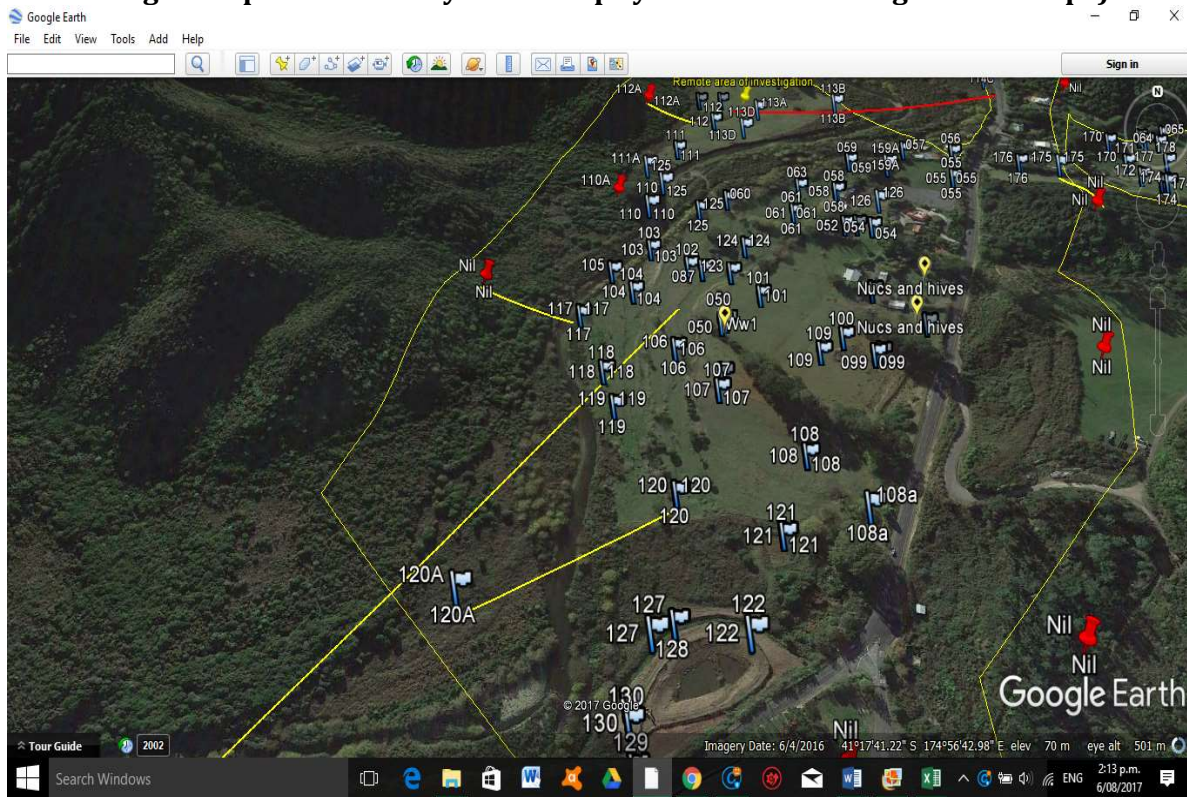
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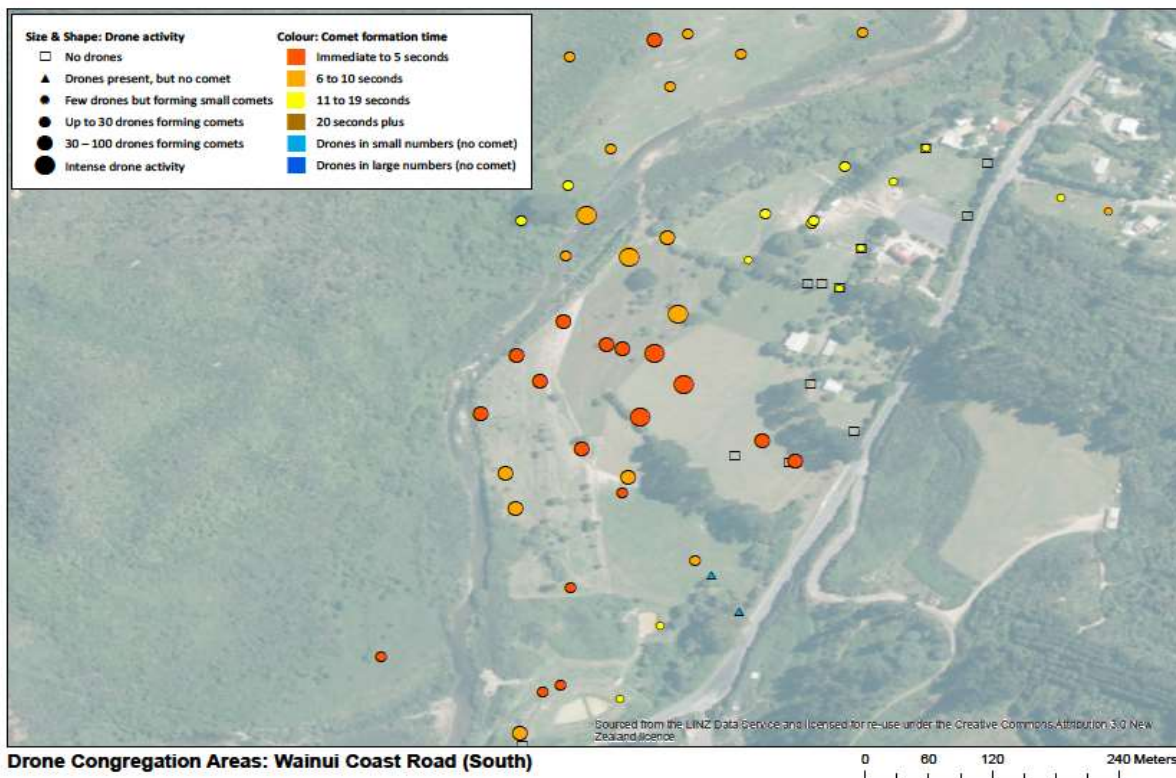
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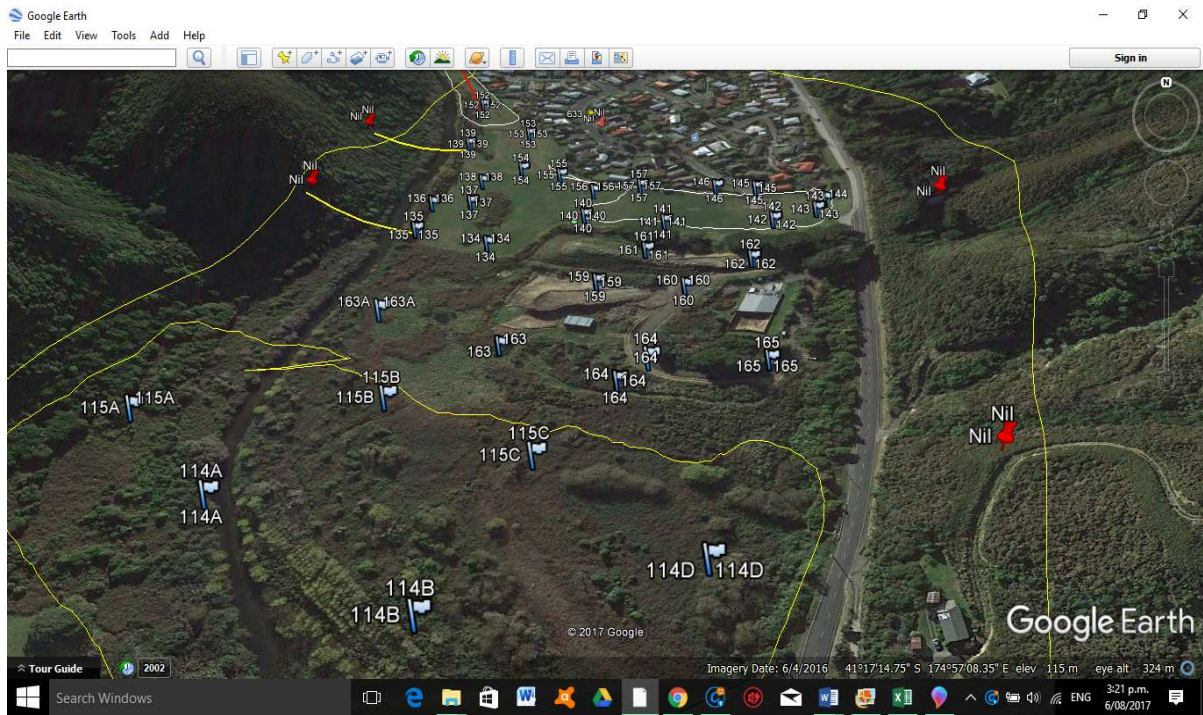
Appendix 1. Maps 1 – 45 of Drone Congregation Areas studies (Refer to Fig 13 to provide the key to the map symbols for the Google Earth maps).



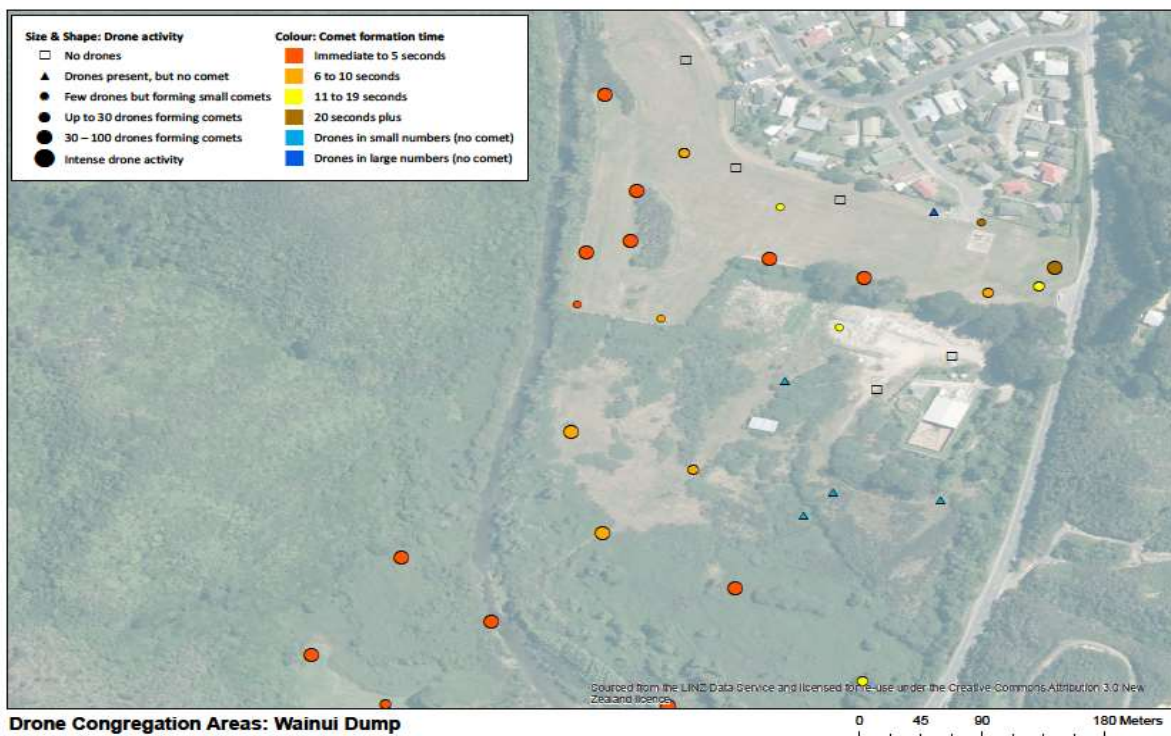
Map 1. Wainuiomata Central area surrounding the apiary.



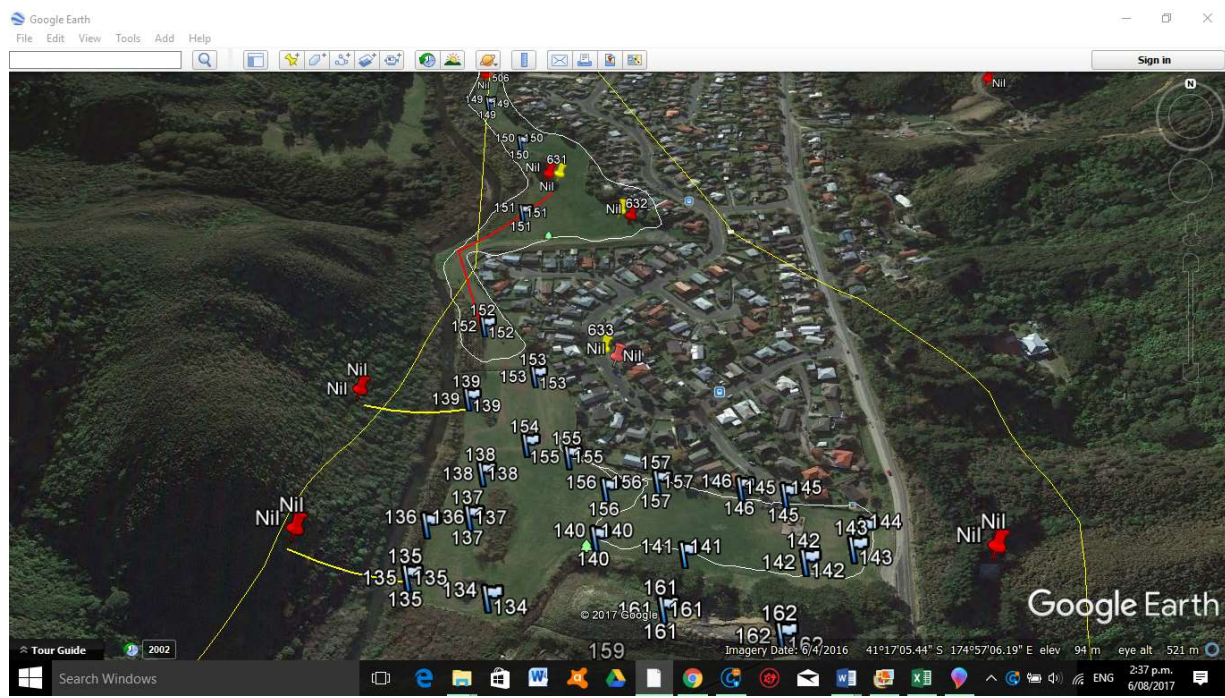
Map 2. Drone Activity map of the same area as Map 1



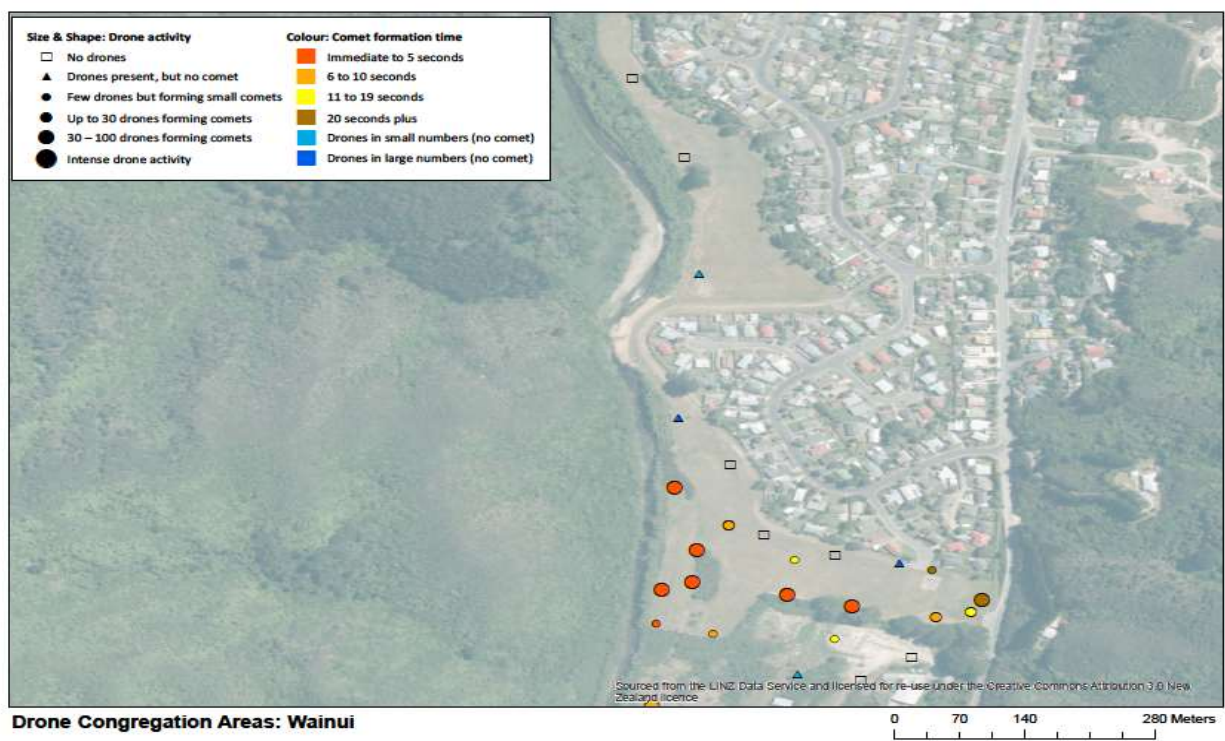
Map 3. Ngatura Park and Dump area Wainuiomata North of the Apiary area



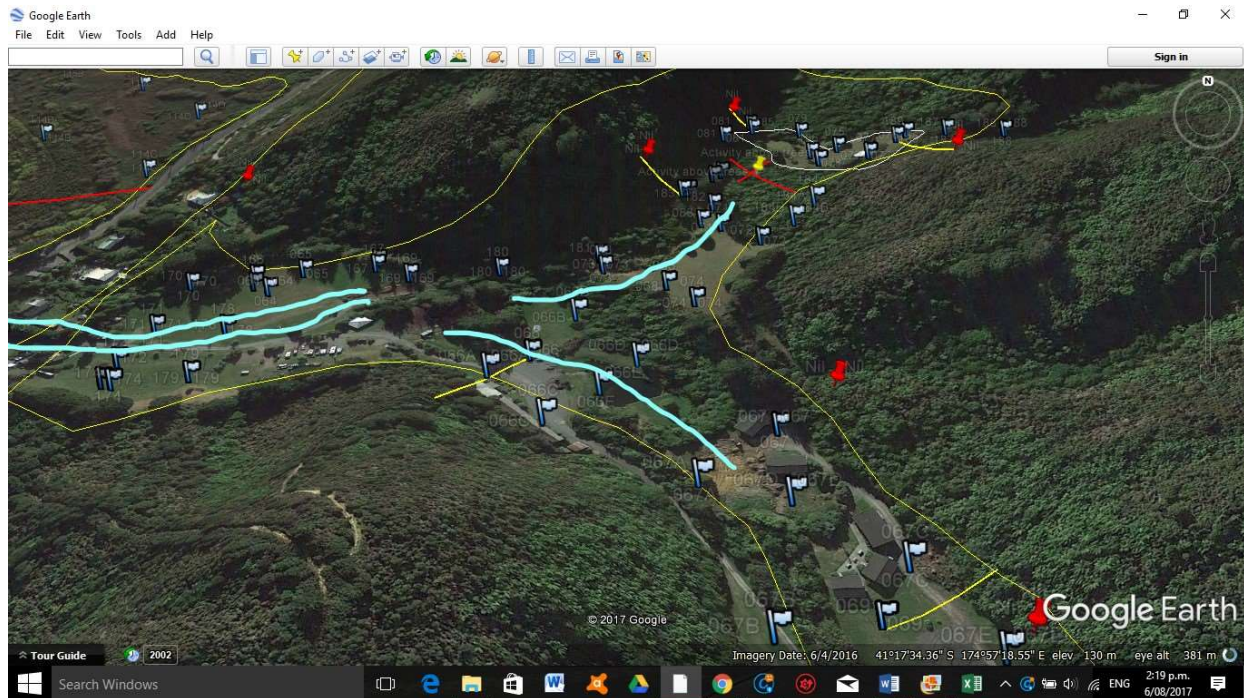
Map 4. Drone Activity Drone Activity map of the same area as Map 3



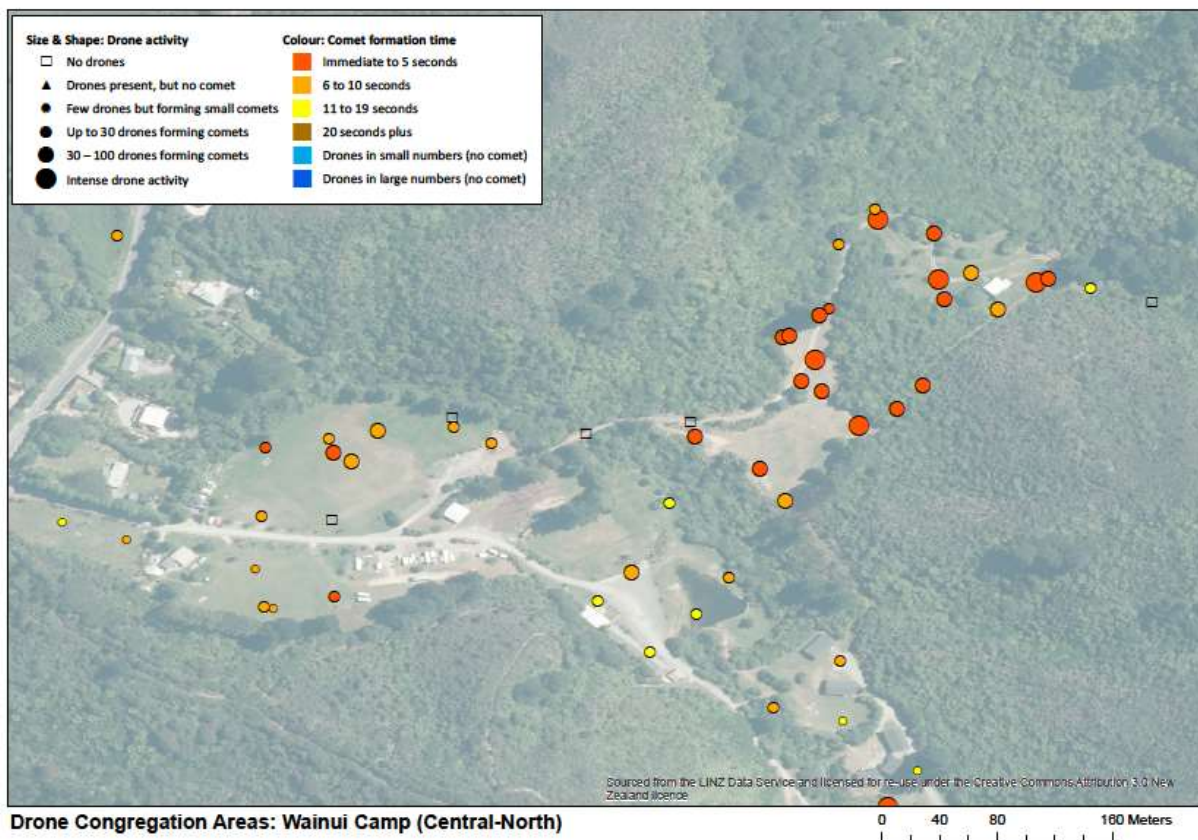
Map 5. The area of Ngatura Park Wainuiomata and North into the built up area of the town showing a diminution of drone activity



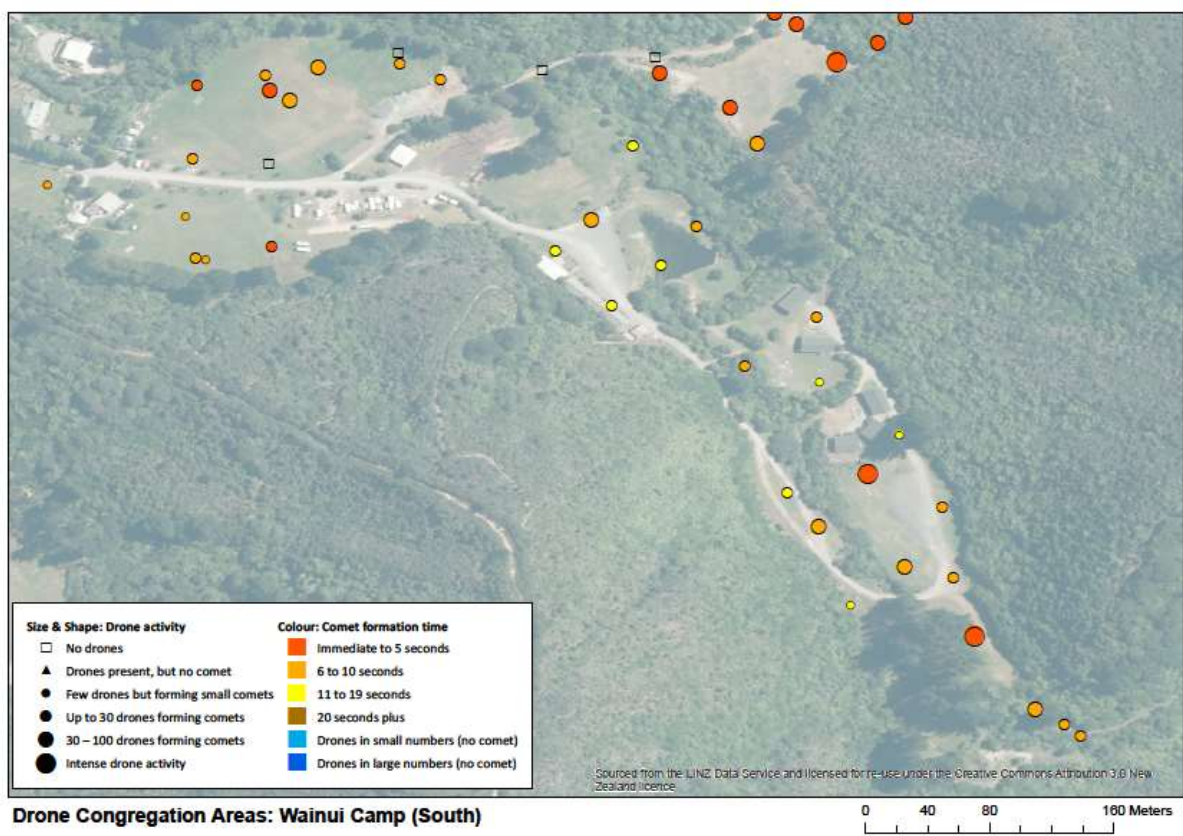
Map 6. Drone Activity map of the same area as Map 5



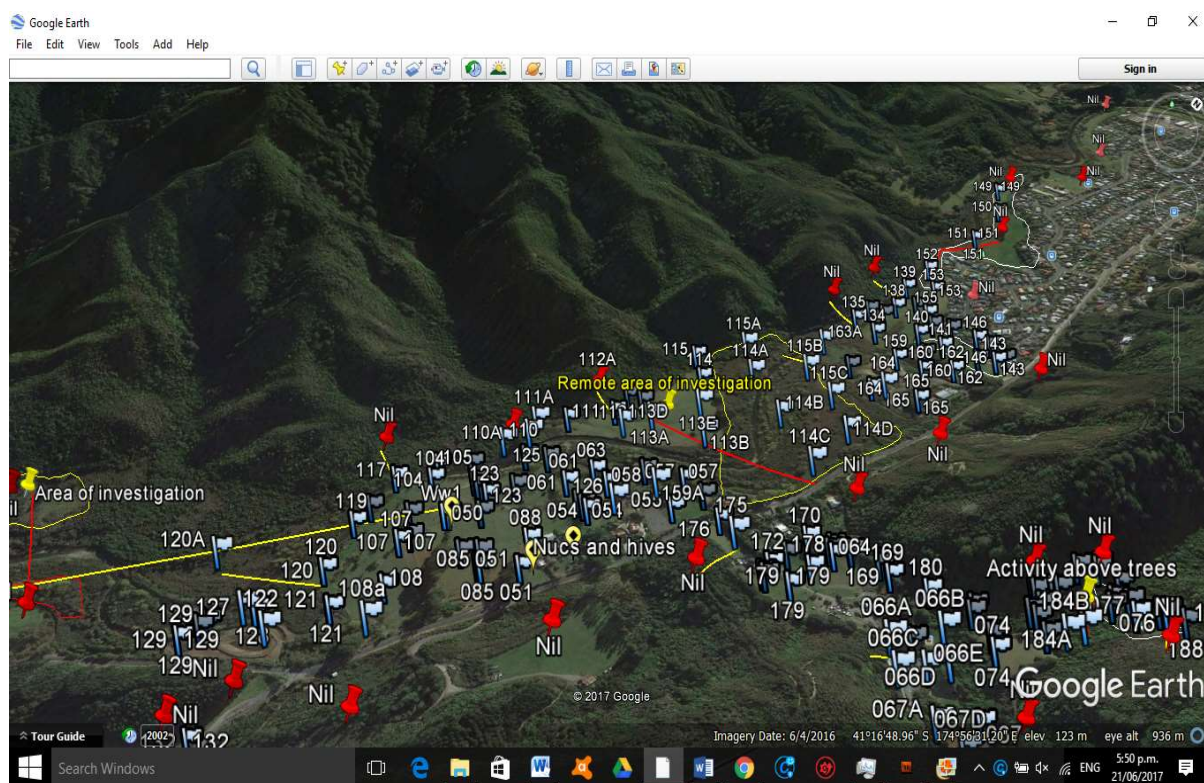
Map 7. Wainuiomata Camp Area North. The blue lines on the picture show areas where drones were flying but appeared to be not interested in the queen or only fleetingly so. These were adjacent to areas where drones did attend the queen and form comets and were possibly flyways between the camp area and the apiary at Wainuiomata (See Map 15).



Map 8. Wainuiomata Camp North. Drone Activity map of the same area as Map 7

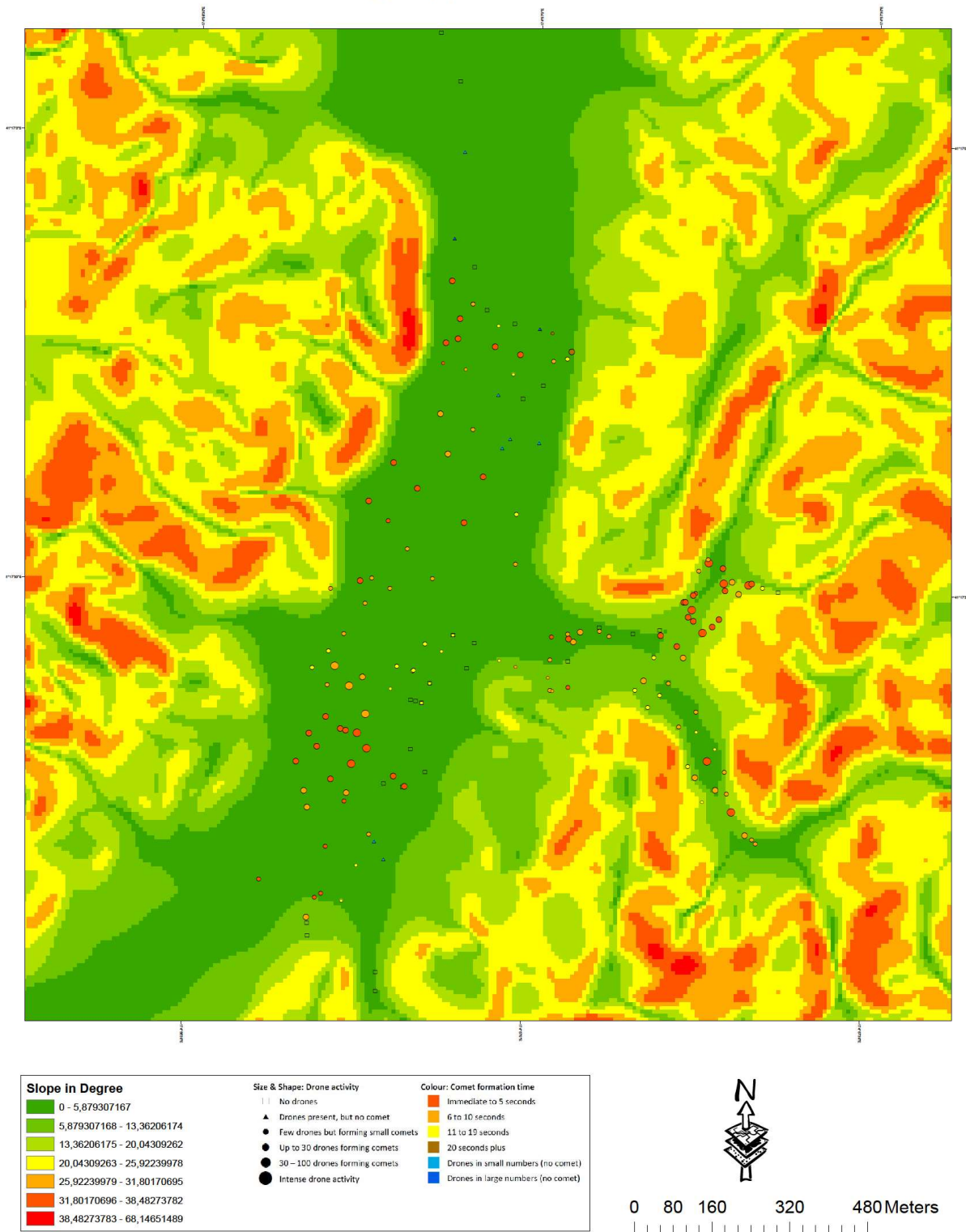


Map 9. Wainuiomata Camp South drone activity extending to the South East Corner map.



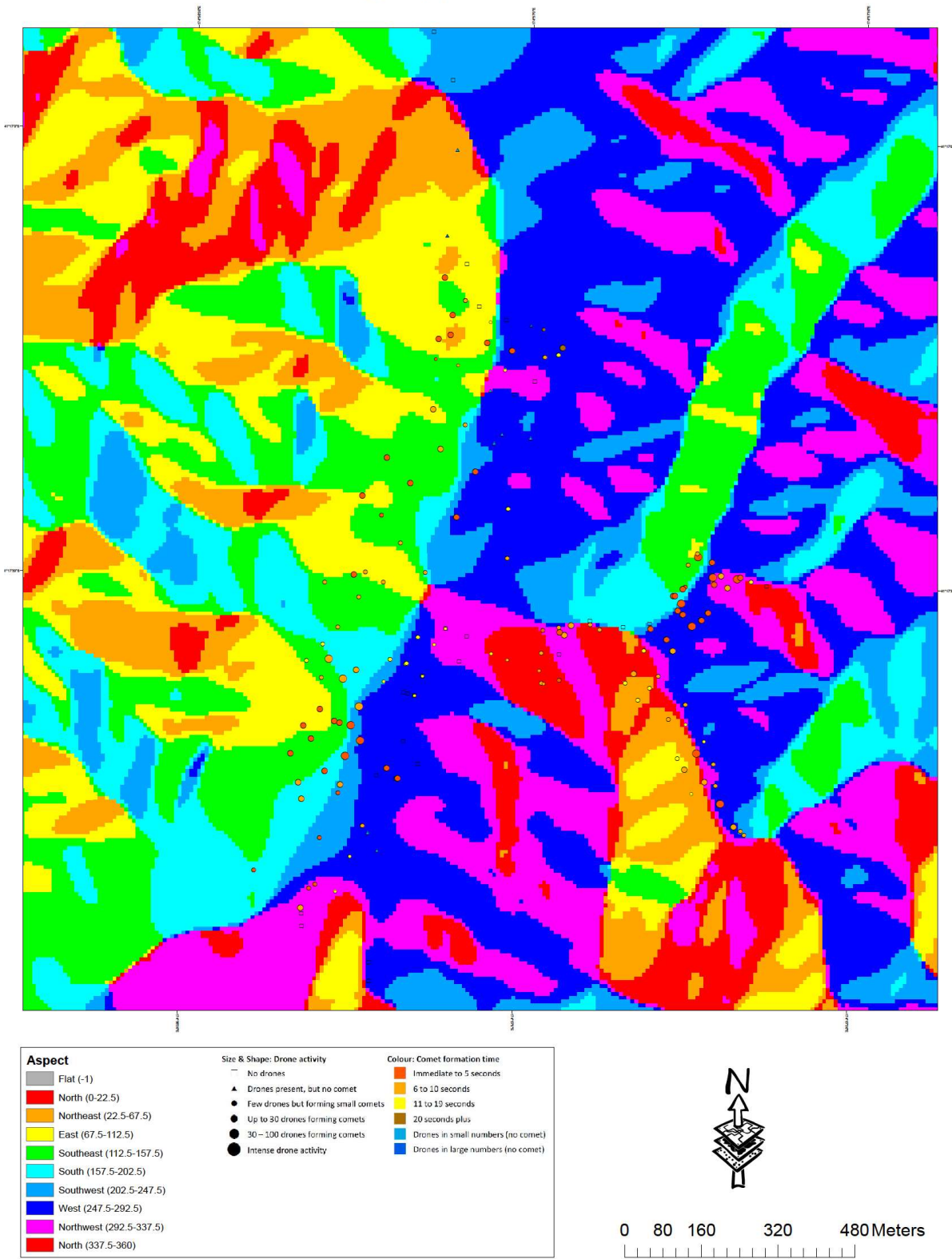
Map 10. Full Wainuiomata area, dump area, Ngatura Park and Wainuiomata Camp area.

Drone Congregation Area: Wainui

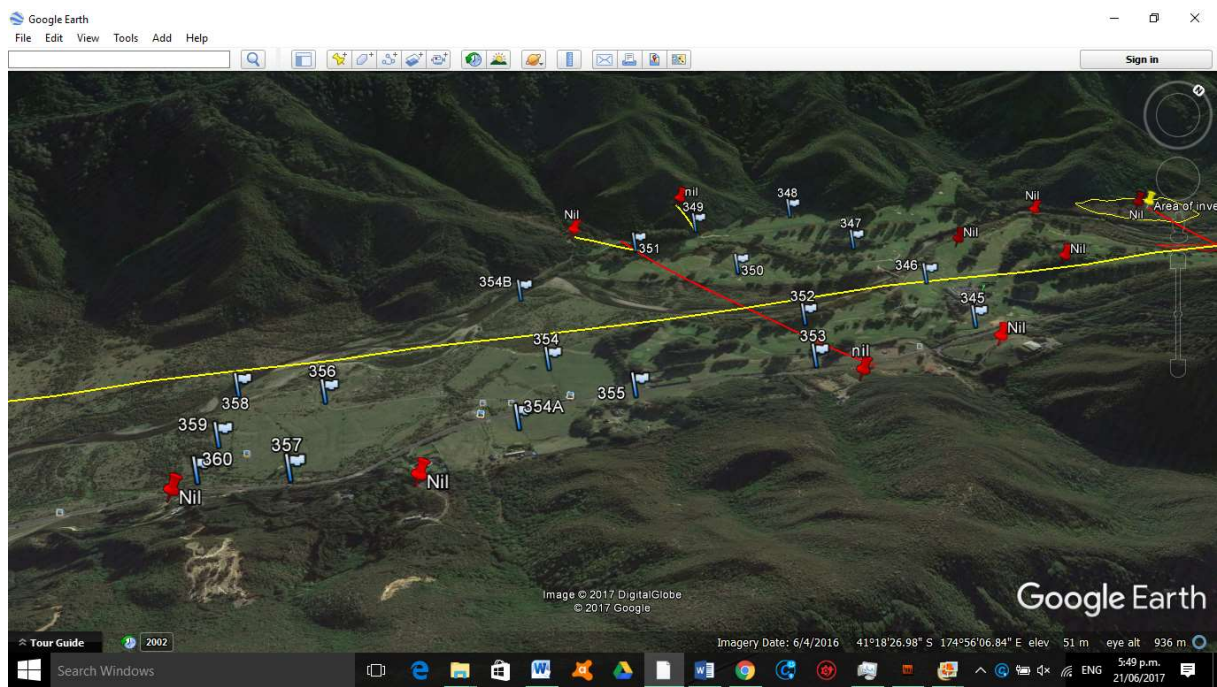


Map 11. Covering the same area as Map 10 with slope overlay

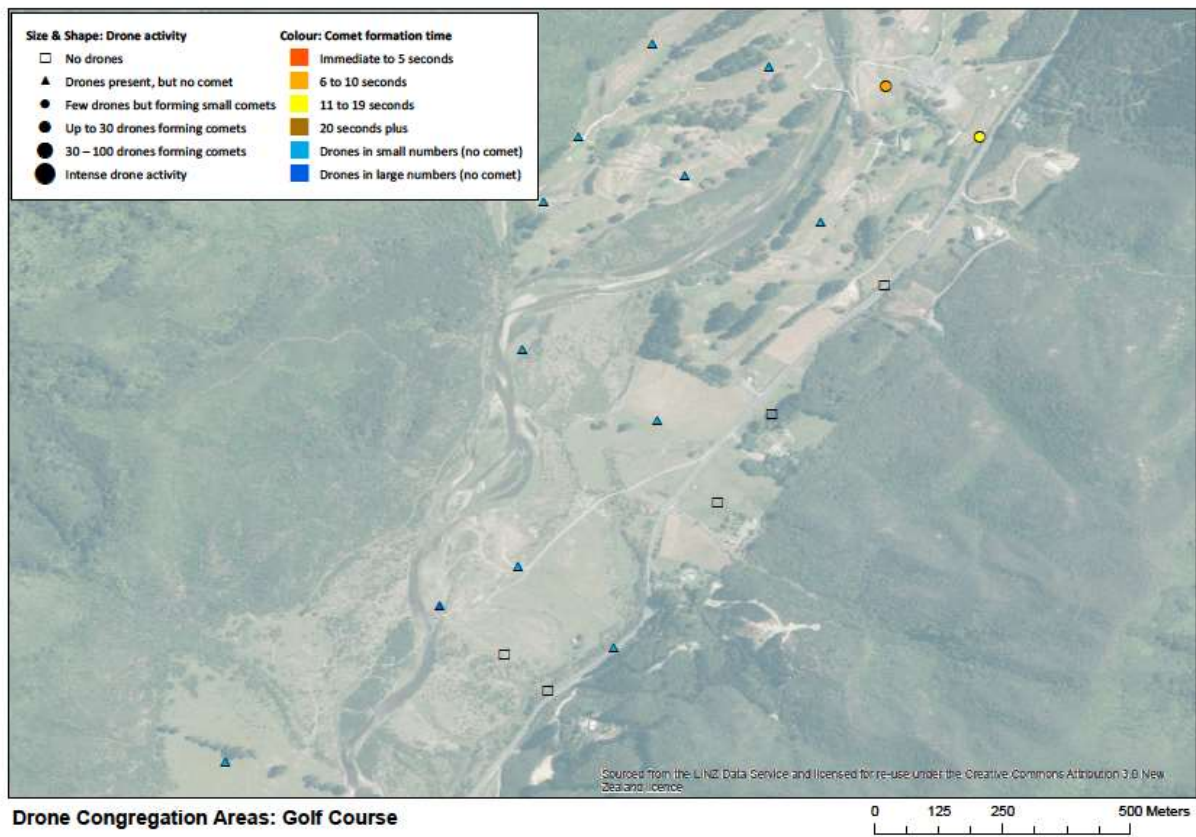
Drone Congregation Area: Wainui



Map 12. Covering the same area as Maps 10 and 11 with aspect overlay

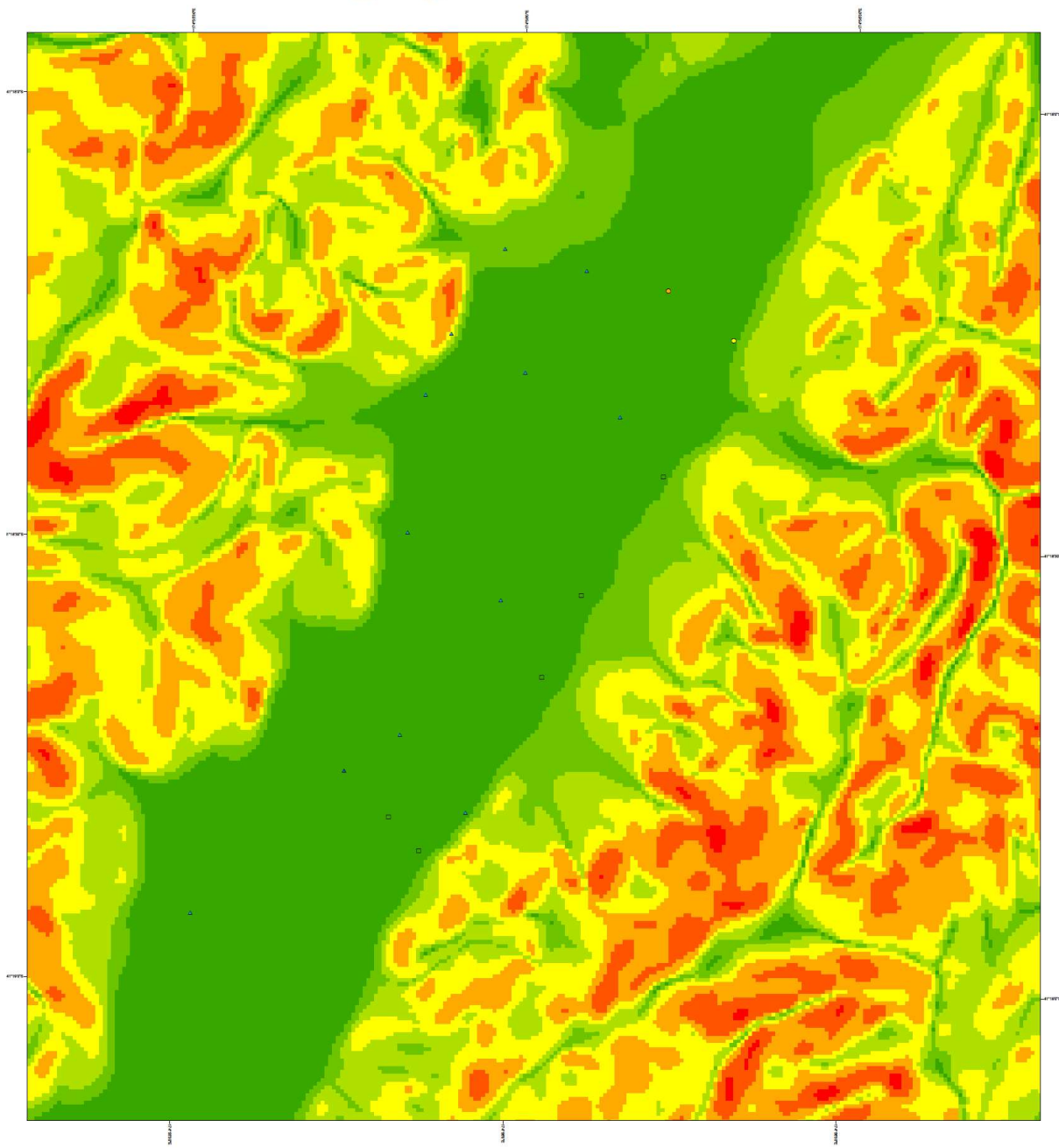


Map 13. Golf Course Wainuiomata – South of the apiary area



Map 14. Limited drone activity at the Golf Course area Wainuiomata

Drone Congregation Area: Golf Course

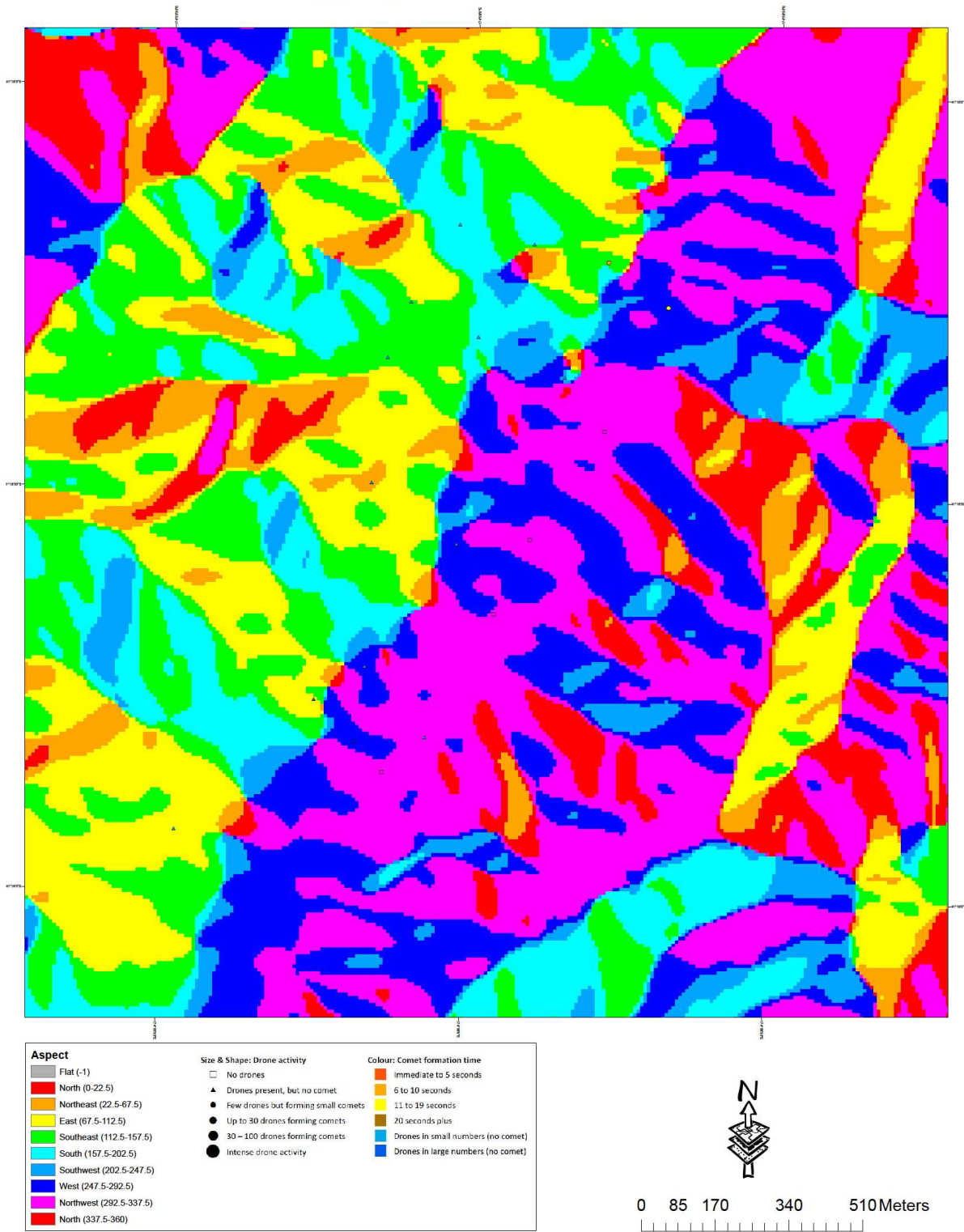


Slope in Degree	Size & Shape: Drone activity	Colour: Comet formation time
0 - 5,879307167	□ No drones	■ Immediate to 5 seconds
5,879307168 - 13,36206174	▲ Drones present, but no comet	■ 6 to 10 seconds
13,36206175 - 20,04309262	● Few drones but forming small comets	■ 11 to 19 seconds
20,04309263 - 25,92239978	● Up to 30 drones forming comets	■ 20 seconds plus
25,92239979 - 31,80170695	● 30 - 100 drones forming comets	■ Drones in small numbers (no comet)
31,80170696 - 38,48273782	● Intense drone activity	■ Drones in large numbers (no comet)
38,48273783 - 68,14651489		

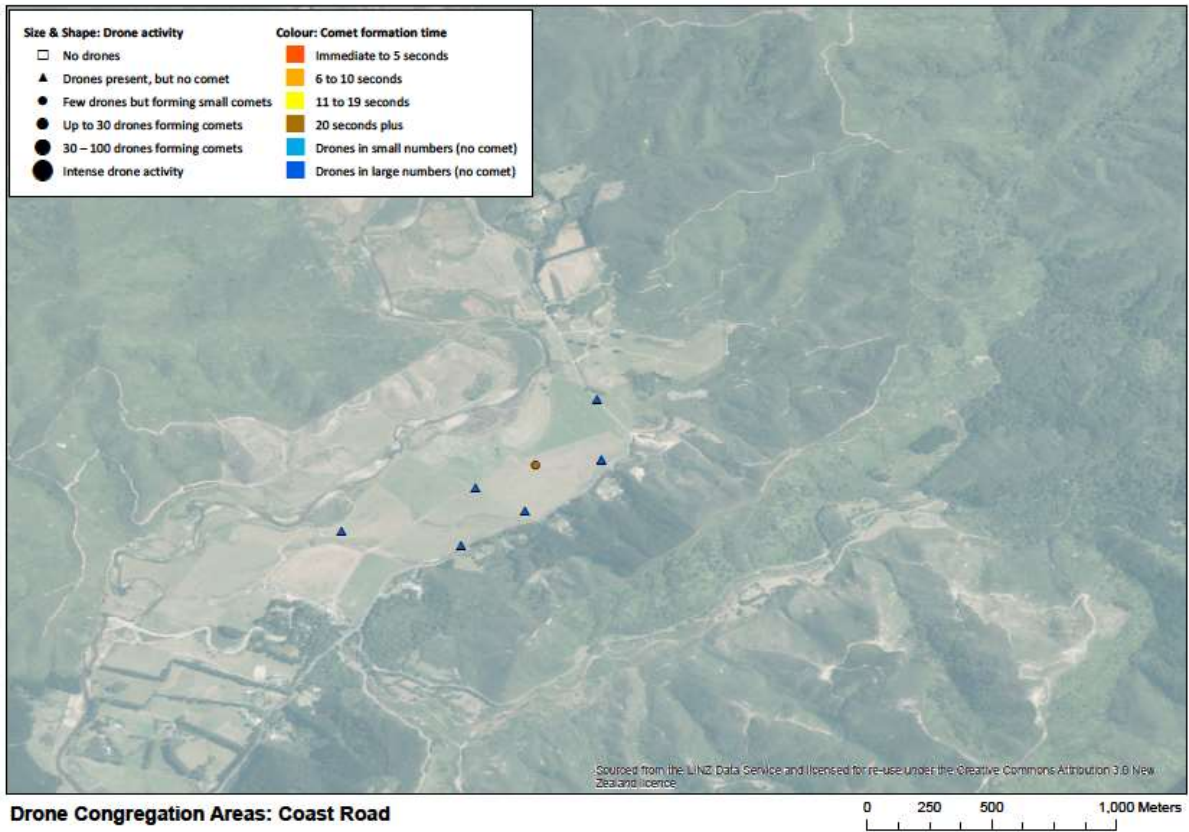


Map 15. Golf Course area with slope overlay

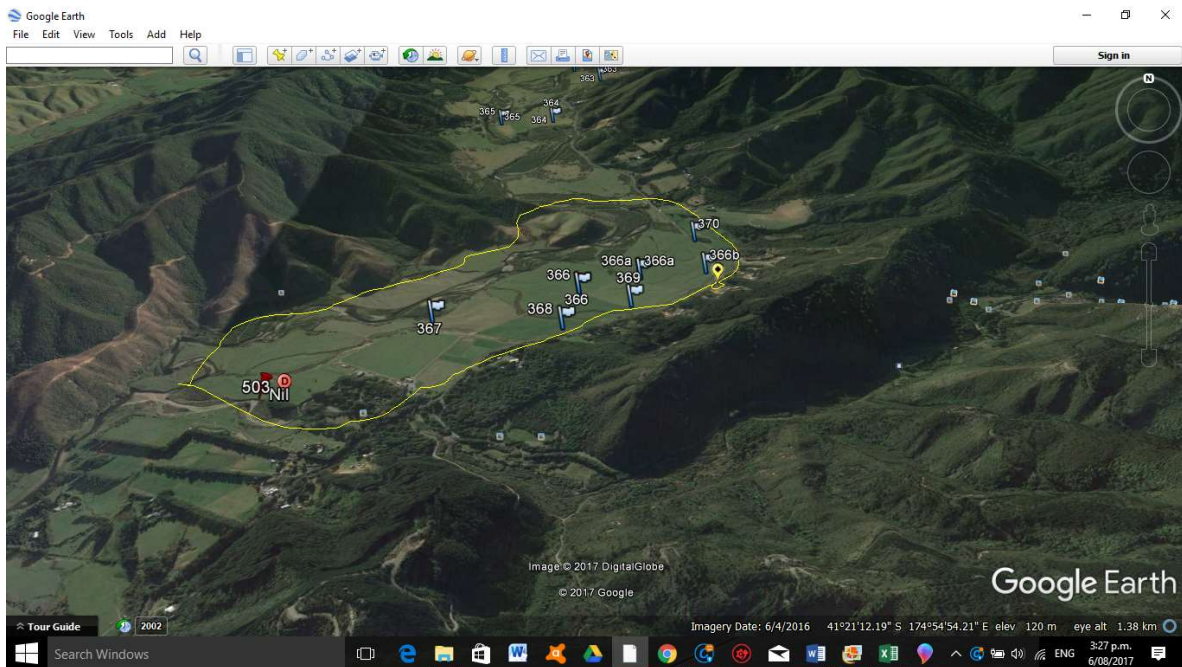
Drone Congregation Area: Golf Course



Map 16. Golf Course area with aspect overlay

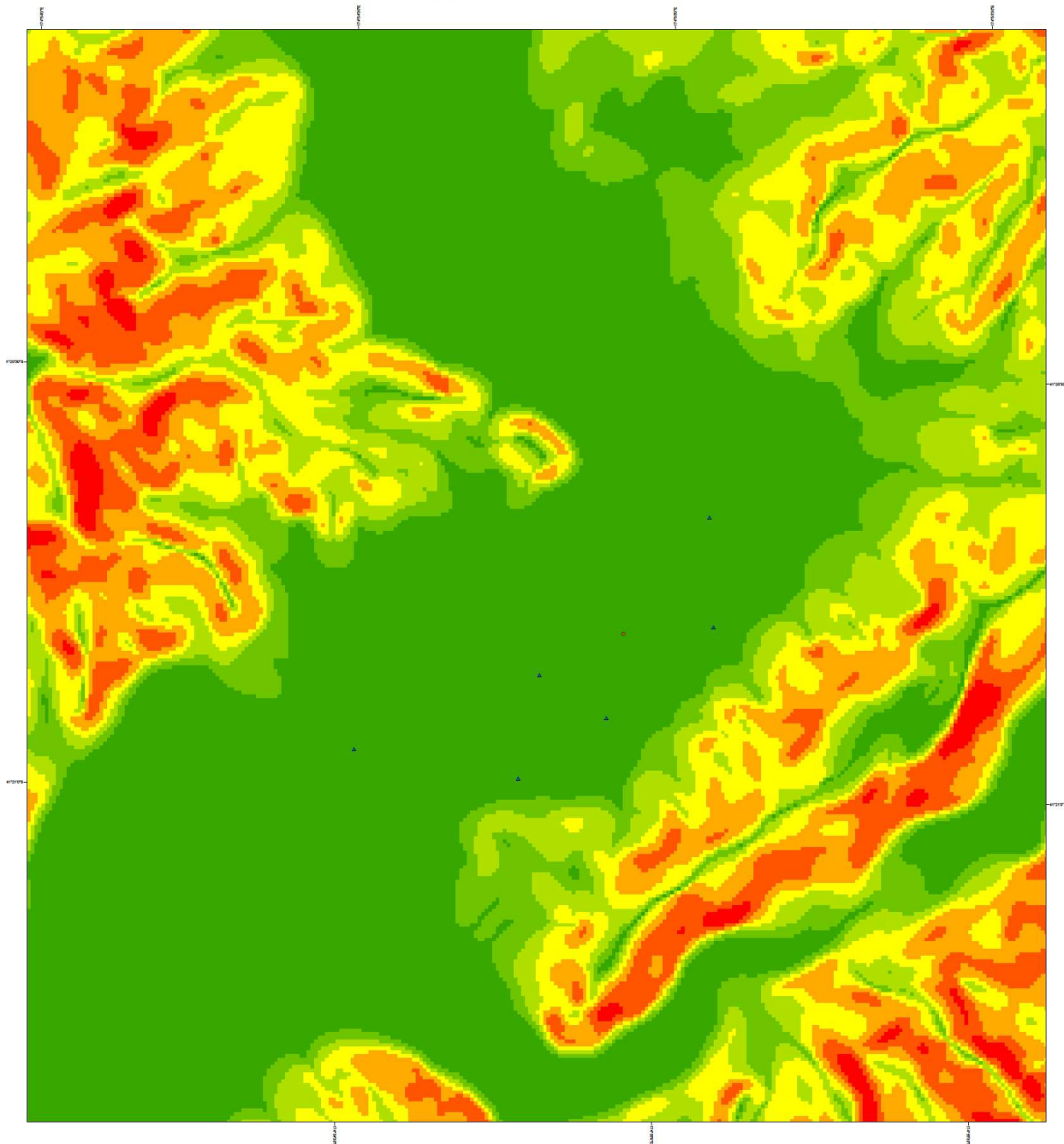


Map 17. Coast Road South area

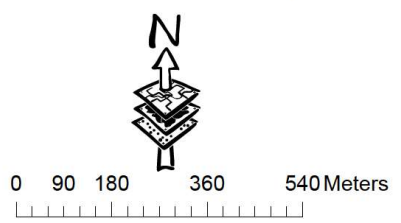


Map 18. Drone Activity Coast Road South area showing very limited drone activity

Drone Congregation Area: Coast Road

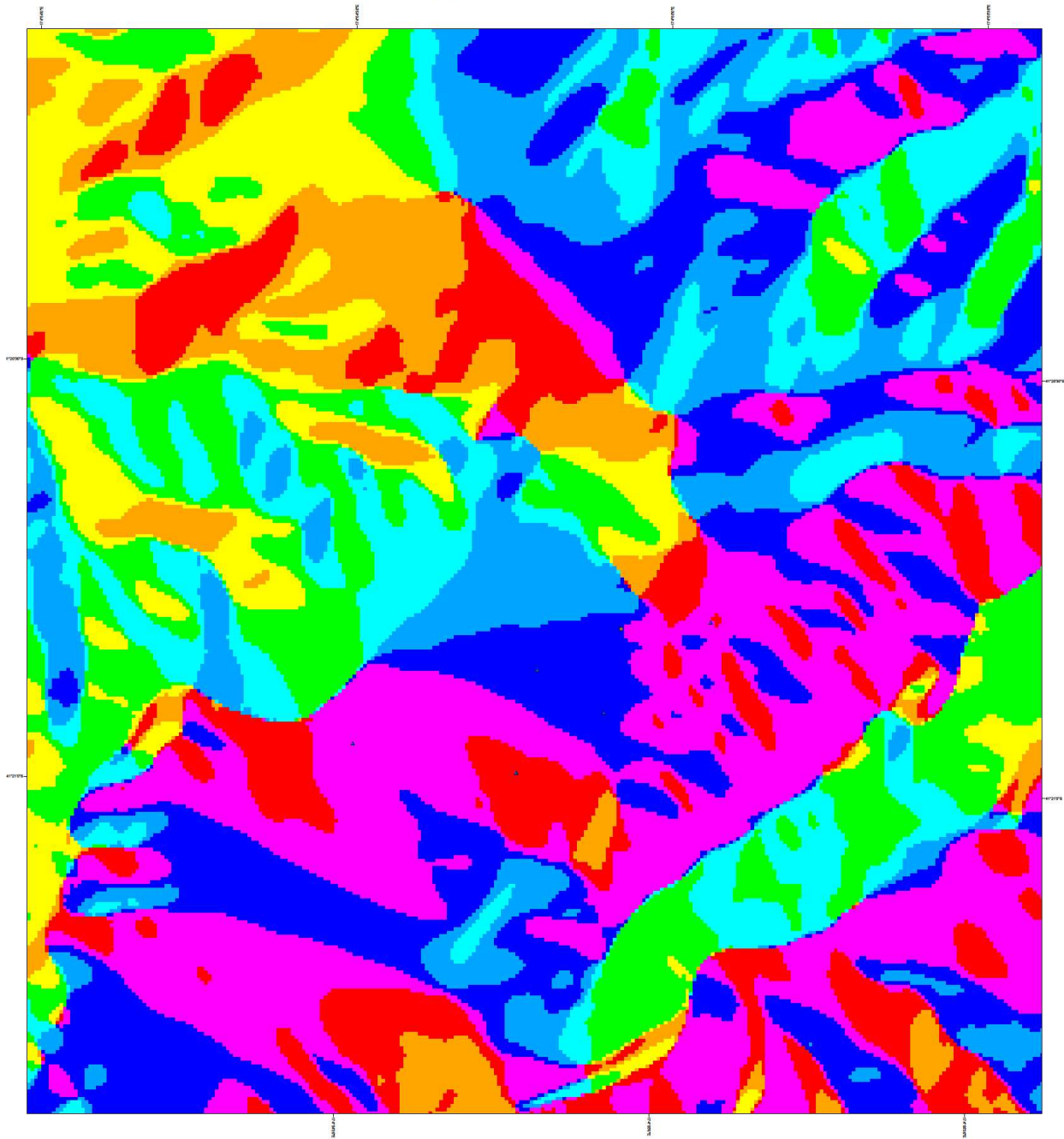


Slope in Degree	Size & Shape: Drone activity	Colour: Comet formation time
0 - 5,879307167	□ No drones	■ Immediate to 5 seconds
5,879307168 - 13,36206174	▲ Drones present, but no comet	■ 6 to 10 seconds
13,36206175 - 20,04309262	● Few drones but forming small comets	■ 11 to 19 seconds
20,04309263 - 25,92239978	● Up to 30 drones forming comets	■ 20 seconds plus
25,92239979 - 31,80170695	● 30 - 100 drones forming comets	■ Drones in small numbers (no comet)
31,80170696 - 38,48273782	● Intense drone activity	■ Drones in large numbers (no comet)
38,48273783 - 68,14651489		

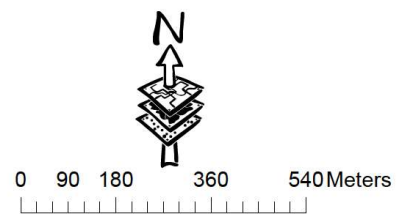


Map 19. Coast Road South area with slope overlay

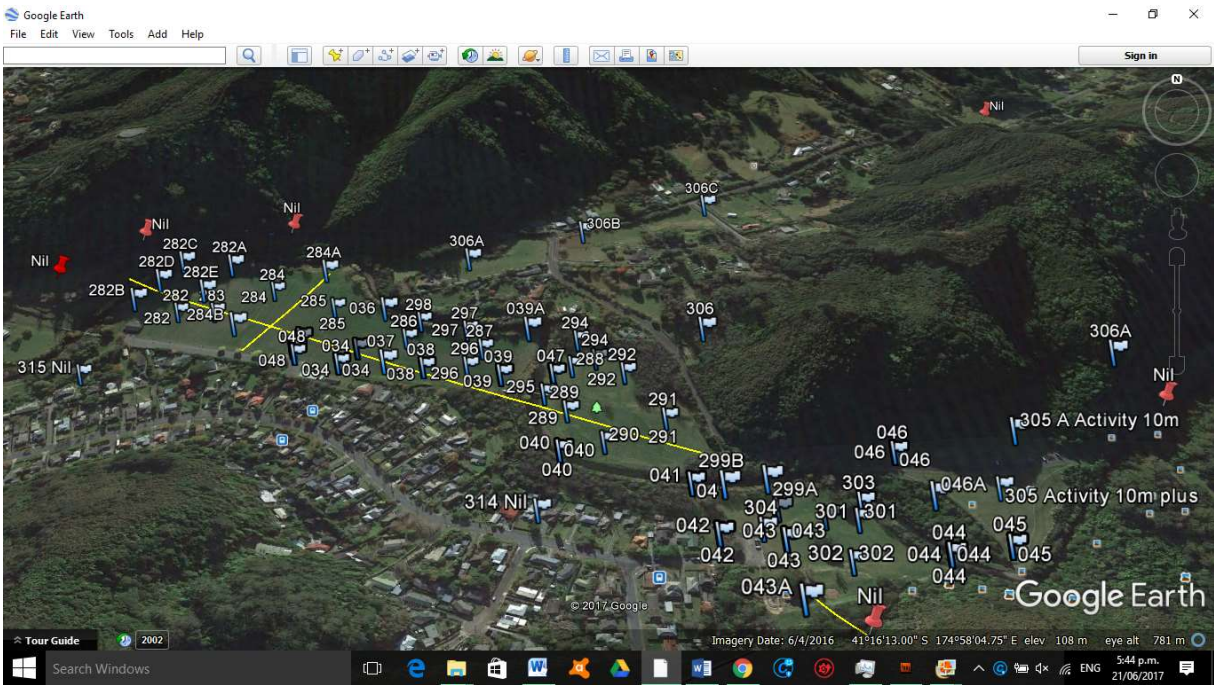
Drone Congregation Area: Coast Road



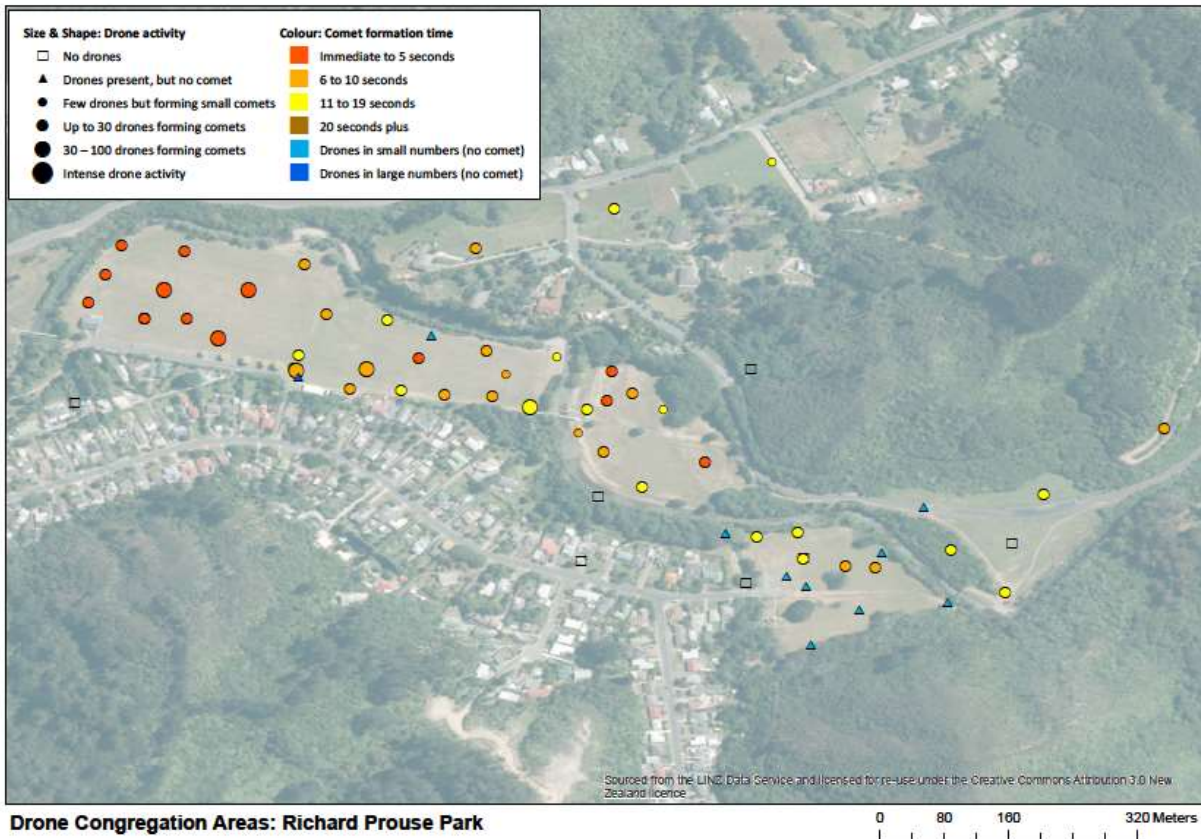
Aspect	Size & Shape: Drone activity	Colour: Comet formation time
Flat (-1)	□ No drones	Orange Immediate to 5 seconds
North (0-22.5)	▲ Drones present, but no comet	Light Orange 6 to 10 seconds
Northeast (22.5-67.5)	● Few drones but forming small comets	Yellow 11 to 19 seconds
East (67.5-112.5)	● Up to 30 drones forming comets	Light Green 20 seconds plus
Southeast (112.5-157.5)	● 30 - 100 drones forming comets	Light Blue Drones in small numbers (no comet)
South (157.5-202.5)	● Intense drone activity	Dark Blue Drones in large numbers (no comet)
Southwest (202.5-247.5)		
West (247.5-292.5)		
Northwest (292.5-337.5)		
North (337.5-360)		



Map 20. Coast Road South area with aspect overlay

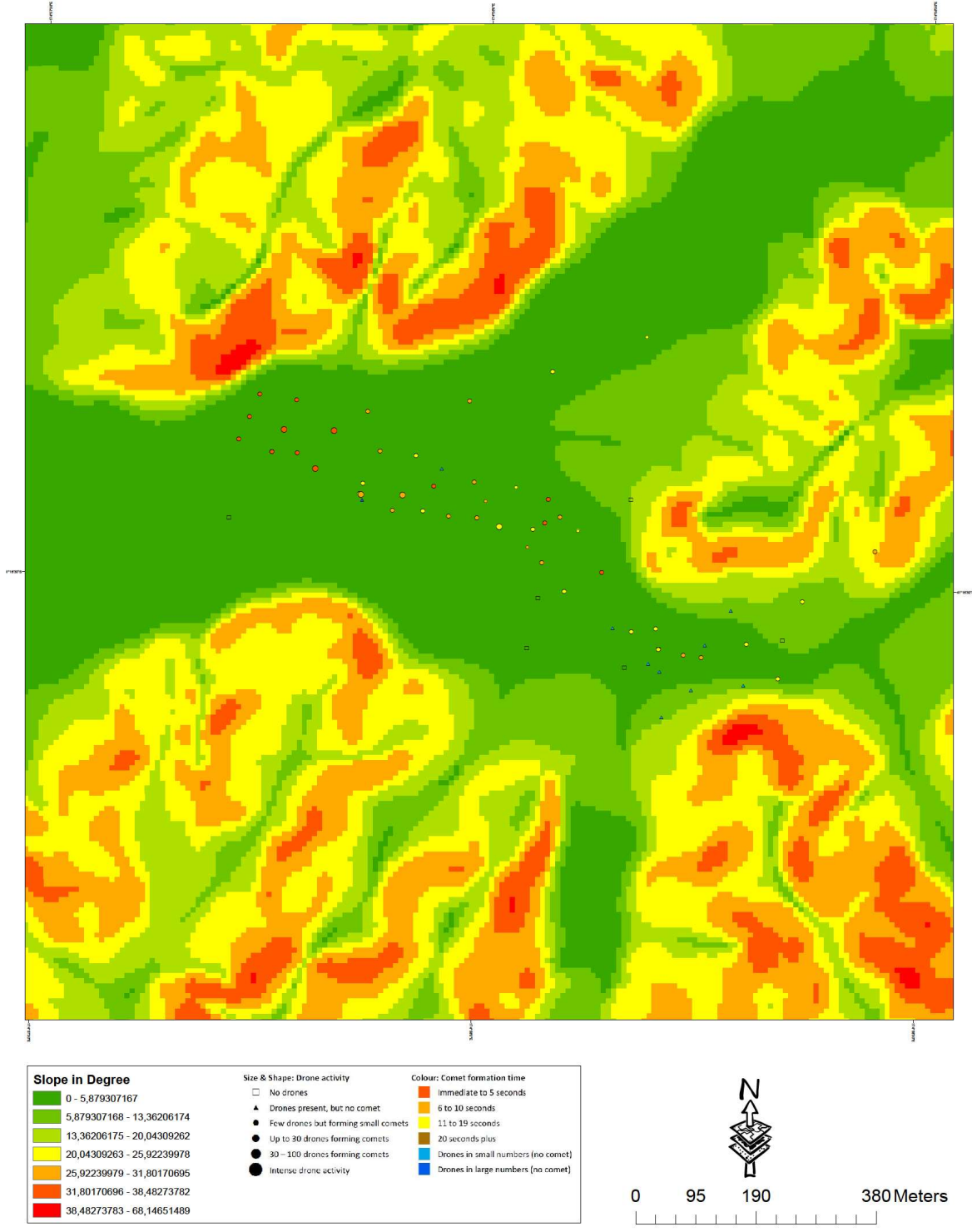


Map 21. Richard Prowse Park area of Wainuiomata



Map 22. Drone activity levels Richard Prowse Park Wainuiomata

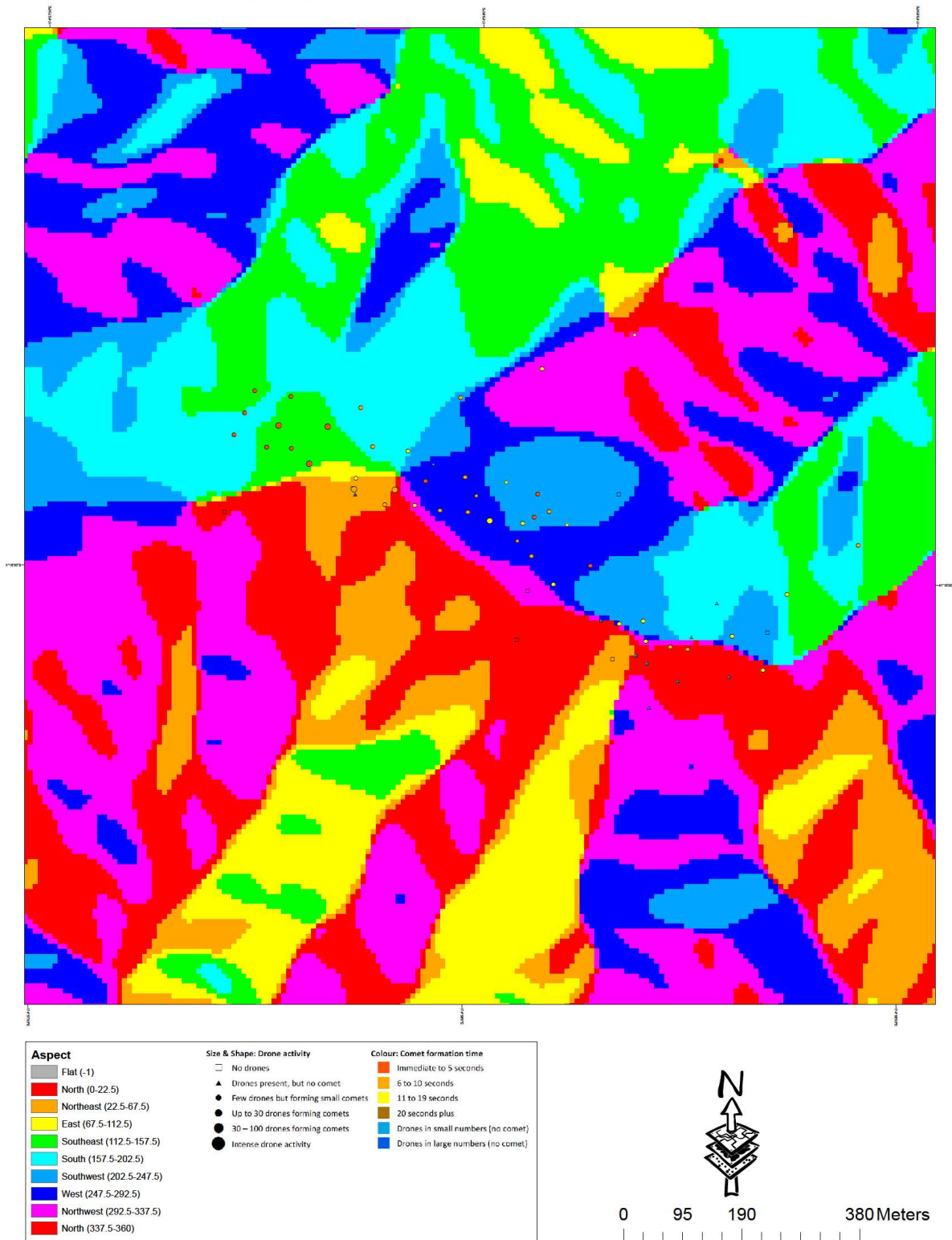
Drone Congregation Area: Richard Prouse Park



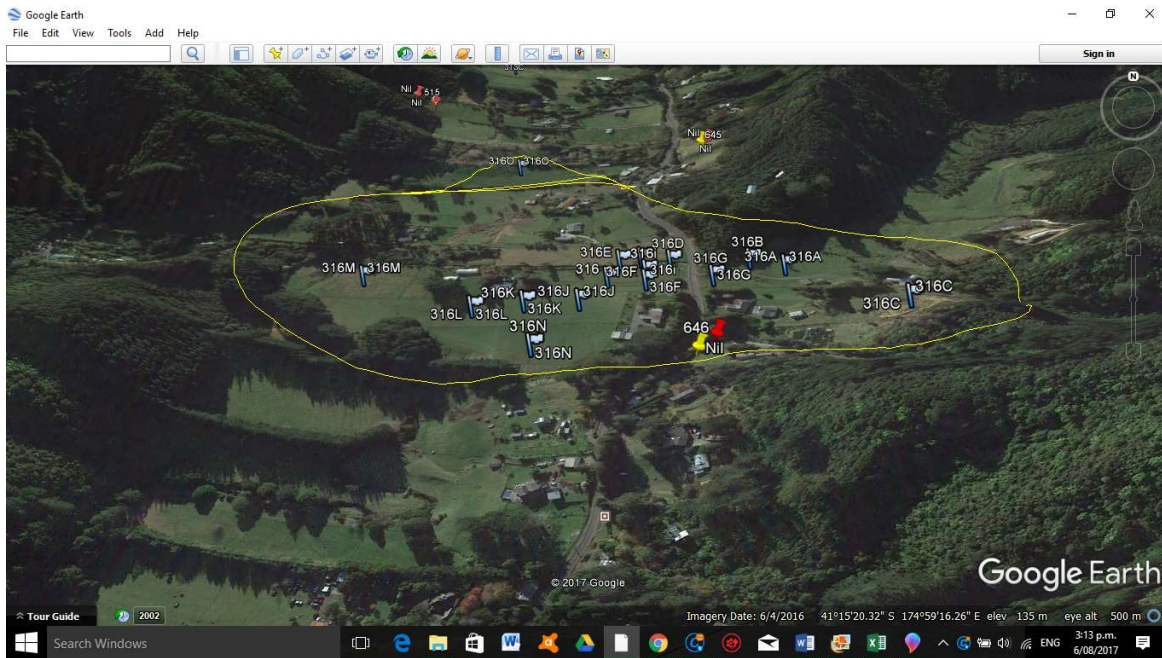
23. Richard Prouse Park area with slope overlay

Map

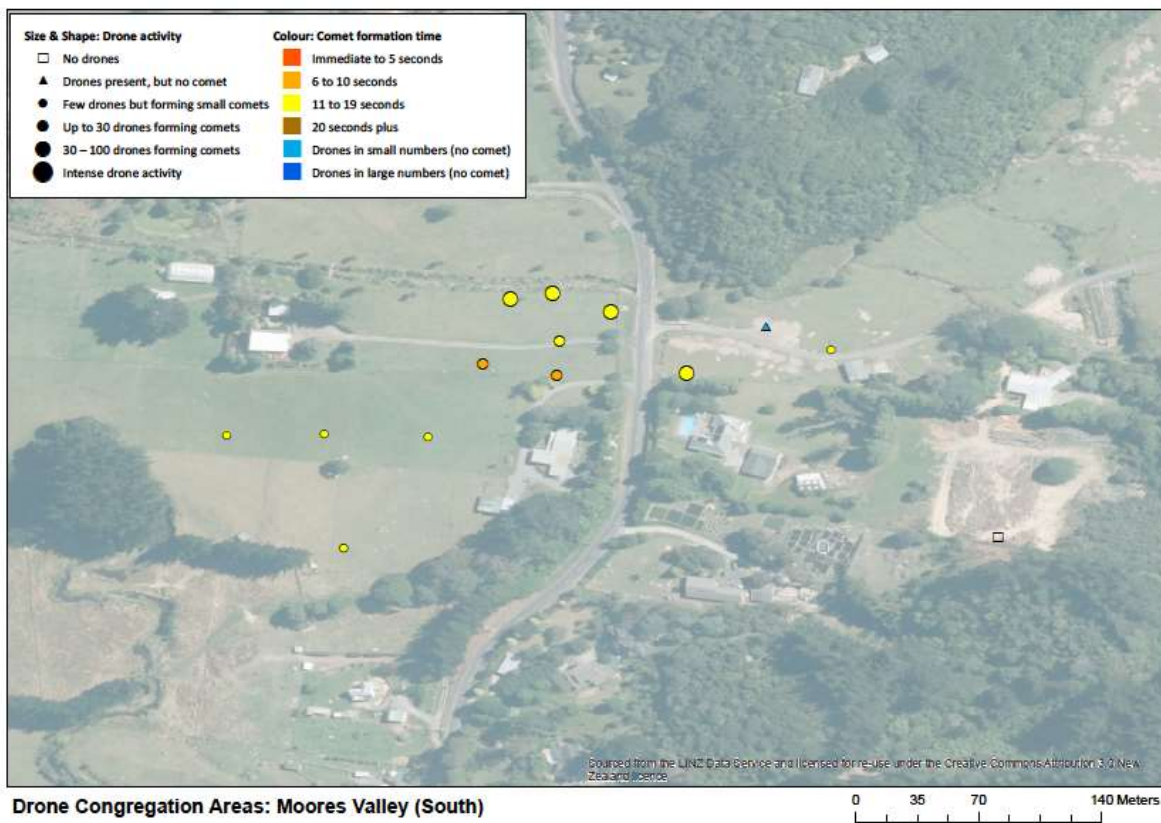
Drone Congregation Area: Richard Prowse Park



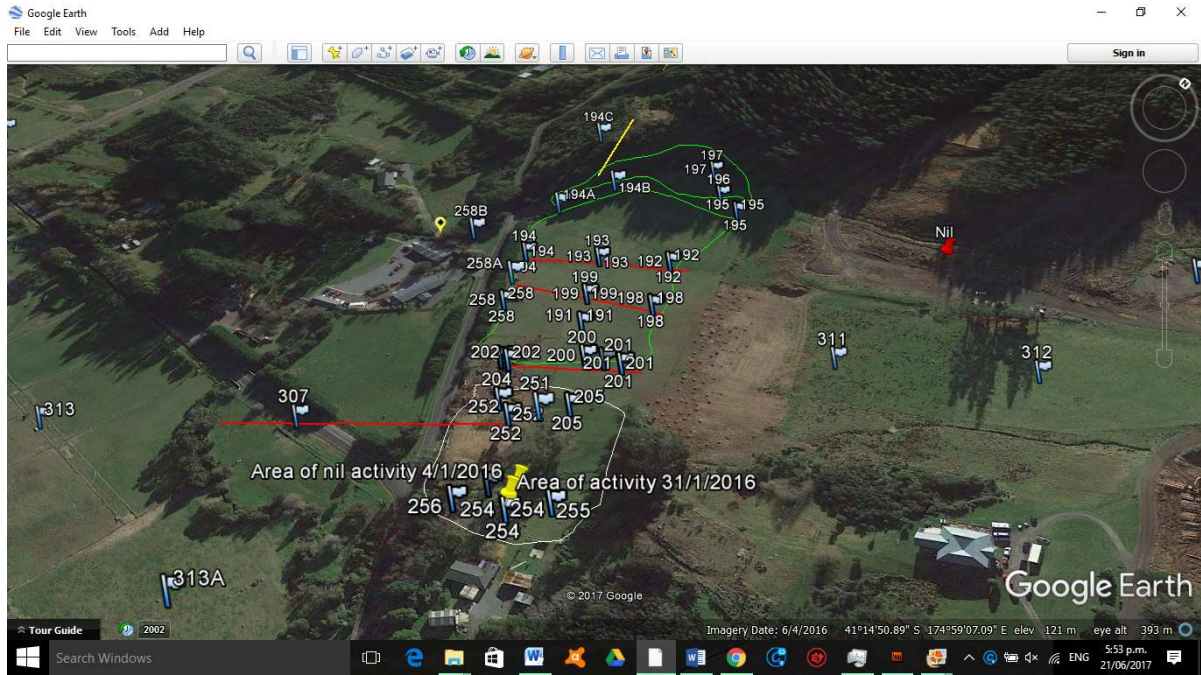
Map 24. Richard Prowse Park area with aspect overlay



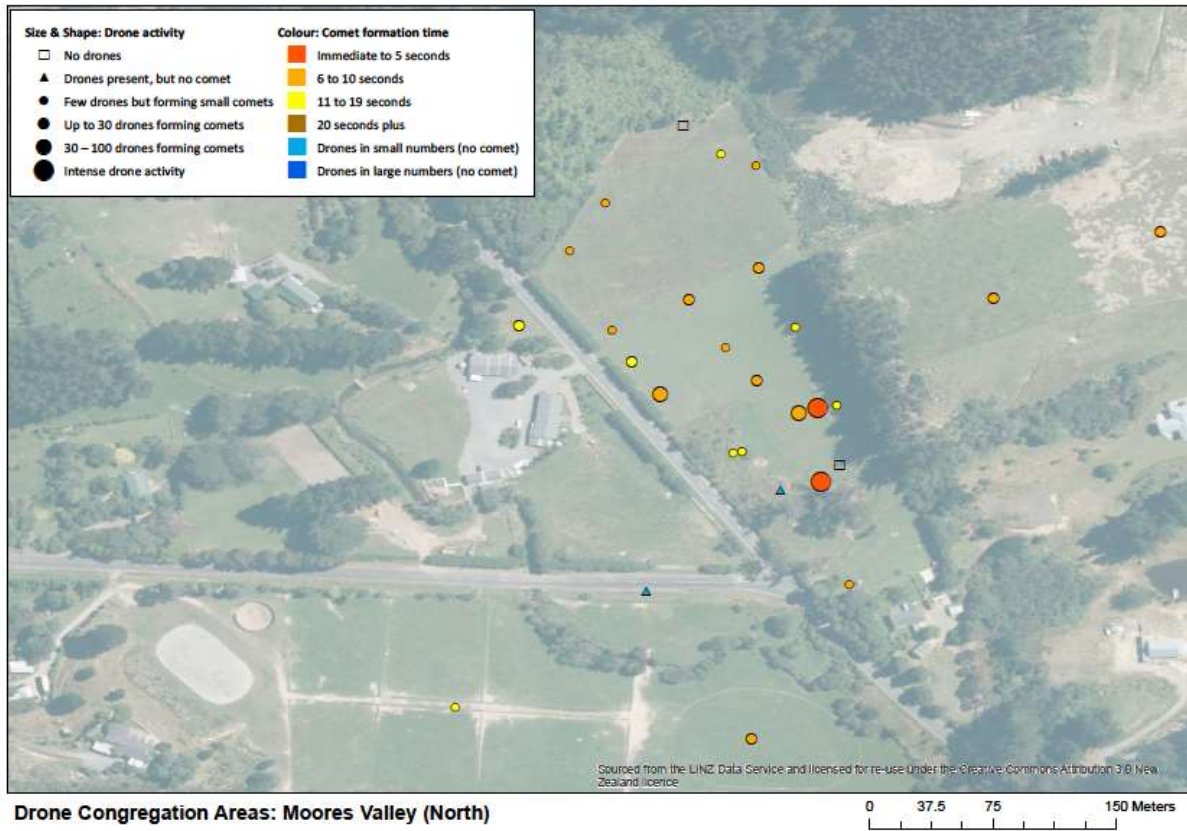
Map 25. Low intensity DCA at Moors Valley South



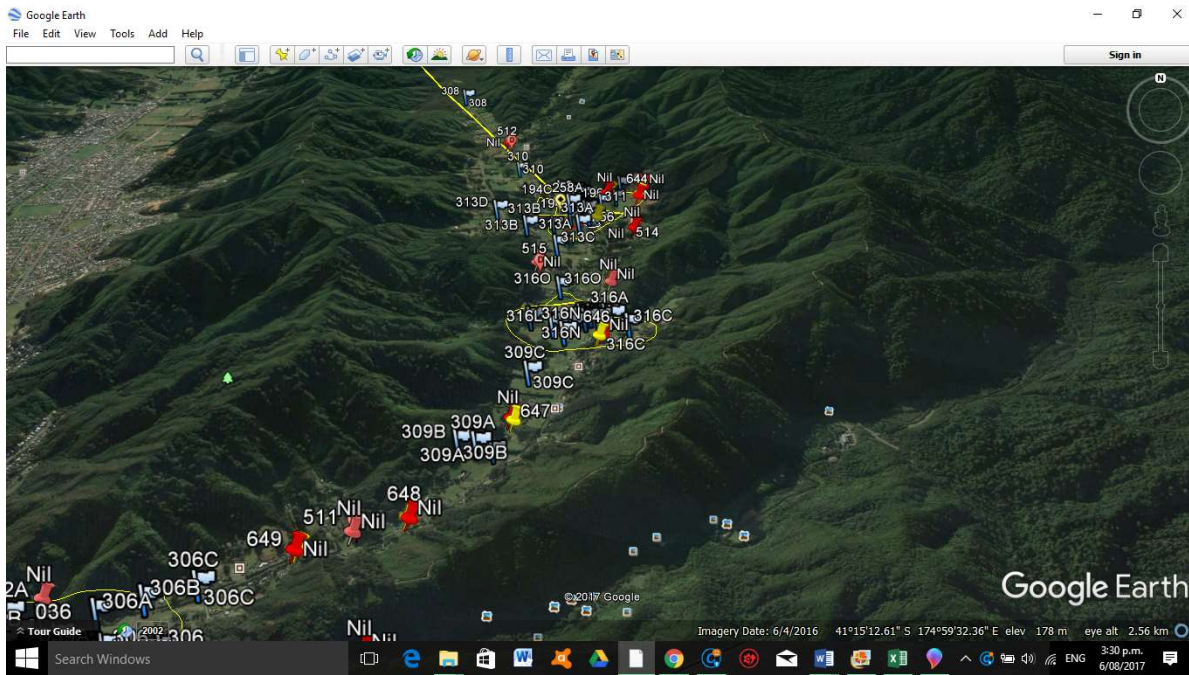
Map 26. Lower density drone Activity at a DCA in Moors Valley South



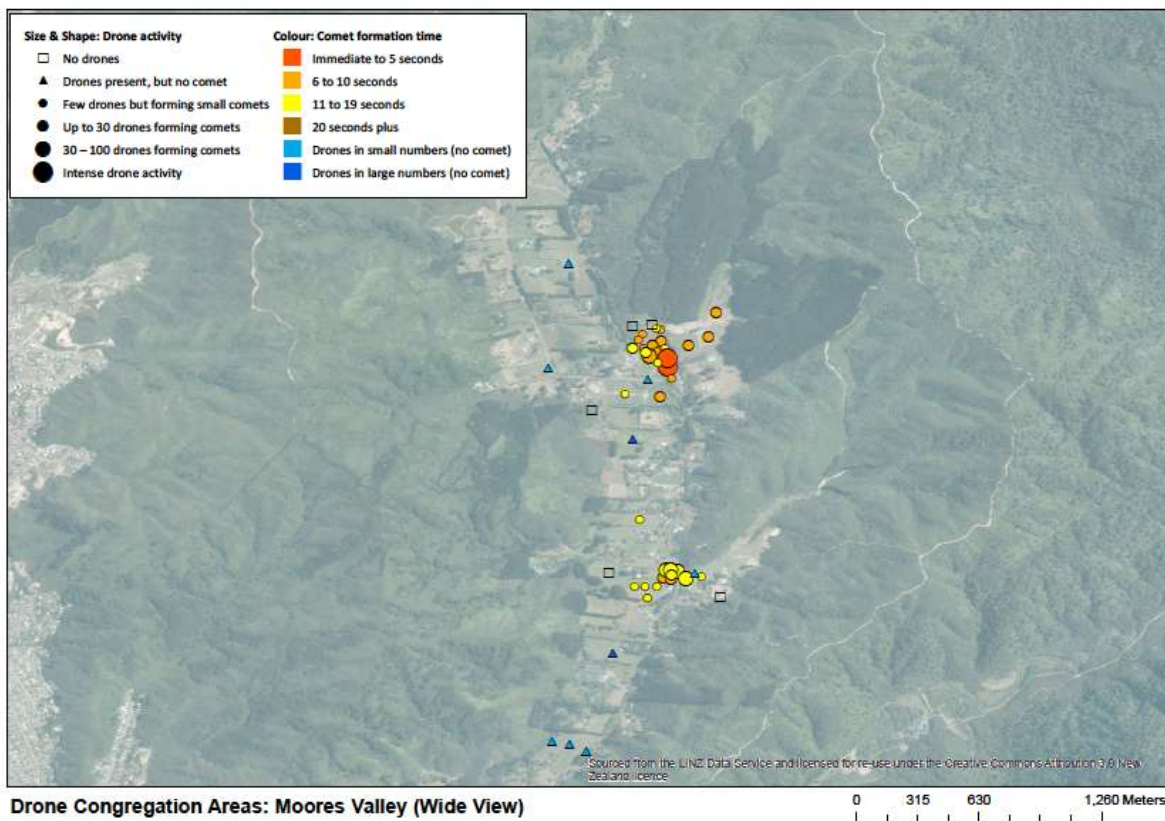
Map 27. DCA at Upper Moores Valley North detail. No apiaries in the vicinity for the first two years of testing.



Map 28. Drone activity at Moores Valley North

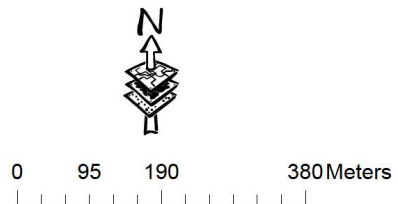
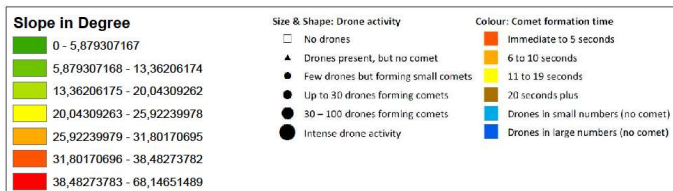
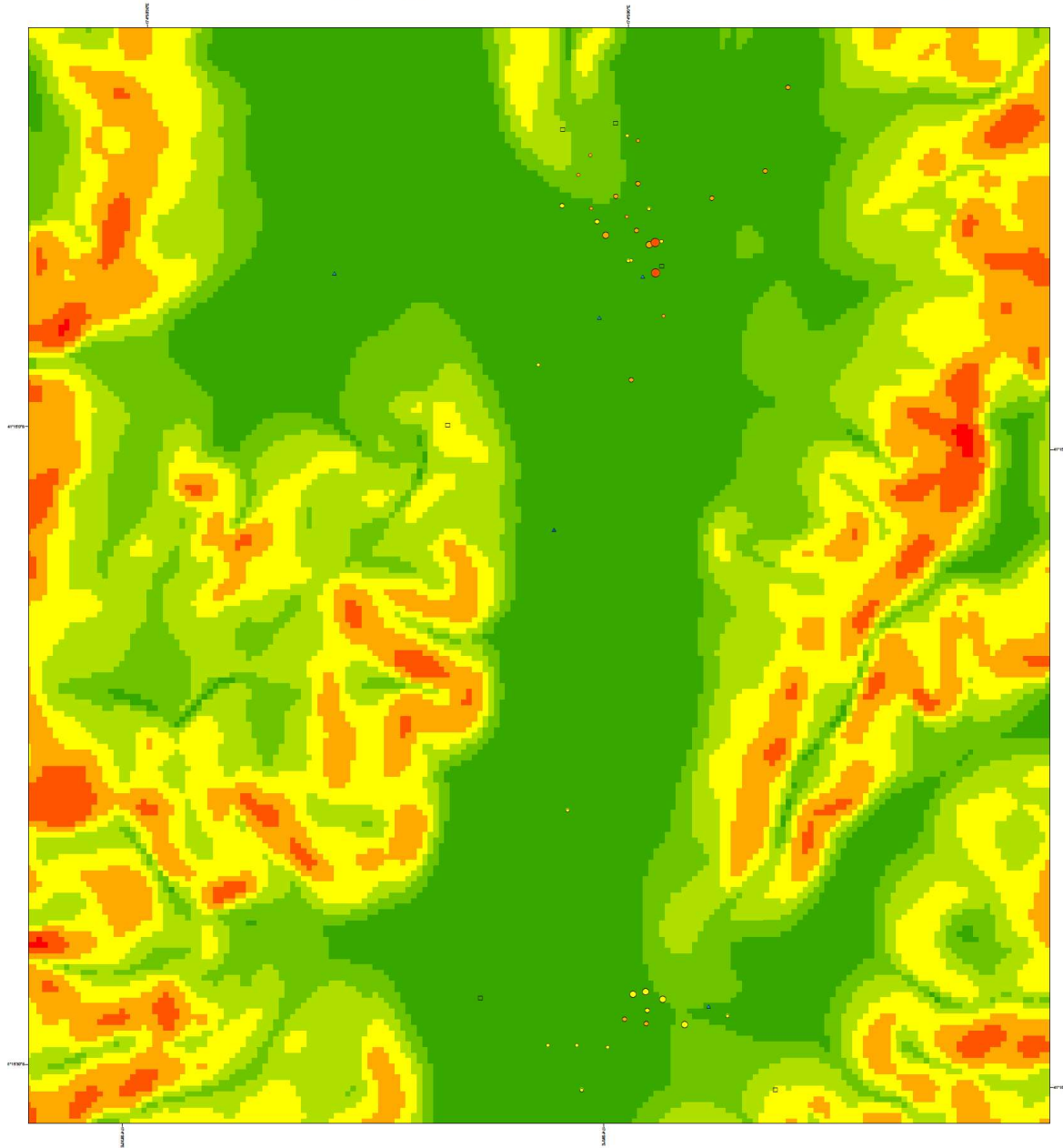


Map 29. Overall view of Moores valley as it leaves Wainuiomata heading North Moores Valley Wainuiomata



Map 30. DCA Activity in Moores Valley Wainuiomata. Overall view

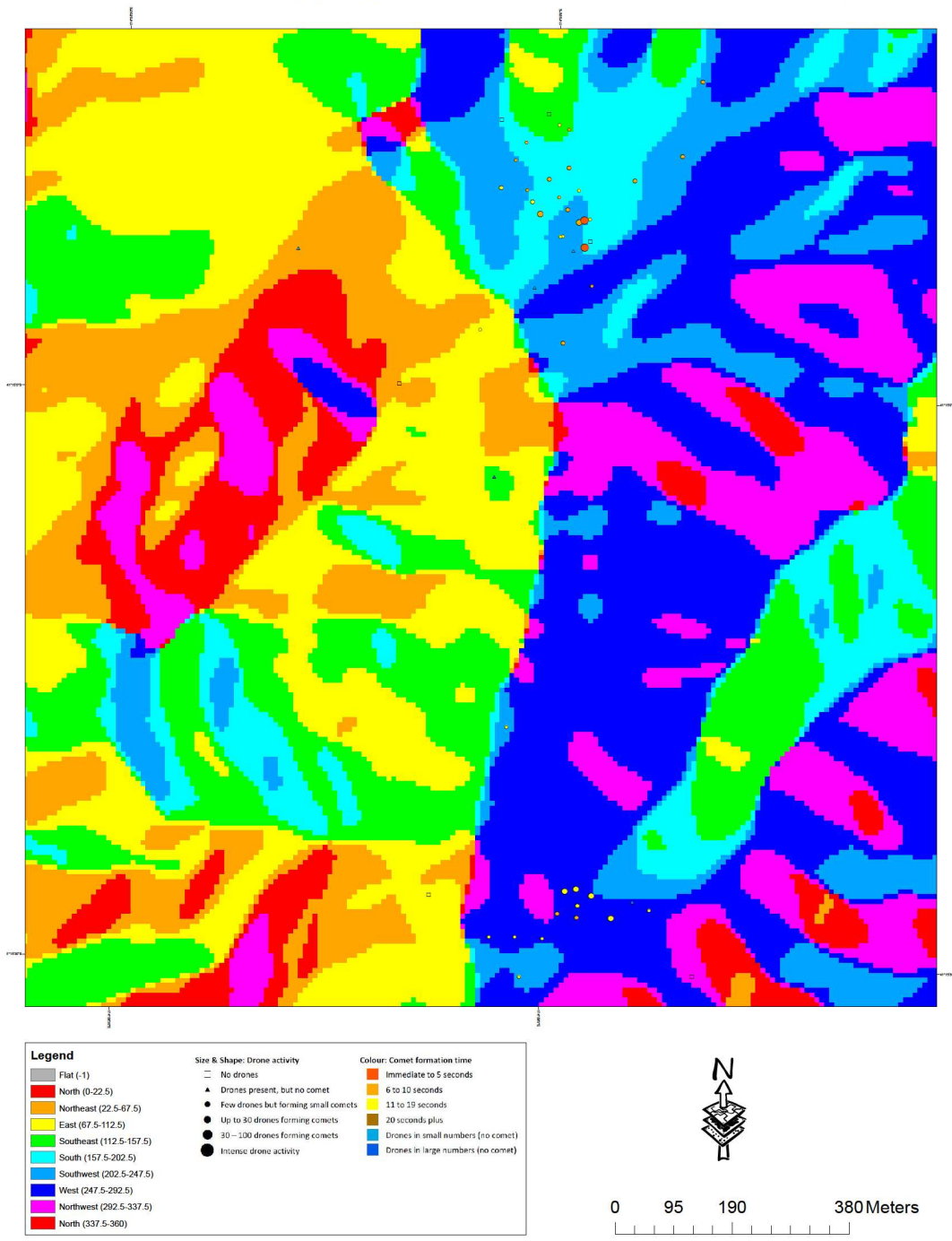
Drone Congregation Area: Moores Valley



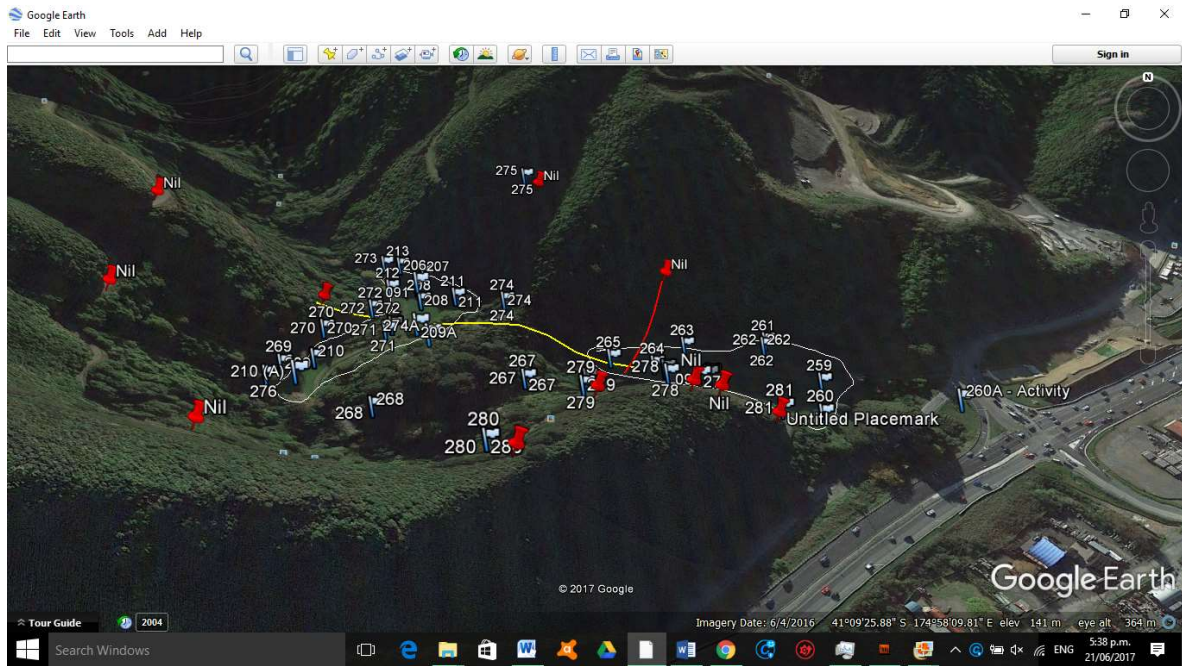
Map

31. Moores Valley with slope overlay

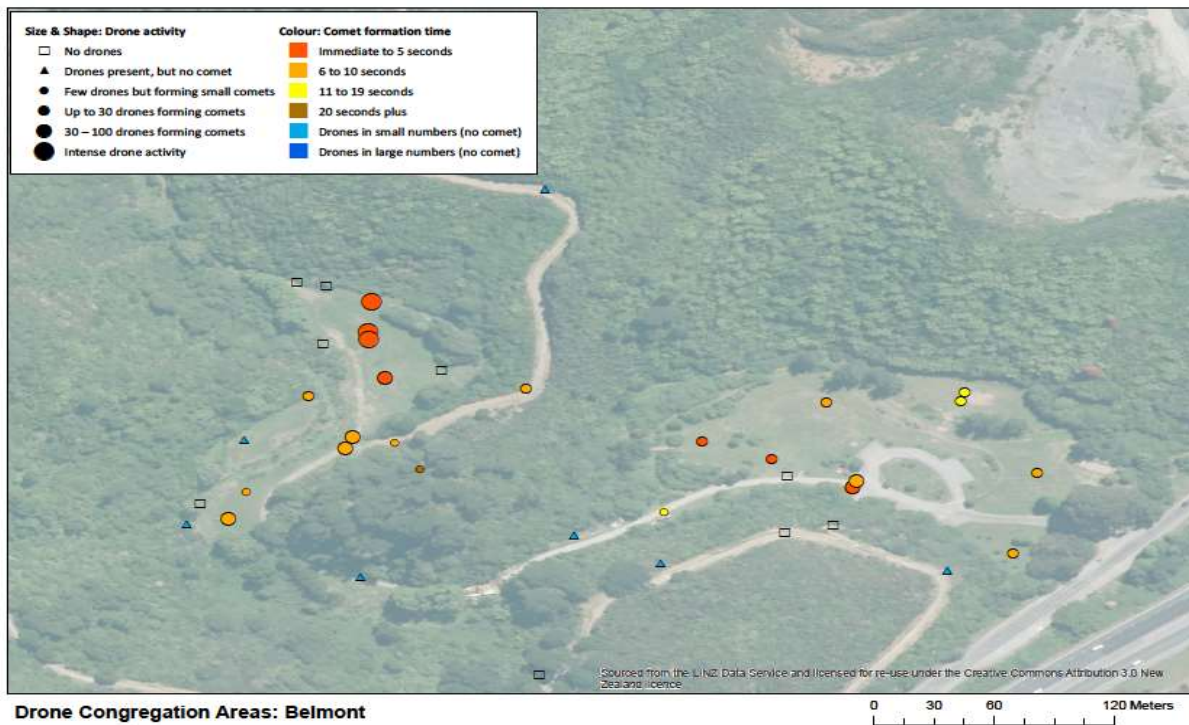
Drone Congregation Area: Moores Valley



Map 32. Moores Valley with aspect overlay

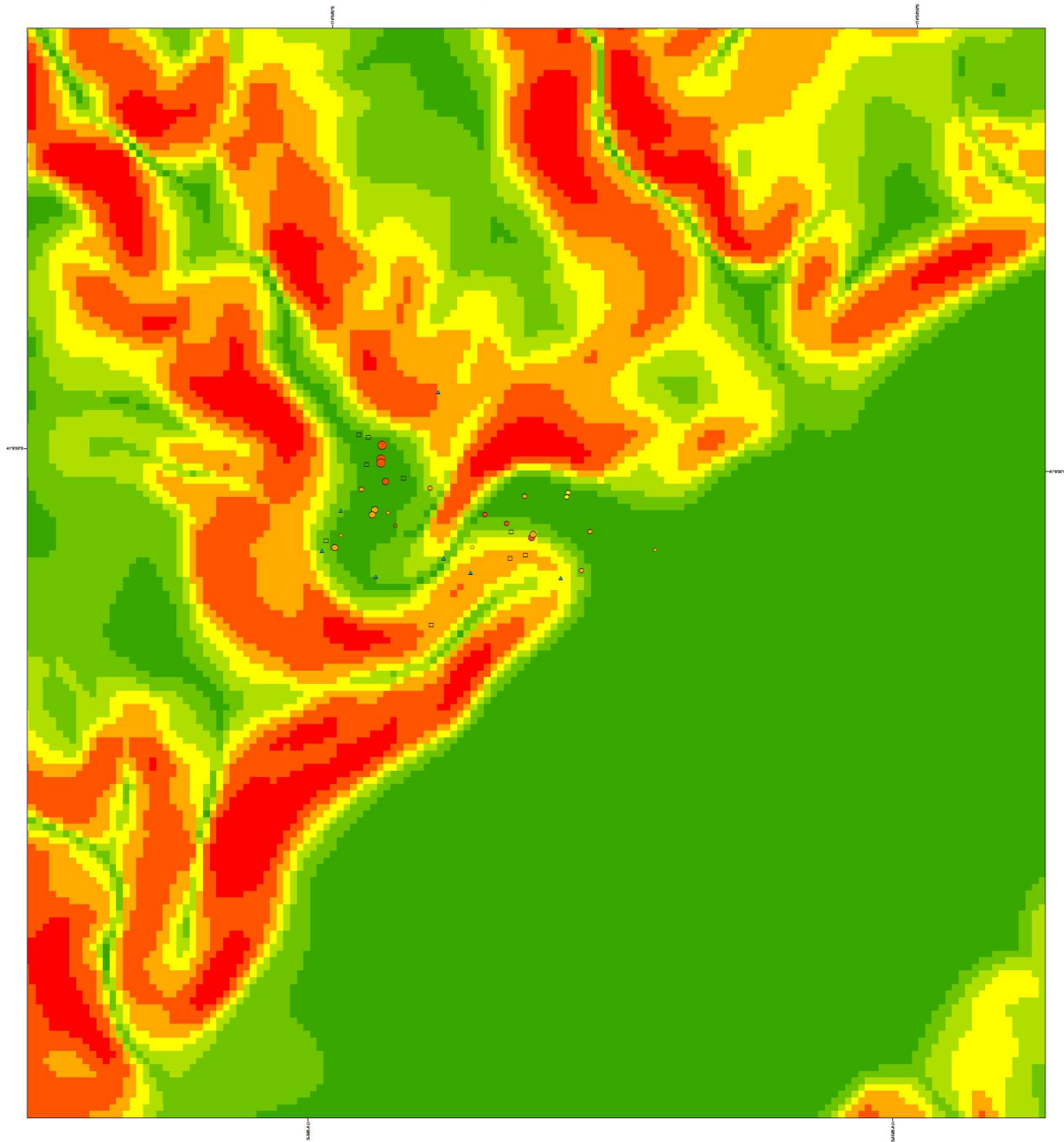


Map 33. Belmont Regional Park – Dry Creek entrance. The yellow line indicates the movement of drones over a col from one area of intense activity to another

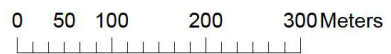


Map 34. Belmont Park Drone activity

Drone Congregation Area: Belmont

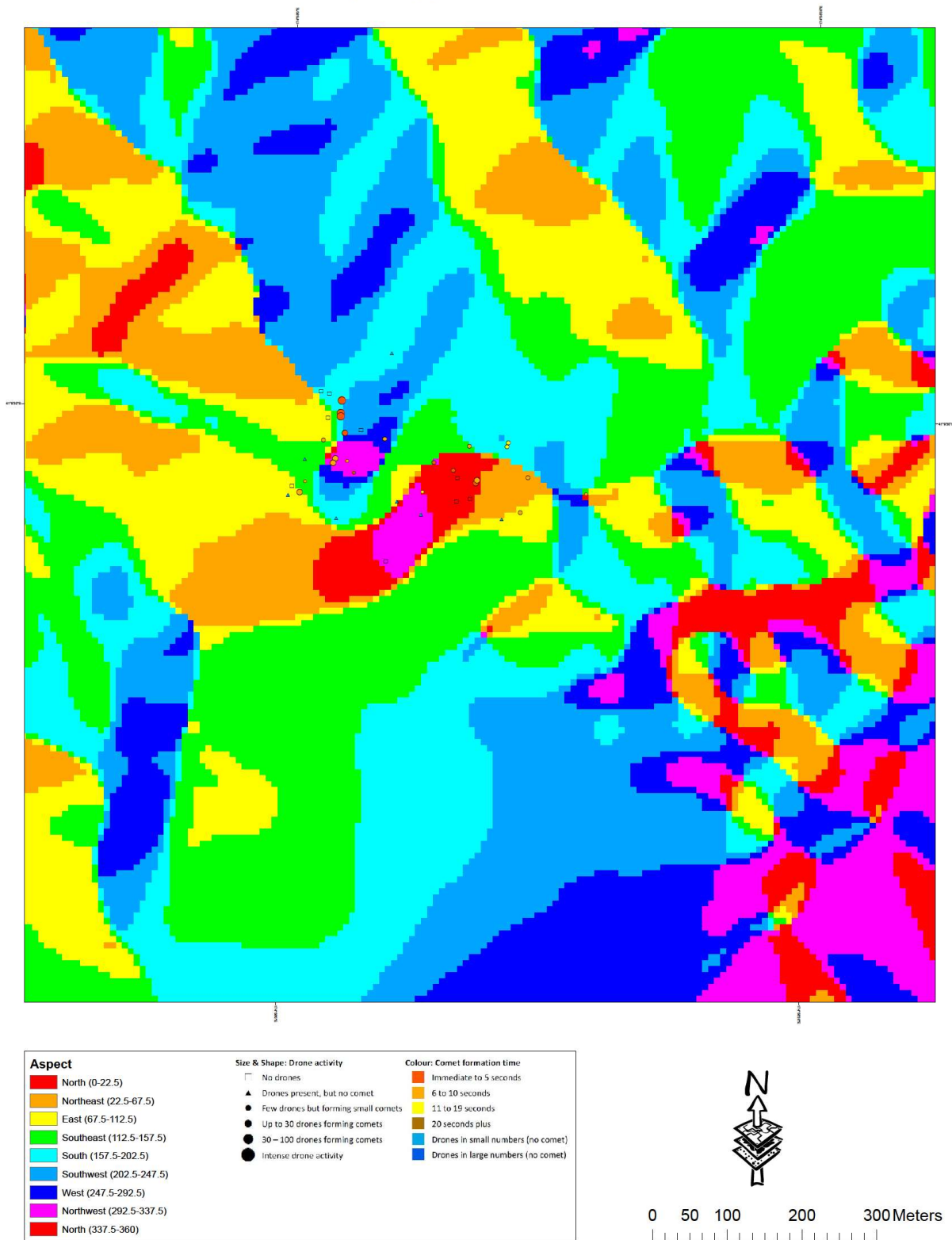


Slope in Degree	Size & Shape: Drone activity	Colour: Comet formation time
0 - 5,531295687	□ No drones	Orange Immediate to 5 seconds
5,531295688 - 12,37956654	▲ Drones present, but no comet	Yellow 6 to 10 seconds
12,37956655 - 18,70104732	● Few drones but forming small comets	Light Green 11 to 15 seconds
18,70104733 - 24,75913307	● Up to 30 drones forming comets	Dark Green 20 seconds plus
24,75913308 - 31,08061386	● 30 - 100 drones forming comets	Light Blue Drones in small numbers (no comet)
31,08061387 - 38,71906981	● Intense drone activity	Dark Blue Drones in large numbers (no comet)
38,71906982 - 67,16573334		

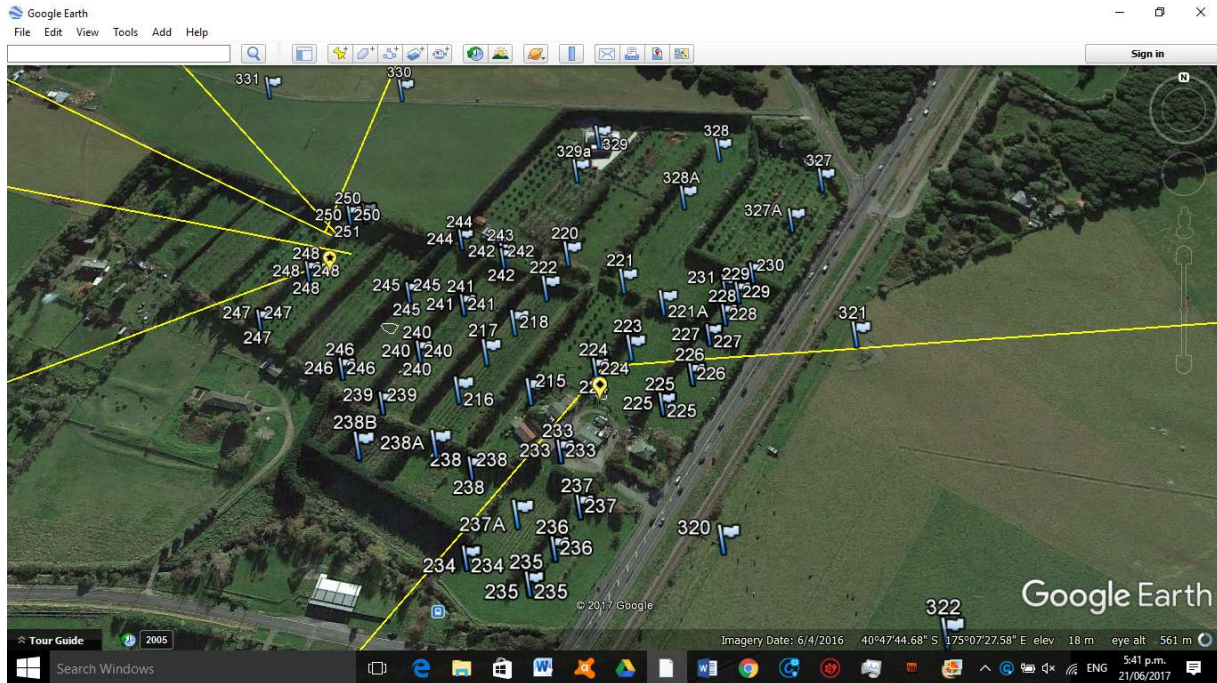


Map 35. Map of Belmont showing slope details overlaid on drone intensity map

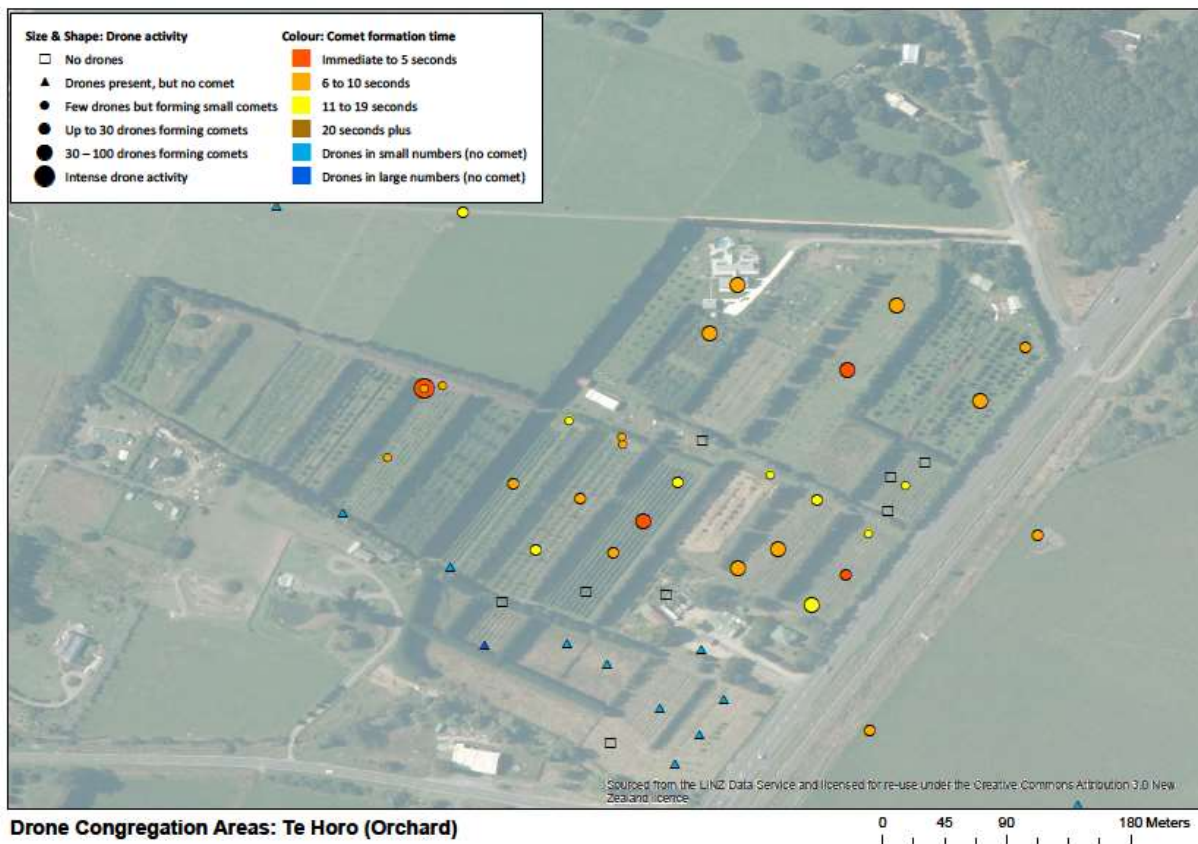
Drone Congregation Area: Belmont



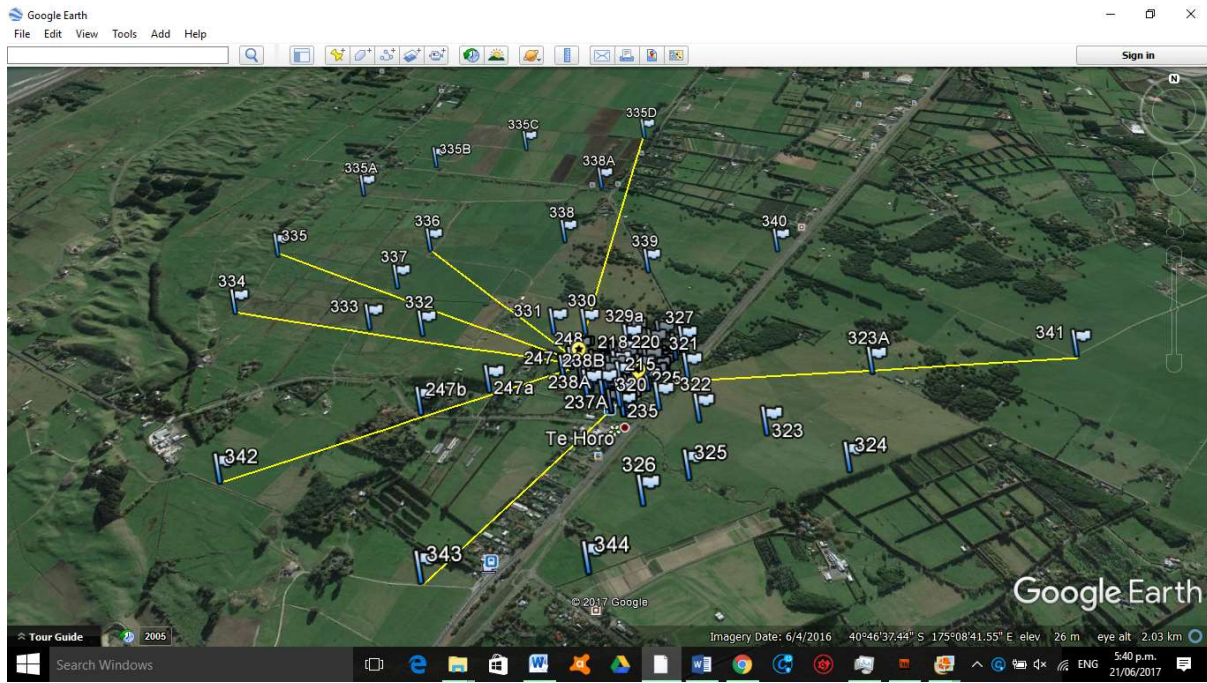
Map 36. Map of Belmont showing aspect details overlaid on drone intensity map



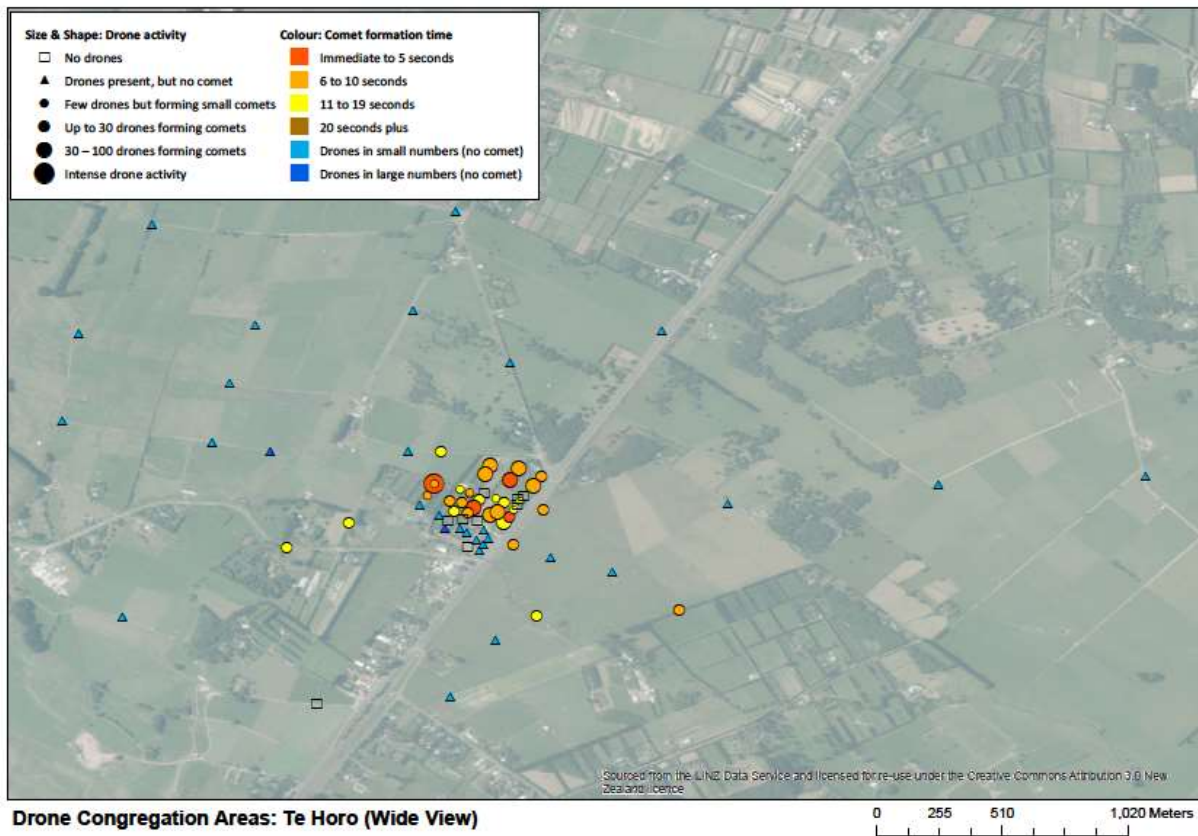
Map 37. Te Horo detailed view of main area of activity



Map 38. Drone activity levels in the Te Horo Berry farm Apiary area

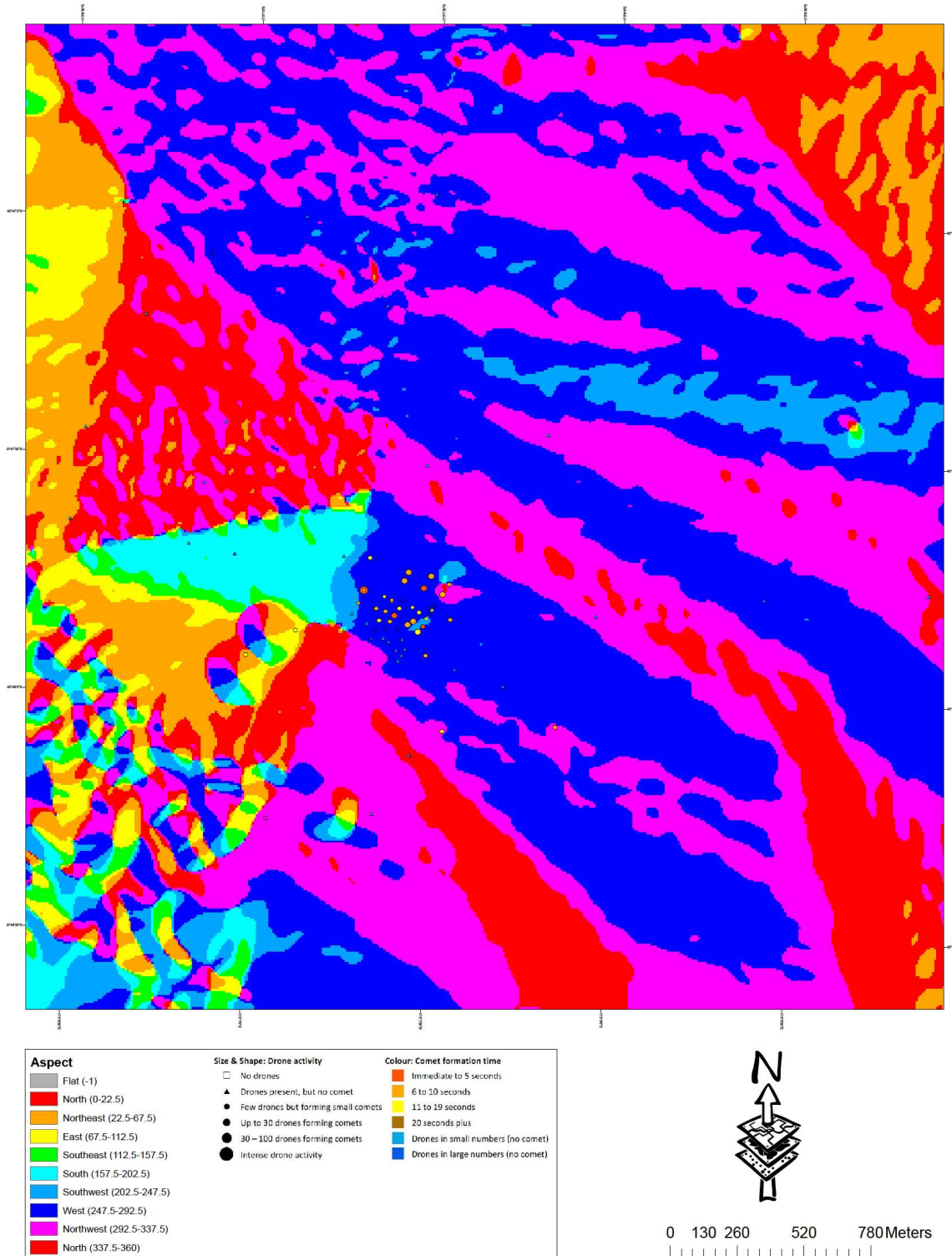


Map 39. Te Horo in the flat, agricultural Kapiti area - expanded view

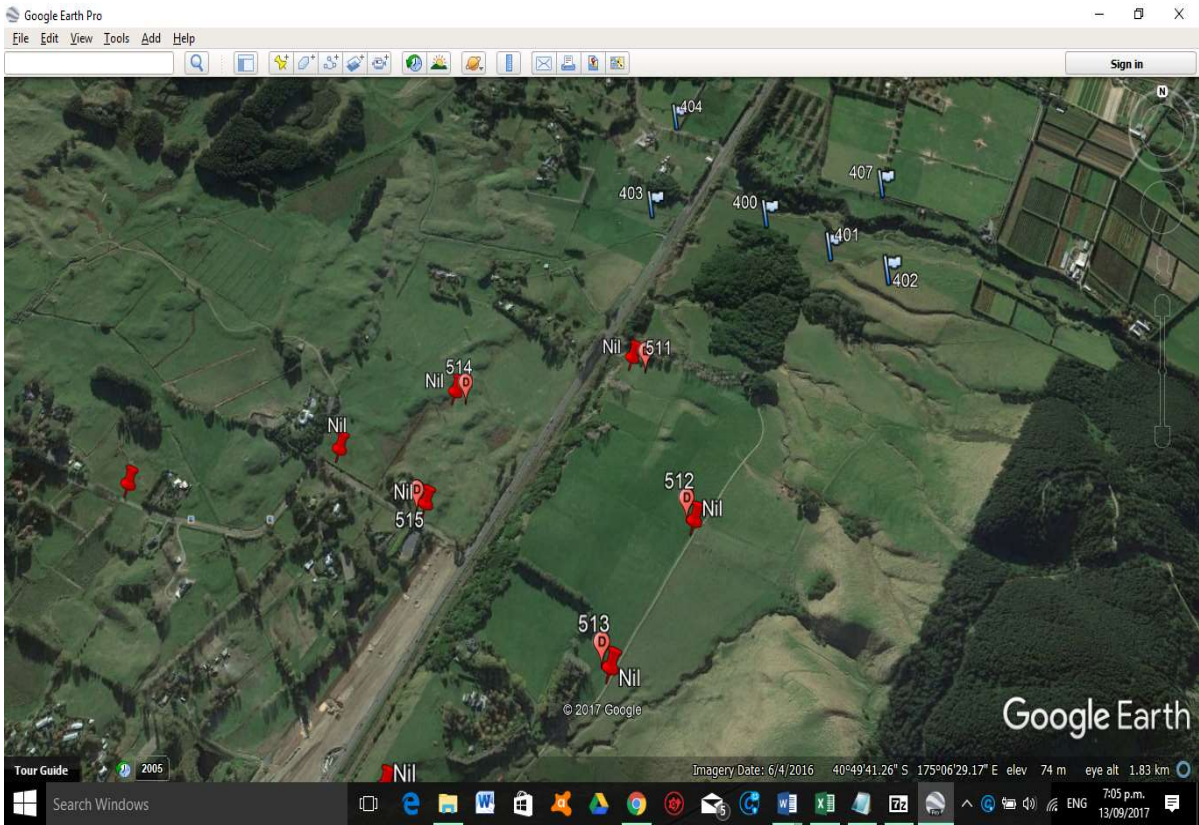


Map 40. Drone activity levels in the Te Horo expanded view area

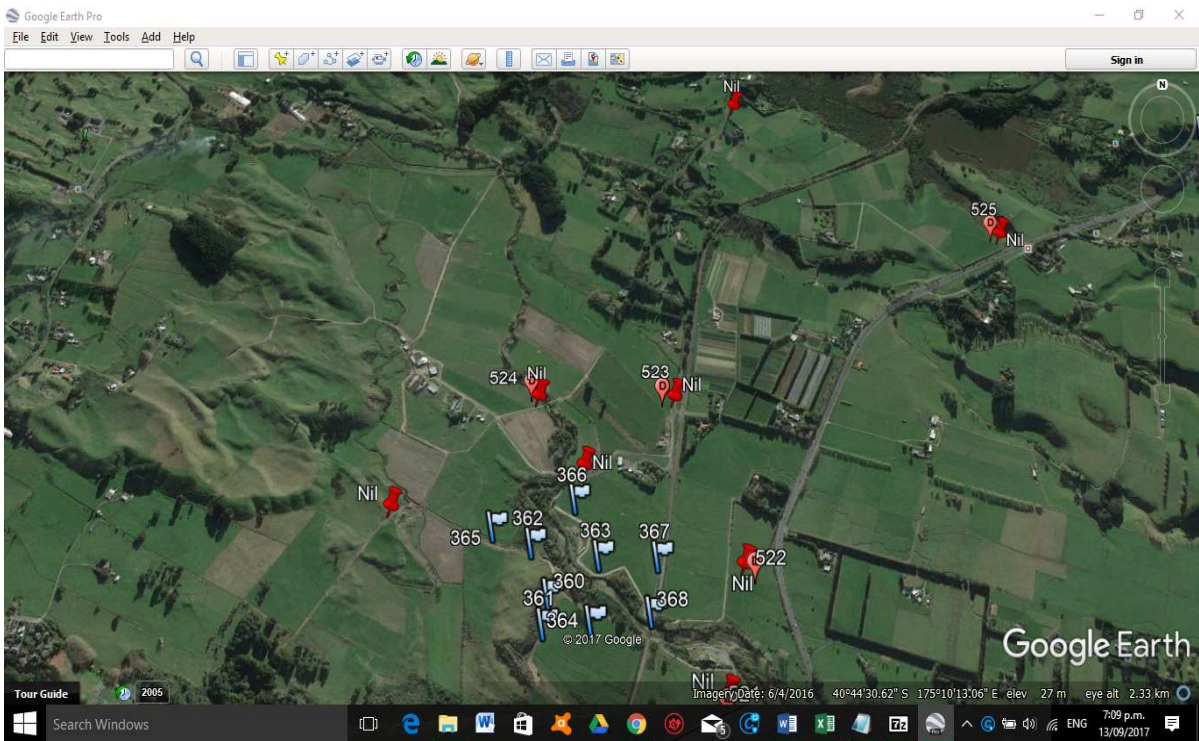
Drone Congregation Area: Te Horo



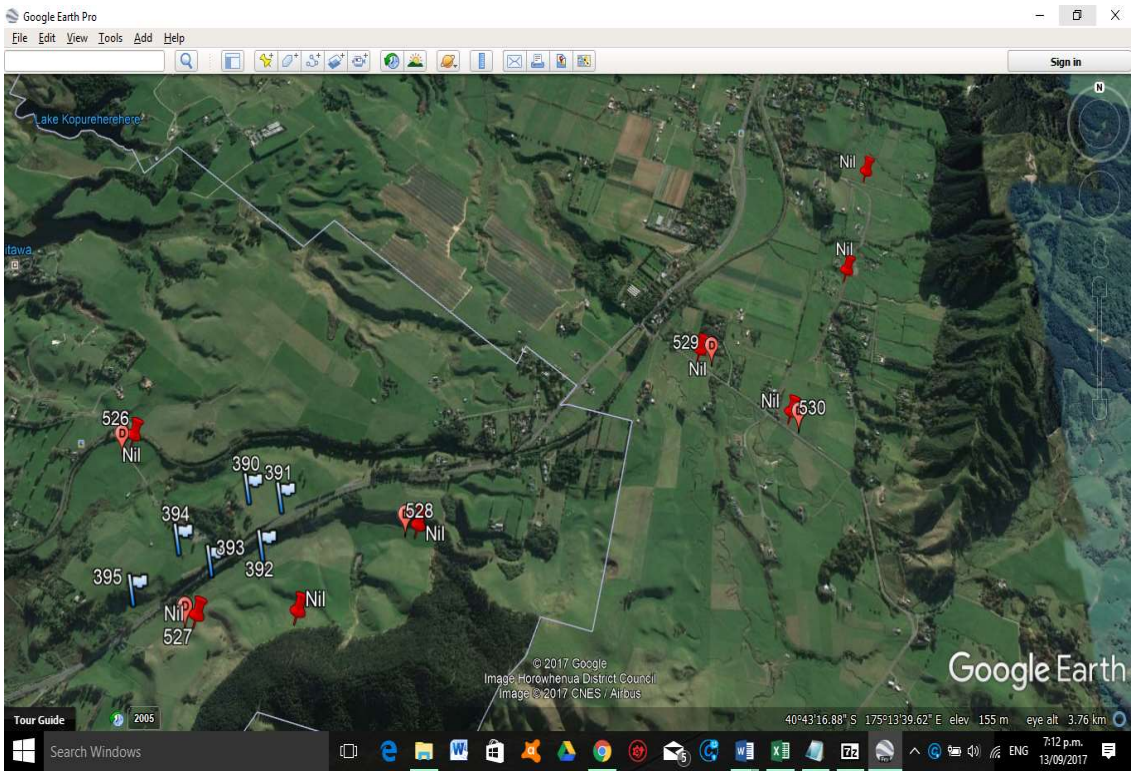
Map 41. Te Horo showing aspect details overlaid on drone intensity map (A slope overlay is not shown as there is no slope differentiation)



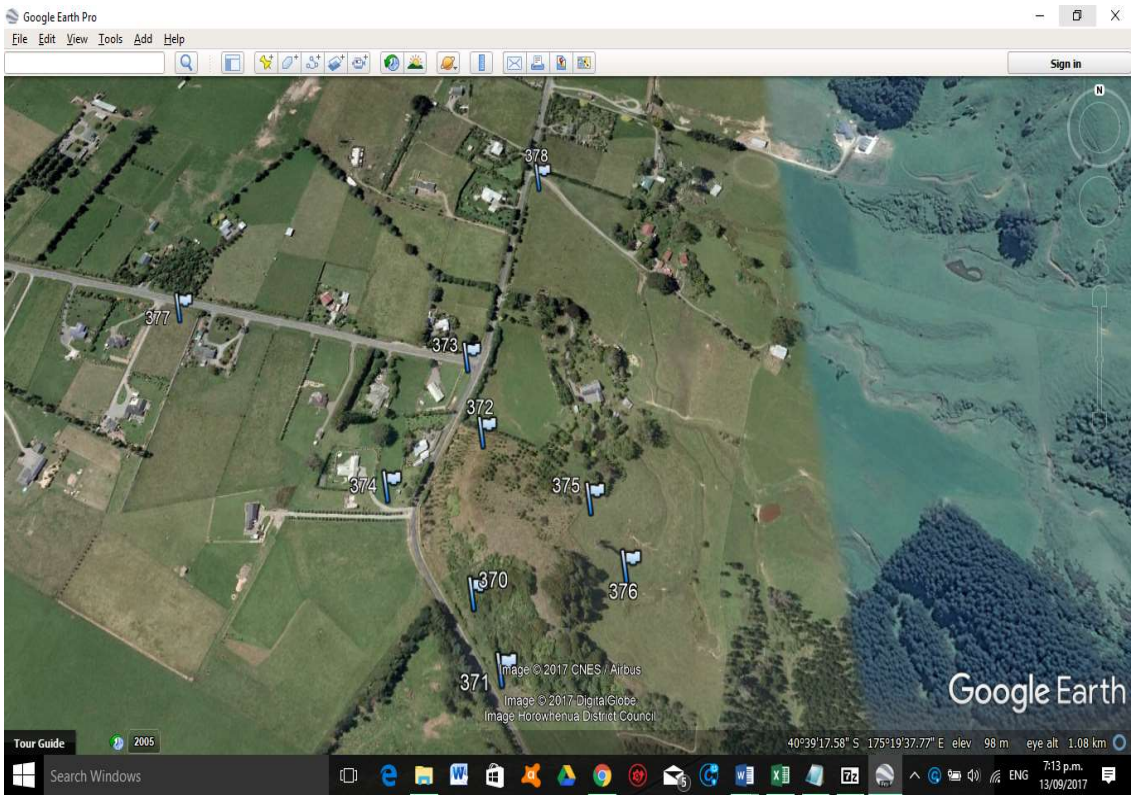
Map 42. Peka Peka Area



Map 43. Otaki area



Map 44. Manukau area



Map 45. Levin area

Appendix 2. Data Tables

Data Table 1. Drone Activity in DCAs

Location	Date	Time	GPS	123	W/S	W/D	Temp	CC
Wainui Camp								
Wainui	13-12-15	15:05	066	221	0 - 6	165	17 - 19	5/8
Wainui	13-12-15	15.08	066A	332	4-5	165	18	5/8
Wainui	13-12-15	15.11	066B	331	3-4	165	19	5/8
Wainui	13-12-15	15.14	066C	332	4-5	165	19	5/8
Wainui	13-12-15	15.16	066D	232	3-4	165	19	5/8
Wainui	13-12-15	15.20	066E	332	3-4	165	18	5/8
Wainui	13-12-15	15.24	067	232	2-3	165	20	5/8
Wainui	13-12-15	15.28	067A	232	3	165	20	5/8
Wainui	13-12-15	15.33	067B	333	2-3	165	20	5/8
Wainui	13-12-15	15.37	067C	342	3-4	165	19	5/8
Wainui	13-12-15	15.42	067D	343	2-3	165	18	5/8
Wainui	13-12-15	15.45	067E	233	4	165	17	5/8
Wainui	13-12-15	15.50	067F	223	3	165	18	5/8
Wainui	13-12-15	15:55	069	111	3- 4	165	17 - 19	5/8
Wainui	13-12-15	16:02	089	111	5-6	165	18 - 20	5/8
Wainui	13-12-15	16:08	090	231	5-6 6	165	19 - 21	5/8
Coast Road								
Wainui	23-12-15	15.05	098	231	1 - 2	185	18 - 19	1/8
Wainui	23-12-15	15.15	099	0	1 - 2	185	17 - 18	1/8
Wainui	23-12-14	15.29	100	121	4	185	19 - 20	1/8
Wainui	23-12-14	15.27	101	111	3 - 4	185	18-19	1/8
Wainui	23-12-14	15.32	054	342	.5 - 1.5	185	2-3	1/8
Wainui	23-12-14	15.35	102	121	3 - 4	185	17-18	1/8

Wainui	23-12-14	15.41	103	121	6	185	16-17	1/8
Wainui	23-12-14	15.44	126	342	2.7	185	19 - 20	1/8
Wainui	23-12-14	15.45	159A	342	0.5 - 2	185	25	1/8
Wainui	23-12-14	15.49	57	342	0.5 - 2	185	26	1/8
Wainui	23-12-14	16.20	104	121	1 - 2	185	17 - 18	1/8
Wainui	23-12-14	16.24	105	121	1 - 2	185	18 - 19	1/8
Wainui	23-12-14	16.27	106	121	4	185	19 - 20	1/8
Wainui	23-12-14	16.33	107	131	3 - 4	185	18-19	1/8
Wainui	23-12-14	16.40	108	231	3 - 4	185	17-18	1/8
Wainui	23-12-14	16.45	108A	*	4	185	18	1/8
Wainui	23-12-14	16.49	109	0	6	185	16-17	1/8
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Wainui	24-12-15	15.25	110	231	3.5	350	21	1/8
Wainui	24-12-15	15.30	110A	332	3.5	350	22	1/8
Wainui	24-12-15	15.35	111	231	3.8	350	22	1/8
Wainui	24-12-15	15.4	111A	332	4	350	21	1/8
Wainui	24-12-15	15.46	112	231	3.6	350	20	1/8
Wainui	24-12-15	15.48	113	231	4.2	350	21	1/8
Wainui	24-12-15	15.55	114	131	0.5	350	21	1/8
Wainui	24-12-15	16.02	115	121	3.5	350	21	1/8
Wainui	24-12-15	16.08	116	121	3	350	21	1/8
Wainui	24-12-15	16.38	117	121	3.2	350	22	1/8
Wainui	24-12-15	15.40	118	221	3	350	22	1/8
Wainui	24-12-15	15.46	119	221	3	350	20	1/8
Wainui	24-12-15	15.48	120	131	3.5	350	21	1/8
Wainui	24-12-15	16.55	121	342	0.5	350	23	1/8
Wainui	24-12-15	15.55	122	342	0.5	350	21	1/8
Wainui	24-12-15	16.45	115A	122	2.5	350	20	1/8

Wainui	24-12-15	16:49	114A	121	3	350	20	1/8
Wainui	24-12-15	16.55	114B	121	3.5	350	19.4	1.8
Wainui	24-12-15	17.02	114C	232	3	350	19	2/8
Wainui	24-12-15	1,708.00	114D	332.00	2.50	350.00	18.80	2/8
Wainui	24-12-15	17.20	115B	222.00	2.80	350.00	18.50	2/8
Wainui	24-12-15	17.25	115C	122.00	3.00	350.00	19.00	3/8
Data Set 26 Dec 2015								
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Wainui	26.12.2015	15.56	088	0	3-4	350	24	1/8
Wainui	26.12.2015	16.10	051	0	1.5	350	24	1/8
Wainui	26.12.2015	16.20	123	111	3	350	24.6	1/8
Wainui	26.12.2015	16.25	124	211	4	350	21	0/8
Wainui	26.12.2015	16.40	060	221	3	350	23	0/8
Data Set 27 Dec								
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Wainui	27/12	3 pm	054	0	0 -1	185	22	0/8
Wainui	27/12	3.05	053	0	0.5	185	23	0/8
Wainui	27/12	3.07	052	0	0 - 1	185	23	0/8
Wainui	27/12	3.15	126	0	0-1	185	25.5	0/8
Wainui	27/12	3.25	061	342	0-2	185	26	0/8
Wainui	27/12	3.30	058	332	0-1	185	26	0/8
Wainui	27/12	3.35	063	332	0-2	185	25.2	0/8
Wainui	27/12	3.42	059	332	0-2	185	26	0/8
Wainui	27/12	3.45	055	0	0-2	185	26	0/8
Wainui	27/12	3.50	056	0	0-2	185	26	0/8

Wainui	27/12	5.54	057	0	0-1	185	26.5	0/8
Data Set 28 Dec S end of Apiary area -								
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Dew pond and old dump	28/12	3.20	127	132	1-2	185	24	1/8
Wainui	28/12	3.24	128	131	0-1	185	28	1/8
Wainui	28/12	3.15	129	0	0-1	185	26	1/8
Wainui	28/12	3.30	130	0	0-1	185	26	1/8
Wainui	28/12	3.33	131	221	0-1	185	26	1/8
Wainui	28/12	3.50	132	0	2-3	185	25	1/8
Wainui	28/12	3.53	133	0	0-2	185	27	1/8
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Ngaturi park area	28/12	4.02	134	242	1-2	185	25	1/8
Wainui	28/12	4.05	135	142	1-2	185	25	1/8
Wainui	28/12	4.09	136	122	0-1	185	26	1/8
Wainui	28/12	4.14	137	122	1-2	185	24	1/8
Wainui	28/12	4.17	138	122	1-2	185	25	1/8
Wainui	28/12	4.22	139	122	0-1	185	25	1/8
Data Set Ngaturi Park Area 29 Dec								
Location	Date	Time	GPS	133	W/S	W/D	Temp	cc
Wainui	28/12	4.18	154	232	2-3	185	20	0/8

Wainui	28/12	4.05	153	0	2-3	185	19	0/8
Wainui	28/12	4.09	154	231	3-4	185	20.1	0/8
Wainui	28/12	4.14	155	0	2-3	185	19	0/8
Wainui	28/12	4.17	156	341	2-3	185	20	0/8
Wainui	28/12	4.22	157	0	1-2	185	22	0/8
Dump Area 30 Dec								
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Wainui	30/12	3.00	158	*	2.8	185	21	1/8
Wainui	30/12	3.15	159	*	3-4	185	21.5	1/8
Wainui	30/12	3.20	160	0	2-3	185	22.6	1/8
Wainui	30/12	3.25	161	342	3-4	185	23	2/8
Wainui	30/12	3.25	162	0	0-1	185	23	2/8
Wainui	30/12	3.30	163	231	0-1	185	23	2/8
Wainui	30/12	3.35	163A	221	0-1	185	24	2/8
Wainui	30/12	3.45	164	*	0-1	185	25	2/8
Wainui	30/12	3.50	165	*	0-1	185	24	2/8
Wainui Camp Entrance Area								
Wainui	30/12	4.05	064	222	0-1	195	23	3/8
Wainui	30/12	4.07	166	232	0-1	195	25	3/8
Wainui	30/12	4.10	167	232	0-1	195	25	3/8
Wainui	30/12	4.11	168	0	0-1	195	25	3/8

Data Moore's Valley Area 4/1/2016								
Location	Date	Time	GPS	123	W/S	W/D	Temp	
MV	4/1/16	4.05	251	112	0 - 0.5	45	24	1/8
MV	4/1/16	4.12	252	Lure	0 - 0.5	45	24	1/8
MV	4/1/16	4.15	253	Lure	0 - 0.5	45	24	1/8
MV	4/1/16	4.16	254	Lure	0 - 0.5	45	23	1/8
MV	4/1/16	4.17	*255	Lure	0 - 0.5	45	24	
MV	4/1/16	4.22	*256	242	1-2	45	22	*In trees
MV	4/1/16	4..32	257	112	1 - 2	45	23	1/8
MV	4/1/16	4.40	258	222	1	45	23	1/8
MV	4/1/16	3.45	258A	333	1	340	28	7/8
MV	4/1/16	3.49	258B	333	1.5	340	28	7/8
		Te Horo Old Berry farm						
Te Horo	24/1/2016	14.28	216	0	0	0	26	6/8
Te Horo	24/1/2016	1432	217	232	0 - 0.5	V	26	6/8
Te Horo	24/1/2016	1440	218	122	0 - 0.5	V	26	6/8
Te Horo	24/1/2016	14.43	219	122	0 - 0.5	V	25	6/8
Te Horo	24/1/2016	14.50	220	0	0 - 0.5	V	26	6/8
Te Horo	24/1/2016	14.55	221	342	0 - 0.5	V	23	6/8
Te Horo	24/1/2016	14.58	223	222	0 - 0.5	V	24	5/8
Te Horo	24/1/2016	15.04	224	222	0 - 0.5	V	24	5/8

Te Horo	24/1/2016	15.08	225	324	1 - 2	240	24	5/8
Te Horo	24/1/2016	15.12	226	131	0 - 0.5	V	25	6/8
Te Horo	24/1/2016	15.20	227	342	0 - 0.5	V	24	6/8
Te Horo	24/1/2016	15.28	228	0	0 - 1	240	24	6/8
Te Horo	24/1/2016	15.41	229	342	0 - 1	240	24	6/8
Te Horo	24/1/2016	15.45	230	0	0 - 1	240	26	6/8
Te Horo	24/1/2016	15.52	231	0	0 - 1	240	26	5/8
Data Te Horo 26/1/2016								
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Te Horo	26/1/2016	14.45	232	*	0 - 1	V	28	1/8
Te Horo	26/1/2016	14.50	233	*	0 - 0.5	V	26	1/8
Te Horo	26/1/2016	14.55	234	0	1 - 1.5	V	26	1/8
Te Horo	26/1/2016	14.57	235	*	1 - 1.5	V	26	1/8
Te Horo	26/1/2016	15.02	236	*	0 - 1	V	28	1/8
Te Horo	26/1/2016	15.05	237	*	0 - 1	V	28	1/8
Te Horo	26/1/2016	15.07	238	*	0 - 1	V	28	1/8
Te Horo	26/1/2016	15.12	239	0	0 - 1	V	28	1/8
Te Horo	26/1/2016	15.15	240	332	0 - 0.5	V	31	1/8
Te Horo	26/1/2016	15.18	241	232	0 - 1.5	240	27	1/8
Te Horo	26/1/2016	15.22	242	243	0 - 1.5	240	28	1/8
Te Horo	26/1/2016	15.26	243	243	0 - 1.5	240	28	1/8
Te Horo	26/1/2016	15.29	244	342	0 - 0.5	V	28	1/8
Te Horo	26/1/2016	15.34	245	232	0 - 0.5	V	30	1/8
Te Horo	26/1/2016	15.40	246	*	0	0	32	1/8
Te Horo	26/1/2016	15.43	247	*	0	0	30	1/8
Te Horo	26/1/2016	15.50	247A	332	0.5	240	31	1/8

Te Horo	26/1/2016	15.59	247B	332	1	240	30	1/8
Te Horo	26/1/2016	16.10	248	243	0 - 0.5	V	32	1/8
Te Horo	26/1/2016	16.15	249	242	0 - 0.5	V	29	1/8
NOTES	Te Horo							
Te Horo	29/1/2016	Orchard and Flats						
Te Horo	29/1/2016	14.30	320	232	3	045	26	4/8
Te Horo	29/1/2016	14.35	321	232	4	045	25	4/8
Te Horo	29/1/2016	14.41	322	*	4	045	26	4/8
Te Horo	29/1/2016	14.45	323	*	4	045	25	4/8
Te Horo	29/1/2016	14.51	324	232	3	045	26	4/8
Te Horo	29/1/2016	14.58	325	332	3	045	26	4/8
Te Horo	29/1/2016	15.04	326	*	3	045	24	4/8
Te Horo	29/1/2016	15.15	327	233	3	045	23	4/8
Te Horo	29/1/2016	15.18	327A	223	2	045	23	4/8
Te Horo	29/1/2016	15.22	328	223	3	045	25	4/8
Te Horo	29/1/2016	15.27	328A	123	3	045	24	4/8
Te Horo	29/1/2016	15.34	329	223	3	045	25	4/8
Te Horo	29/1/2016	15.40	329A	223	3-4	045	25	4/8
Te Horo	29/1/2016	Flats to NW						
Te Horo	29/1/2016	15.46	330	332	4	045	26	4/8
Te Horo	29/1/2016	15.55	331	*	3-4	045	24	4/8
Te Horo	29/1/2016	16.00	332	**	4-5	045	23	4/8
Te Horo	29/1/2016	16.10	333	*	4	045	23	4/8

Flats around Orchard	12/3/2016							
Te Horo	12/3/2015	15.00	334	*	3-4	350	24	5/8
Te Horo	12/3/2015	15.05	335	*	5-6	350	24	5/8
Te Horo	12/3/2015	15.05	335A	*	5	350	25	5/8
Te Horo	12/3/2015	15.35	335B	0	4-5	350	24	5/8
Te Horo	12/3/2015	15.40	335C	*	5-7	350	22	5/8
Te Horo	12/3/2015	15.45	335D	*	5	350	24	5/8
Te Horo	12/3/2015	15.15	336	*	5-7	350	24	5/8
Te Horo	12/3/2015	15.18	337	*	3-4	350	23	5/8
Te Horo	12/3/2015	15.24	338	*	4	350	24	5/8
Te Horo	12/3/2015	15.30	338A	*	4-5	350	24	5/8
Te Horo	12/3/2015	15.30	339	*	3-5	350	23	5/8
Te Horo	12/3/2015	15.55	340	*	4	350	23	5/8
Te Horo	12/3/2015	16.05	341	*	4-5	350	24	5/8
Te Horo	12/3/2015	16.15	341A	*	5	350	22	4/8
Te Horo	12/3/2015	16.30	342	*	3-4	350	24	3/8
Te Horo	12/3/2015	16.30	343	0	3-4	350	24	3/8
Te Horo	12/3/2015	16.30	344	*	3-4	350	24	3/8
Otaki 30/1/2016								
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Otaki	30/1/2016	14.05	360	0	4	350	23	5/8
Otaki	30/1/2016	14.08	361	0	3-4	350	24	5/8
Otaki	30/1/2016	14.15	362	*	5-6	350	24	5/8
Otaki	30/1/2016	14.26	363	*	4-5	350	25	5/8

Otaki	30/1/2016	14.32	364	0	5-7	350	24	5/8
Otaki	30/1/2016	14.42	365	*	5-7	350	22	5/8
Otaki	30/1/2016	14.52	366	0	5-7	350	22	5/8
Otaki	30/1/2016	14.58	367	0	5-7	350	22	5/8
Otaki	30/1/2016	15.05	368	0	5-7	350	22	5/8
Manakau	31/01/2016	14.01	390	**	3	45	22	4/8
Manakau	31/01/2016	14.05	391	**	4	45	23	4/8
Manakau	31/01/2016	14.11	392	*	4	45	22	4/8
Manakau	31/01/2016	14.15	393	*	4	45	24	4/8
Manakau	31/01/2016	14.25	394	0	2	45	22	4/8
Manakau	31/01/2016	14.28	395	0	2	45	22	4/8
Manakau	31/01/2016	14.32	396	*	2	45	22	4/8
Manakau	31/01/2016	14.45	397	*	2	45	22	4/8
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
SH1 PekaPeka 30/01/2016								
SH1 PekaPeka	31/01/2016	15.05	400	0	1	45	24	5/5
SH1 PekaPeka	31/01/2016	15.12	401	*	1	45	24	5/5
SH1 PekaPeka	31/01/2016	15.15	402	0	3	45	24	5/5
SH1 PekaPeka	31/01/2016	15.32	403	0	3	45	24	5/5
SH1 PekaPeka	31/01/2016	15.42	404	0	3	45	23	5/5
SH1 PekaPeka	31/01/2016	16.05	405	**	4	45	23	5/5
SH1 PekaPeka	31/01/2016	16.15	406	**	2	45	23	5/5
SH1 PekaPeka	31/01/2016	16.32	407	**	2	45	23	5/5
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc

Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
Park	21/2/2016	15.25	271	223	1-3	N	24	1/8
Park	21/2/2016	15.32	272	231	0-0.5	N	22	0/8
Park	21/2/2016	15.40	273	0	0.5	N	21	1/8
Park	21/2/2016	15.48	274	231	1-3	N	23	1/8
Park	21/2/2016	15.55	274A	442	1-3	N	24	1/8
Park	21/2/2016	16.15	275	*	1	N	22	0/8
Park	21/2/2016	16.20	276	*	1	N	23	0/8
Park	21/2/2016	16.24	277	0	.5 - 1	N	23	1/8
Park	21/2/2016	16.28	278	0	1	N	22	1/8
Park	21/2/2016	16.35	279	*	1-2	N	24	1/8
Park	21/2/2016	16.38	280	0	1-2	N	23	1/8
	21/2/2016	16.47	281	*	0.5	N	22	1/8/2 016
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
RPP	12/12/2015	14.30	034	233	0-1	45	24	5/8
RPP	12/12/2015	14.34	035	223	0-1	45	22	5/8
RPP	12/12/2015	14.40	036	333	0-1	45	24	5/8
RPP	12/12/2015	14.45	037	333	0-1	45	24	5/8
RPP	12/12/2015	14.52	038	233	0-1	45	23	5/8
RPP	12/12/2015	14.56	039	323	0-1	45	23	5/8
RPP	12/12/2015	14.56	039A	343	0-1	45	23	5/8
RPP	12/12/2015	14.59	040	0	0-1	45	25	5/8
RPP	12/12/2015	15.06	041	*	0-1	45	24	5/8
RPP	12/12/2015	15.11	042	0	0-1	45	24	5/8
RPP	29/02/2016	15.15	043	*	0-1	45	23	5/8
RPP	29/02/2016	15.22	043A	*	0-1	45	22	4/8
RPP	12/12/2015	15.45	044	*	0-1	45	22	4/8

RPP	12/12/2015	15.50	045	333	0-1	45	22	4/8
RPP	12/12/2015	15.55	046	*	0-1	45	23	4/8
RPP	12/12/2015	16.05	046A	333	0-1	45	23	3/8
Richard Prowse Park 28/2/ 2016								
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
RPP	28/02/2016	15.00	282	131	2-4	45	24	4/8
RPP	28/02/2016	15.05	282	131	2-4	45	24	4/8
RPP	28/02/2016	15.08	282	131	2-4	45	24	4/8
RPP	28/02/2016	15.13	283	131	2-4	45	25	4/8
RPP	28/02/2016	15.18	284	121	1-2	45	25	4/8
RPP	28/02/2016	15.23	284A	232	1-2	45	25	4/8
RPP	28/02/2016	15.29	284B	121	1-2	45	25	4/8
RPP	28/02/2016	15.39	285	231	0-3	45	24	4/8
RPP	28/02/2016	15.50	286	131	0-3	45	26	4/8
RPP	28/02/2016	15.55	287	241	1-2	45	23	4/8
RPP	28/02/2016	16.05	288	131	0-1	45	24	4/8
RPP	28/02/2016	16.10	289	231	0-1	45	25	4/8
RPP	28/02/2016	16.20	290	332	2-3	45	24	4/8
RPP	28/02/2016	16.45	291	131	1-2	45	25	4/8
RPP	28/02/2016	16.50	292	343	0-0.5	45	29	4/8
RPP	28/02/2016	16.54	293	231	0-1	45	26	4/8
RPP	28/02/2016	17.00	294	131	0-0.5	45	27	4/8
RPP	28/02/2016	17.02	295	241	0-2	45	24	4/8
RPP	28/02/2016	17.09	296	231	2-3	45	23	4/8
RPP	28/02/2016	17.12	297	232	1-2	45	24	4/8
RPP	28/02/2016	17.14	298	*	2-3	45	24	4/8

Golf Course Area				Warm gusty, windy conditions				
Location	Date	Time	GPS	123	W/S	W/D	Temp	cc
GC	13/3/2016	14.45	345	333	3-4	185	24	5/8
GC	13/3/2016	14.50	346	233	3	185	24	5/8
GC	13/3/2016	14.58	347	*	2-4	185	23	5/8
GC	13/3/2016	15.05	348	*	3-5	185	23	5/8
GC	13/3/2016	15.12	349	*	6-7	185	22	5/8
GC	13/3/2016	15.18	350	*	3-4	185	22	5/8
GC	13/3/2016	15.25	351	*	3	185	22	4/8
GC	13/3/2016	15.45	352	*	4	185	21	4/8
GC	13/3/2016	15.58	353	0	3-5	185	21	5/8
GC	13/3/2016	16.15	354	*	5	185	22	5/8
GC	13/3/2016	16.15	354A	0	5	185	22	5/8
GC	13/3/2016	16.15	354B	*	5	185	22	5/8
GC	13/3/2016	16.22	355	0	5-6	185	21	5/8
GC	13/3/2016	16.40	356	*	4-5	185	21	5/8
GC	13/3/2016	16.50	357	*	4-5	185	20	5/8
GC	14/3/2016	14.00	358	**	3-4	185	25	4/8
GC	14/3/2016	14.10	359	0	3-4	185	25	4/8
GC	14/3/2016	14.16	360	0	4	185	25	4/8
GC	14/3/2016	15.05	361	*	3	185	26	4/8
15/3/2016								
CR	15/3/2016	14.05	362	*	2.5	185	24	3/8
CR	15/3/2016	14.1	363	*	2.4	185	23	3/8
CR	15/3/2016	14.18	364	0	2.6	185	24	3/8

CR	15/3/2016	14.34	365	0	2.5	185	25	3/8
CR	15/3/2016	14.45	366	**	3	185	24	3/8
CR	15/3/2016	14.52	366a	442	2.5	185	24	3/8
CR	15/3/2016	14.57	366b	**	3	185	23	3./8
CR	15/3/2016	15.12	367	**	2	185	24	3/8
CR	15/3/2016	15.22	368	**	2.6	185	24	3/8
CR	15/3/2016	15.4	369	**	2	185	23.5	3/8
CR	15/3/2016	16.08	370	**	2.4	300	23	4/8

Data Table 2: Showing the data used to perform regression analysis on four variables

Key:

Y Intensity of Drone Activity 1-9 based on the size of drone comets and the speed of appearance.

X1 Landscape slope in degrees

X2 Landscape aspect degrees converted to Radians

X3 Landscape cover as follows:

1 – Pasture

2 – Bush

3 – Margin bush/pasture

4 – Low forest

5 – Forest

6 – Urban/waste area

7 – Urban grass/park

8 – Trackways

X4 – Whether the spot was adjacent to a tree line which could provide shelter

Intensity (Y)	Slope (X1)	Aspect (X2)	Cover (X3)	Adjacent (X4)	Location
6	2.76	3.14	1	0	Belmont Park
6	2.76	3.93	1	0	Belmont Park
5	2.76	5.5	8	1	Belmont Park
5	2.76	5.5	8	1	Belmont Park
5	2.76	1.57	1	1	Belmont Park
6	21.73	0.2	8	1	Belmont Park
3	8.95	5.5	1	0	Belmont Park
3	15.54	0.2	1	0	Belmont Park
2	2.76	1.57	1	1	Belmont Park
2	2.76	4.71	8	1	Belmont Park
2	2.76	2.36	1	0	Belmont Park
2	2.76	0.78	1	0	Belmont Park
2	8.95	1.57	8	1	Belmont Park
1	2.76	3.14	1	0	Belmont Park
1	2.76	3.14	1	0	Belmont Park
4	15.54	0.2	1	0	Belmont Park
6	2.76	3.14	1	1	Moore's Valley

6	2.76	3.14	1	1	Moores Valley
5	2.76	3.14	1	0	Moores Valley
5	2.76	3.93	1	1	Moores Valley
4	2.76	4.71	1	0	Moores Valley
4	2.76	4.71	1	0	Moores Valley
4	2.76	4.71	1	0	Moores Valley
4	2.76	4.71	1	1	Moores Valley
2	2.76	3.93	1	0	Moores Valley
2	2.76	3.14	1	0	Moores Valley
2	2.76	3.14	1	0	Moores Valley
2	2.76	3.14	1	0	Moores Valley
2	2.76	3.93	1	0	Moores Valley
2	2.76	4.71	1	0	Moores Valley
2	2.76	4.71	1	0	Moores Valley
6	15.54	5.5	1	1	Wainui Camp
6	8.95	4.71	1	1	Wainui Camp
6	8.95	2.36	1	1	Wainui Camp
6	8.95	4.71	1	1	Wainui Camp
6	15.54	4.71	1	1	Wainui Camp
6	2.76	4.71	4	1	Wainui Camp
6	15.54	4.71	4	1	Wainui Camp
6	15.54	4.71	4	1	Wainui Camp
6	15.54	5.5	4	1	Wainui Camp
6	8.95	3.14	4	1	Wainui Camp
6	8.95	3.14	4	1	Wainui Camp
6	8.95	3.14	4	1	Wainui Camp
6	8.95	4.71	1	1	Wainui Camp
6	8.95	4.71	1	1	Wainui Camp
6	2.76	3.93	4	1	Wainui Camp

6	8.95	4.71	1	1	Wainui Camp
6	2.76	5.5	1	0	Wainui Camp
5	8.95	5.5	1	0	Wainui Camp
5	8.95	5.5	1	1	Wainui Camp
5	8.95	4.71	4	1	Wainui Camp
5	8.95	0.2	8	0	Wainui Camp
5	8.95	5.5	1	0	Wainui Camp
5	8.95	5.5	1	0	Wainui Camp
3	2.76	5.5	1	1	Wainui Camp
3	8.95	0.2	1	0	Wainui Camp
2	2.76	0.2	1	0	Wainui Camp
2	8.95	0.2	1	1	Wainui Camp
2	2.76	5.5	4	1	Wainui Camp
2	2.76	4.71	8	1	Wainui Camp
2	8.95	0.78	4	1	Wainui Camp
1	15.54	5.5	4	1	Wainui Camp
1	15.54	0.78	8	1	Wainui Camp
1	15.54	0.78	8	1	Wainui Camp
1	2.76	0.78	8	1	Wainui Camp
1	15.54	0.2	4	1	Wainui Camp
6	2.76	0.76	1	1	Wainui Camp
6	2.76	4.71	4	1	Wainui Camp
5	15.54	1.57	8	1	Wainui Camp
5	15.54	5.5	1	0	Wainui Camp
5	15.54	4.71	4	1	Wainui Camp
2	15.54	4.71	8	1	Wainui Camp
2	15.54	4.71	8	1	Wainui Camp
2	8.95	4.71	4	1	Wainui Camp
2	8.95	4.71	4	1	Wainui Camp

1	15.54	1.57	8	1	Wainui Camp
5	8.95	5.5	2	0	Wainui Valley
3	2.76	5.5	2	0	Wainui Valley
3	2.76	5.5	2	0	Wainui Valley
3	2.76	3.14	2	0	Wainui Valley
3	2.76	3.14	1	1	Wainui Valley
2	2.76	4.71	1	0	Wainui Valley
3	2.76	3.93	1	1	Wainui Valley
5	2.76	3.93	1	1	Wainui Valley
5	2.76	3.14	3	0	Wainui Valley
5	2.76	3.14	3	0	Wainui Valley
6	2.76	3.14	1	0	Wainui Valley
6	2.76	4.71	1	1	Wainui Valley
6	2.76	4.71	1	1	Wainui Valley
6	2.76	2.36	3	1	Wainui Valley
6	2.76	3.14	1	1	Wainui Valley
6	2.76	4.71	1	0	Wainui Valley
6	2.76	3.14	3	0	Wainui Valley
6	2.76	2.36	3	0	Wainui Valley
6	2.76	1.57	3	1	Wainui Valley
6	2.76	1.57	3	1	Wainui Valley
6	2.76	3.93	3	0	Wainui Valley
6	2.76	3.14	3	1	Wainui Valley
2	2.76	3.14	1	1	Wainui Valley
6	2.76	2.36	2	1	Wainui Valley
4	2.76	2.36	2	0	Wainui Valley
4	2.76	3.14	2	0	Wainui Valley
4	2.76	3.14	1	0	Wainui Valley
4	2.76	4.71	1	1	Wainui Valley

5	2.76	4.71	1	1	Wainui Valley
5	2.76	1.57	1	0	Wainui Valley
5	2.76	1.57	1	0	Wainui Valley
5	2.76	2.36	1	0	Wainui Valley
5	2.76	1.57	1	1	Wainui Valley
5	15.54	2.36	2	0	Wainui Valley
5	2.76	2.36	2	0	Wainui Valley
6	2.76	2.36	1	1	Wainui Valley
6	2.76	1.57	1	1	Wainui Valley
2	2.76	4.71	1	1	Wainui Valley
1	2.76	4.71	1	1	Wainui Valley
6	2.76	2.36	1	1	Wainui Valley
6	2.76	2.36	2	0	Wainui Valley
6	2.76	2.36	2	0	Wainui Valley
6	2.76	4.71	2	1	Wainui Valley
6	2.76	2.36	3	0	Wainui Valley
6	2.76	4.71	3	1	Wainui Valley
2	2.76	3.14	3	0	Ngatura Park
6	2.76	1.57	7	1	Ngatura Park
6	2.76	0.78	7	1	Ngatura Park
6	2.76	1.57	7	1	Ngatura Park
6	2.76	1.57	7	1	Ngatura Park
6	2.76	1.57	7	1	Ngatura Park
6	2.76	4.71	7	1	Ngatura Park
6	2.76	1.57	7	0	Ngatura Park
2	2.76	2.36	7	0	Ngatura Park
2	2.76	4.71	7	1	Ngatura Park
1	2.76	4.71	7	0	Ngatura Park
5	21.73	3.14	8	1	R Prowse Park

4	8.95	3.14	8	1	R Prowse Park
4	2.76	0.2	8	1	R Prowse Park
4	2.76	3.93	2	0	R Prowse Park
2	2.76	0.2	7	0	R Prowse Park
2	2.76	0.2	7	1	R Prowse Park
4	2.76	5.5	7	0	R Prowse Park
4	2.76	3.93	7	1	R Prowse Park
4	2.76	4.71	7	1	R Prowse Park
4	2.76	4.71	7	0	R Prowse Park
6	2.76	4.71	7	0	R Prowse Park
5	2.76	4.71	7	0	R Prowse Park
1	2.76	3.93	7	1	R Prowse Park
2	2.76	4.71	8	1	R Prowse Park
4	2.76	3.14	8	0	R Prowse Park
6	2.76	3.14	7	0	R Prowse Park
5	2.76	3.14	7	0	R Prowse Park
6	2.76	3.14	7	1	R Prowse Park
6	2.76	4.71	7	1	R Prowse Park
1	2.76	3.14	7	1	R Prowse Park
4	2.76	5.5	7	0	R Prowse Park
1	2.76	5.5	8	0	R Prowse Park
5	2.76	3.14	7	0	R Prowse Park
5	2.76	4.71	7	1	R Prowse Park
2	2.76	4.71	7	0	R Prowse Park
5	2.76	4.71	7	1	R Prowse Park
5	2.76	4.71	7	1	R Prowse Park
4	2.76	5.5	7	1	R Prowse Park
6	2.76	4.71	7	0	R Prowse Park
5	2.76	0.78	7	1	R Prowse Park

6	2.76	0.2	7	0	R Prowse Park
4	2.76	3.14	7	1	R Prowse Park
6	2.76	0.78	7	1	R Prowse Park
4	2.76	0.78	7	0	R Prowse Park
5	2.76	3.14	7	0	R Prowse Park
5	2.76	3.14	7	0	R Prowse Park
6	2.76	2.36	7	0	R Prowse Park
6	2.76	2.36	7	0	R Prowse Park
6	2.76	2.36	7	0	R Prowse Park
6	2.76	2.36	7	0	R Prowse Park
6	2.76	2.36	7	0	R Prowse Park
6	2.76	2.36	7	0	R Prowse Park
6	2.76	3.14	7	0	R Prowse Park
6	2.76	3.14	7	0	R Prowse Park
6	2.76	3.14	7	1	R Prowse Park

Data table 3. Single Lift points to test drone abundance in hill/valley and flat areas (Points noted if drones appeared)

Golf Course/Coast Road				
Location	Point	D time	C Time	WD
GC	500	139	205	185
CR	501	155	170	185
CR	502	100	135	185
CR	503	122	154	185
GC	504	150	180	185
GC	505	140	205	185
GC	506	120	150	185
Location	Point	D time	C Time	WD
MV	511	190	245	45
MV	512	120	210	45
MV	513	98	170	45
MV	514	160	230	45
PCR Poole Crescent Reserve				
Location	Point	D time	C Time	WD
PCR	516	90	120	45
PCR	517	83	98	45
PCR	518	85	113	45
PCR	519	125	134	45
PCR	520	163	180	45
PCR	521	159	173	45
Ngaturi Park Wainuiomata				
Location	Point	D time	C Time	WD
NP	506	160	213	185
NP	507	145	145	185

NP	508	178	209	185
NP	509	182	220	185
Kapiti				
Location	Point	D time	C Time	WD
Kapiti	511	65	72	45
Kapiti	512	70	75	45
Kapiti	513	68	75	45
Kapiti	514	60	71	45
Kapiti	515	55	68	45
Kapiti	516	45	58	45
Kapiti	517	48	62	45
Kapiti	518	55	71	45
Kapiti	519	62	68	45
Kapiti	520	84	92	45
Kapiti	521	48	58	45
Kapiti	522	55	71	45
Kapiti	523	78	92	45
Kapiti	524	75	85	45
Kapiti	525	82	102	45
Kapiti	526	63	68	45
Kapiti	527	45	45	45
Kapiti	528	58	63	45
Kapiti	529	85	92	45
Kapiti	530	55	71	45

Data Table 4. Test A. Queen/drone attraction tests

Queen	Test	Time in start Q	Time in other quadrant	Time to home	Notes
			4/4. 24 C. RH 68. CC 5/8		
1	1	10 A	15 W2	93 W1	Fell in well for 52 seconds VIDEO
1	2	7 D	11 W1	18 W1	
1	3	6 D	16 W1	22 W1	
1	4	8 D	21 W1	29 W1	
2	1	4 D	8 W2	12 W2	
2	2	3 D	7 W1	18 W2	
2	3	7 A	5 W2	12 W2	
2	4	4 D	10 W2	14 W2	
			9/4. 22C. RH 72 CC 5/8		
3	1	3 A	10 W2	12 W2	
3	2			W1	Video

3	3	3 D	11 W1	14 W1	
3	4	9 W2	12 W1	21W1	
4	1	3 D	15 W1	18 W1	
4	2			W1	VIDEO
4	3	8 W1		8 W1	
4	4	11 W1		11 W1	
5	1			W2	VIDEO
5	2	2 D	3 A	6 W2	
5	3	3 D	9 W2	19 W1	
5	4	6 W2	6W1	12 W1	
			15/4. 23C. RH68 CC4/8		
6	1	4 D	9 W1	13 W1	
6	2	8 A	8 W2	16 W2	

6	3	4 A	3 D	12 W2	
6	4	8 A	7 W2	15 W2	
7	1	2 A	8 D In tunnel for 9	20 W2	Stayed in D tunnel for 9 secs
7	2	2 A	6 D	17 W1	
7	3	5 D	8 W1	13 W1	
7	4	7 A	6 D	18 W1	
8	1	2 D	7 W1	9 W1	
8	2	5 A	3 D	12 W1	
8	3	9 A	D3	19 W1	
8	4		Queen lost		Queen fell in well and lost during rescue.
			16/4/2017 24C. RH 72 CC 6/8		
9	1	5 A	6 W2	11 W2	

9	2	7 W2		7 W2	
9	3	8 A	5 W2	13 W2	
9	4	6 W2		6 W2	Direct in
10	1	4 A	11 D	19 W1	Lingered in D near exit. Almost went in
10	2	6 D	5 W 1	11 W1	
10	3	9 W1	4 D	16 W1	
10	4	14 A	8 D	31 W1	

Key to illustrate the figures in the tables

1	1	7 A	31 W1	38 W1	
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Drone 1. Test 1 7 seconds in start quadrant Air. 31 Seconds in Worker 1 quadrant before disappearing from sight, at which point the final time is given.

Data Table 5. Test B. Queen/drone only attraction tests (no worker odour source present)

Queen	Test	Time in start Q	Time in other quadrant	Time to home	Notes
			5/4. 24 C. RH 68. CC 5/8		
1	1	33 A	13 D	46D	Moved between air quadrants
1	2	25 D	9A	42D	
1	3	11 A	15 A	31D	
1	4	9 A	11 A	23D	
2	1	14 A	17D	31D	
2	2	18 A		22D	
2	3	25 A		37D	
2	4	18 D		18D	
			9/4. 22C. RH 72 CC 5/8		
3	1	21A		28A	
3	2	8 A	13 D	21D	

3	3	23D		23D	
3	4	19 A		29D	
4	1	28 D		28D	
4	2	18A	9D	27D	
4	3	24A		24A	Left A and fell in well.
4	4	17A	9D	26D	
5	1	28A	13D	41D	
5	2	7D	19A	32D	Moved between D and a quadrants
5	3	19A	15D	34D	
5	4	25A	9D	34D	

Data Table 6. Test C. Drone/drone attraction tests

Test	Drone	Time in start Q	Time in other quadrant	Time to home	Notes
			8/4/17. 18 C. RH69 2.45 – 3.20		
1	1	7 A	31 W1	38 W1	
1	2	5 A	9 W1	14 W1	
1	3	4 D	18 W2	36 W1	
1	4	1 D	9 W2	30 W1	
2	1	2 A	22 D	40 W1	
2	2	2 A	12 D	38 W1	
2	3	30 D	30 D	32 W1	
2	4	15 D	15W1	36 W1	Moved between D and W
3	1	2 D	14 W2	28 W1	
3	2	8 W2	10 W1	18 W1	

3	3	14 W1	11 W2	25 W2	
3	4	40 W1	28 W2	68 W2	Drone tiring
4	1	4 A	14 W2	18 W2	
4	2	14 A	11 W2	25 W2	
4	3	5 D	15 W2	38 W1	
4	4	2 A	8 D	48 W2	Drone tiring
5	1	3 A	13 W1	37 W2	
5	2	7 W1	23 W2	30 W2	
5	3	8 A	20 D	48 W1	
5	4	4 A	18 D	45 W1	
6	1	9 A	18 D	60 W1	
6	2	8 A	9 D	25 W1	

6	3	10 D	10 D	68 W1	Many stops
6	4	4 A	15 D	48 W1	
7	1	A4	8 W1	25 W2	
7	2	14 W1	14 W1	38 W2	
7	3	32 W1	32 W1	58 W2	
7	4	16 A	16 A	40 W2	
8	1	7 D	7 D	34 W1	
8	2	8 A	15 D	60 W1	
8	3	4 D	A 6	38 W2	
8	4	18 A	A 18	64 W2	
9	1	D 18	8 A	42 W1	
9	2	25 W2	25 W2	45 W1	

9	3	54 W1	54 W1	54 W1	
9	4	9 A	15 D	40 W1	
10	1	38 D	38 D	60 W1	
10	2	15 D	15 D	15 D	
10	3	38 W1	38 W1	49 D	
10	4	14 D	14 D	45 W1	
			13/4. 19 C 72RH 2.15 – 15.45		
11	1	45 D	10 W1	55 W1	
11	2	20 W1	20 W1	20 W1	
11	3	10 W2	15 W1	25 W1	
11	4	5 W2	30 W1	60 D	
12	1	30 W1	30 W1	45 D	
12	2	9 A	26 D	35 D	

12	3	40 D	8 W1	60 W2	Drone stayed in D quadrant
12	4	15 D	15 D	15 D	
13	1	8 A	3 D	25 W1	
13	2	9 D	9 D	30 W1	
13	3	10 A	5 D	45 W1	
13	4	20 A	5 D	40 W1	
14	1	16 W2	22 A	63 D	
14	2	38 W1	38 W1	38 W1	
14	3	5 D	15 W1	20 W1	
14	4	21 A	27 W2	62 W1	
15	1	4 A	14 W2	18 W2	
15	2	25 W2	25 W2	25 W1	

15	3	17 W2	25 W1	42 W1	
15	4	16 A	4 D	38 W1	
			22/4 22 C. RH70 14.30 – 15.50		
16	1	7 W2	9 A	28 D	Then left D after 30 secs to centre and fell in well
16	2	17 W1	17 W1	39 W2	
16	3	20 A	20 A	42 D	
16	4	20 W1	20 W1	20 W1	
17	1	17 A	6 W1	38 W2	
17	2	15 A	33 W2	48 D	
17	3	9 D	4 D	27 W2	
17	4	24 W1	24 W1	24 W1	

18	1	9 W1	13 W2	22 W2	
18	2	4 A	11 W2	15 W2	
18	3	8 D	4 W1	38 W2	
18	4	6 D	22 W1	28 W1	Fell in well on first attempt
19	1	11 A	18 D	52 W1	
19	2	7 D	21 W1	28 W1	
19	3	14 W1		14 W1	
19	4	28 W1		28 W1	
20	1	4 D	23 A	48 W2	
20	2	5 D	7 A	28 W2	
20	3	14 W1		22 W2	
20	4	18 D		18 D	

Data Table 7. Test D. Drone/drone attraction tests - no worker influence

Test	Drone	Time in start Q	Time in other quadrant	Time to home	Notes
1	1	11 A1	9 D	20 D	
1	2	12 D	13 A	23 D	
1	3	14 A2	8 A1	31 D	
1	4	12 A1		17 D	
2	1	9 A1		18 D	
2	2	9 A2	7 A3	24 D	
2	3	11A3		18 D	
2	4	9 A2	17 A1	35 D	
3	1	13 D	14 W2	13 D	
3	2	9 A2	11 A3	28 D	

3	3	11 A1		20 D	
3	4	9 A3		25 D	
4	1	4 A2	9 A3	21 D	
4	2	19 A3		28D	
4	3	7 A2	9 A3	29 D	
4	4	39 A3	8 D	60 D	Drone tiring
5	1	17 D		17 D	
5	2	9 A3		18 D	
5	3	11 A1	8 D	30 A3	*
5	4	19 A1		29 D	
6	1	9 A3	18 D	22 D	
6	2	11 A2	9 A3	28 D	

6	3	14 A1		21 D	
6	4	8D	7 A1	23 D	
7	1	12 D		12 D	
7	2	15 A1		23 D	
7	3	9 A3		D 20	
7	4	7 A2		28 A3	
8	1	14 A3		29 D	
8	2	2 A2	7 A3	29 D	
8	3	A2	0	0	Drone on Perspex ceiling squashed whilst attempting to remove.
8	4	0	0	0	No test Drone
9	1	17 D		17 D	
9	2	9 D		9 D	
9	3	18 A2		24 D	

9	4	18 A2	9 A1	39 D	
10	1	9D	11 A3	31 D	
10	2	11 A3		11 A3	
10	3	17 A2	19 A1	58 D	
10	4				Drone falls in well. Damaged on exit