University of Gloucestershire

NS6244 Ecological and Environmental Science Dissertation

Topographical and Plant Density Effects on the Rates of Hybridisation of Bluebells (*Hyacinthoide*)

Ecology and Environmental Science Assessment 02

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Guy Copperthwaite

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Declaration: This dissertation is the product of my own work and does not infringe the ethical principles set put in the University's Handbook for the Research Ethics. I agree that it may be made available for reference at the discretion of the University of Gloucestershire.

Signed: ……..

Guy Copperthwaite

Dated: 1st of March 2019

Abstract

The United Kingdom currently maintains the world's highest distribution of *Hyacinthoide non-scripta* (*Lilacease*) of approximately 25% to 50%, but now sees this species under threat from anthropogenic mediated introductions primarily from the Iberian Peninsula (Kohn *et al.,* 2009; Grundmann *et al.,* 2010; Allum, 2016). Recent civilian science based surveys by the Natural History Museum and the Woodland Trust suggest the balance between the invasive *Hyacinthoide hispanica* and native species now places the endemic *H. non-scripta* under threat of national and international (Europe) elimination through hybridisation (Grundmann *et al.,* 2010; Marquardt, 2016). Surveys and studies have clarified a genetic drift (Kohn *et al.,* 2009; Grundmann *et al.,* 2010; Allum, 2016; Marquardt, 2016) towards the potential prevalence of the *Hyacinthoide x massartiana* by assessing competitiveness and hybridising interactions between native and alien taxa through co-occurrence and abundance in relation to habitat occupied, land type cover and phenotypic variables on a large geographic scale (Kohn *et al.,* 2009; Allum, 2016). The aim of this research/study is to establish whether hybridisation is limited by natural topographical features and flora density of a deciduous woodland or alternatively, demonstrate that no natural feature is able to limit the gradual silent extinction of the *H. non-scripta* from the United Kingdom. To do this a woodland was selected based on its variability of flora and topographical features. An area of 4.8km² was surveyed using geographical system positioning to plot every group of bluebells by position, area and elevation. Each group was counted using a random cluster sampling method and an in depth analysis was conducted at all elevations with the use of a random generator to determine area to be surveyed. All explanatory variables: density, flora types, atmospheric and ground temperatures, incline, pH, wind speed, luminosity and soil construct were recorded. Results showed (1) native species were only 42.17% of all bluebells recorded, (2) distribution of both species types were recorded at all elevations, (3) *H. hispanica* related to variable associated to areas of human habitation. The distribution of data suggests that through the introduction of invasive species the flow of genetic gradualisation is turning towards a landscape saturated by hybrids, (4) that elevation was not the determining factor that limited the complete hybridisation of *H. non-scripta*.

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My grateful thanks also goes to all staff that have listened to my proposals, wishes, desires and aspirations in the pursuit of my education but also more importantly, my wish to make a difference in the world of ecology and environmental science. I appreciate that not all requests by me, and consequently, support given, fell within the remit of the university. However, support was given and for that, I will be eternally thankful.

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Chapter 1: Introduction

1.1 Evolution and Distribution of *Asparagaceae Scilloideae*

Bluebells are a bulbous (monocotyledon) perennial that is part of the genus Hyacinthoide Heist. Ex Fabr. Presently there eleven species within the group with one known hybrid. Recently their taxonomy and relationship were phylogenetically revised from the group known as *Hyacinthaceae* (Grundmann *et al.*, 2010) into the family Asparagaceae s.I (APG III, 2009), sub-family, Scilloideae, of the genus Hyacinthoides (Pierre Chouard: 1934) (Pfosser and Speta, 1999). Prior to the bluebells new classification, bluebells were commonly referred to as *Endymion non-scriptus* or *Scilla non-scripta* (Johann Centurius von Hoffmannsegg and Johann Heinrich Friedrich Link transferred species to Scilla in 1803 and Christian August Friedrich Garcke transferred species to Endymion in 1849).

The origin of the bluebell through the family *Hyacinthoideae sensu* (Chase *et al.*, 2009) has been traced back to sub-Saharan-Africa where it is thought to have been established around the Miocene period (Ali *et al.,* 2012; Buerki *et al.,* 2012). Dispersal into the Mediterranean region was thought to have started around 20 million years ago (mya) (Buerki *et al.,* 2012).

With the aid of molecular clock dating, using plastid sequences, it is suggested that the origins of the Iberian species originated approximately 5.81 million years ago (mya) (Ali *et al.,* 2012) prompted by the start of diversification initiated through the large scale desiccation of the Mediterranean sea during the Messinian salinity crisis of around 5mya (Grundmann *et al.,* 2010). Further diversification was thought to have occurred with the split of the H. non-scripta and H. hispanica clade by Pleistocene glaciation cycles around 2.58mya (Grundmann *et al.,* 2010).

The family *Hyacinthoideae* has since divided into three monophyletic tribes; with one group restricted to the French/Italian Alps and two groups overlapping in the southern Iberian Peninsula and Northern Morocco. The *Hyacinthoideae* variants (reclassified as *Scilloideae Hyacinthoides*) are now considered to have a strict Mediterranean origin.

Western Iberian Peninsula maintains a widespread distribution of five species within the genus *Hyacinthoides.* Grundman *et al.,* (2010) confirmed the geographical distribution of the five variants using DNA extraction, PCR (Polymerase Chain Reaction) amplification and sequencing. These analysis confirmed the following species: *H. non-scripta, H. hispanica, H. paivae*, *H. reverchonii* and *H. mauritanica* (Figure 1). Grundman *et al.,* (2010) also confirmed within that study, the existence of an unknown species located in north east of Spain.

The distribution of these five species are separated into two clades. The south western/central clade (southern Portugal and south eastern Spain) are comprised of the *H. mauritanica* (ma) and *H. reverchonii* (re). It is suggested that both species types (*H. mauritanica and H. reverchonii*) have achieved phenotypical variation as a consequence of speciation by geological separation due to no range overlap. This is in comparison to the second clade which covers the north east and mid northern area of Spain. Species variants includes: *H*. *non-scripta* (ns), *H. paivae* (pa) and *H. hispanica* (hi). The second clade (north east and mid northern Spain) comprises a widespread distribution resulting in two haplotypes of the *H. non-scripta* and *H. hispanica* plus an unknown phylogenetic origin for the *H. paivae* (Ortiz and Rodrguez-Oubi~na, 1996; Ortiz *et al.,* 1999). Like the *H. mauritanica* and *H. reverchonii*, the unknown origin of the *H. paivae* is due to geographical separation.

1.2 Distribution of *H. non-scripta* and *H. hispanica*

Currently, the British form of the *H. non-scripta* (L.) Chouard ex Rothm., ranges from the north west of Spain, spreading along the Atlantic coast (northern France, northern Belgium and south west Holland) into the British Isles (Grundman et al., 2010). Expansion into the British Isles is thought to have occurred post glacial (cca., 8 TYA) from northern France using the English Channel land bridge that is thought to have been present during the that time (Hewitt, 1999); alternatively, it is postulated that bluebells were transported by migrating/trading humans that used the bluebell bulbs for their ethno-medicinal effects (Hodkinson and Thompson, 1997).

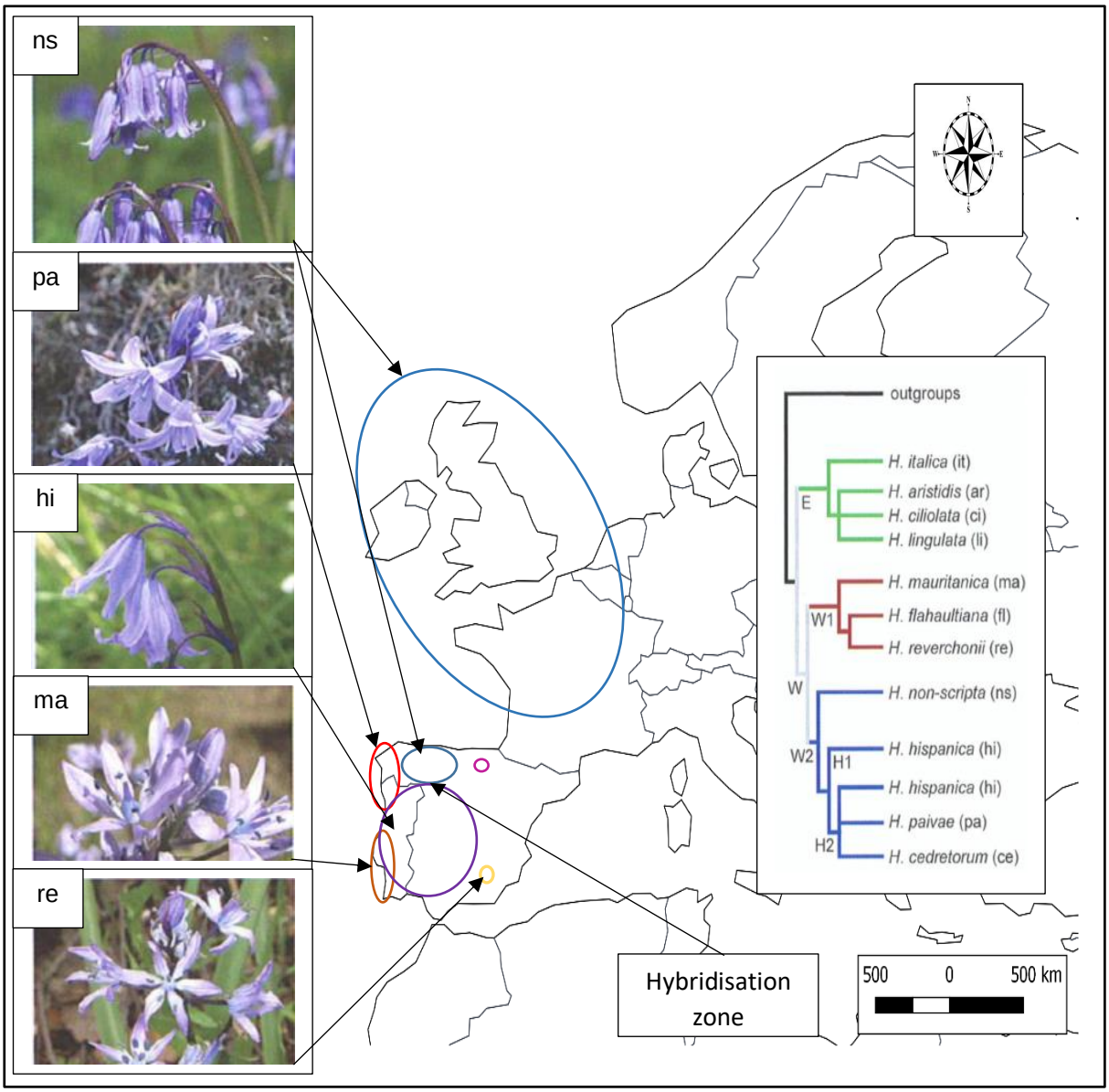

Figure 1. Geographical distribution showing flower variation with a simplified phylogeny of Hyacinthoides species. Dark pink circle indicates an area in northern Spain indicates with a known distribution of unidentified species.

(Distribution and phytogenic information by Grundman *et al.,* 2010) (Images from Grundman *et al.,* 2010)

Map: 1:250,000 Raster (TIFF Geo-Spatial) 1:250,000 OS Global Outline, Updated 2019, Using; Edina Digi-map Ordnance Survey Service < https://digimap.edina.ac.uk/roam/download/os> Downloaded: March 2019.

Maps created using QGIS 2.16.1 Nødebo.

Distribution of *hyacinthoides* is via two forms, seed germination and distribution through loculicidal dehiscence and natural vegetative reproduction. Bluebells prefer slightly acid soils as within the same woodland niche, alkaline conditions would normally be occupied by other species (Packham, 1992). As an adaptive woodland species, the young seed radicle is able to penetrate through a thick layer of leaf litter. Seed germination in both the *H. non-scripta* and *H. hispanica* is determined by a

two-phase temperature treatment due to their need to adapt to their distribution range (Blackman and Rutter, 1954; Thompson and Cox, 1978; Vandelook and van Assche, 2008). Distribution is based on temperature with the highest and lowest seasonal peaks prompting either seeding or natural vegetative reproduction (Blackman and Rutter, 1954). Bulbs have contractile roots and over the season draw themselves deeper into the soil. Primary bulbs produce daughter bulbs with each new bulb giving rise to a new plant.

1.3 Ethno-medicinal and Industrial Use

The bulb of the bluebell contains reserves of carbohydrate and sucrose in the form of fructose (fructan) (Brocklebank and Hendry, 1989). Throughout history the sticky sap produced by the bulb has been used in the manufacture of arrow-heads, the fixing of book bindings and as a starch that created a rigidness to the cuffs and collars of Elizabethan clothing (Simmonds and Sims, 2004). Medicinally, bluebells have supported the treatment of leprosy, snake bites (Simmonds and Sims, 2004), and leucorrhoea (discharge of mucus from the vagina) (Mulholland *et al.,* 2013). Bluebells contain glycosidase-inhibiting alkaloids (Watson *et al.,* (1997) and are known to cause abdominal pain, dysentery, lethargy and dullness in mammals once consumed (Simmonds and Sims, 2004; Watson *et al.,* 1997).

1.4 Overview of Bluebells in the British Isles

The United Kingdom currently maintains an estimated 25 to 50% of the world's population (Ingrouille, 1995) of *Hyacinthoide non-scripta* (L) Chouard ex Rothm. In Scotland the native/British bluebell (*H. non-scripta*) is referred to as the harebell, Campanula rotundifolia. The distribution of the *H. hispanica* (Mill.) Rothm., in contrast, only occurs naturally in the central to western regions of the Iberian Peninsula (Grundmann et al., 2010).

The *H. non-scripta* has come under threat due to over grazing, changes in land use, over exploitation due to commercialisation and more importantly, through the introduction of non-native horticultural varieties (Kohn *et al.,* 2009) such as the *Hyacinthoide hispanica* (Mill) Rothm. This has led to the hybridisation of the

indigenous *H. non-scripta*, creating a newly recognised sub-species, *Hyacinthoide 'x' massartiana* (Geerinck).

Selling of *H. hispanica* on a large scale commercial basis has allowed the sub-species to become naturalised, and with pollination compatibility, ubiquitous on a national scale. Concerns have been expressed regarding the future of *H. hispanica* and *H. 'x' massartiana* and its ability to outcompete and replace native species through a degradation of the native bluebells genetic integrity (Kohn *et al.,* 2009). Due to the *H. hispanica* and *H. 'x' massartiana* larger taxa (Figure 1), both species are now considered prevalent (Huxel, 1999 and Pilgrim and Hutchinson, 2004).

Further decline in the population of the *H. non-scripta* has to be considered, however, it is currently not clear in how that reversal will be achieved. Currently, restorative actions include the removal of invasive variants, reseeding from reliable seed sources, the possible introduction of Wild Boar (*Sus scrofa*) (Sims *et al.,* 2014) and a study into natural ecological breaks or hybrid zones (Marquardt, 2017). To understand if a natural barrier such as topography and flora density are able to protect natural bluebell stocks is important for the future integrity of the *H. non-scripta*.

1.5 Status and Protection of the *H. non-scripta* within the British Isles

Bluebells are a well-known plant with an iconic status within the British Isles. It is highly regarded for the swathes of violet-blue flowers and sweet scent (similar to sweet pea) that it produces in the ancient woodlands of the United Kingdom during the month of cca; April (Pigott, 1984). Rose (1999) has suggested that the *H. non-scripta* is an indicator species often denoting areas of ancient (British) woodlands; even once the woodland has been removed.

Bluebells are not very resilient and are prone to damage, especially through cutting, trampling, and over grazing either by cattle (*Bos tauros*) and/or wild boar (*Sus scrofa*). This is substantiated through leaf regeneration after Short-term response and recovery of bluebells (*H. non-scripta*) after rooting by wild boar (Sus scrofa) (Sims *et al.,* 2014). Bluebell leaves are redefined prior to season growth and once damaged cannot regrow during that season (Allun, 2016). As a consequence, the damage leads to a resource deficit in the present and subsequent year (Cooke,

1997; Grime *et al.,* 1988; Sims *et al.,* 2014); affecting overall flora density and sustainability. Areas of bluebells in the past have been decimated due to exploitation whether that was for commercial or private use. Due to this, the *H. non-scripta* became a protected species 1998 (Kohn *et al.,* 2009).

For over a decade, the advancement geographically in hybridisation has been studied. It is clear that the primary variable as to why hybridisation has increased significantly, is the modern retail ability to import invasive plant species into a country in such large quantities, that those plants once planted, have as a consequence, altered the natural balance (Kohn *et al.,* 2009).

In 1998 the native *H.non-scripta* became a protected species and was listed under the Wildlife and Countryside Act (WCA, 1981).

1.6 Studies and Surveys to Date

The hybrid *H. 'x' massartiana* was first noted in 1963 and since then has proved to be a highly aggressive and adaptable sub-species (Pilgrim and Hutchinson, 2004). A study in southern Scotland has seen a discernible increase in hybrid bluebells in as little as three years, with research in 2009 noting how prolific the *H. hispanica* is compared to the native *H. non-scripta* (Kohn *et al.,* 2009).

Due to reports of the decline in the density population of native bluebells, surveys have been conducted by established organisations such as the; Natural History Museum in conjunction with the Royal Botanic Garden Edinburgh (survey - 2006 to 2015), the Woodlands Trust (mapping - 2017) and the Royal Botanical Society for the British Isles (explore and record - 2019) (Rumsey 2006), to support research and gather information in regards to the potential loss of the native bluebell from all aspects of the United Kingdom's landscape.

Reports and surveys have primarily dealt with the distribution of the three taxa, and information has been collated and analysed from sightings gathered on a social science basis. However, through a lack of clarification (Kohn et al., 2009, Rix, 2004 and Grundman *et al.,* 2010) it cannot be concluded that these data has not considered the subtle genetic variations in its results.

This research has considered all variations and based on current genetic analysis by Grundman *et al.,* (2010) and Rix (2004), accepted the phenotypic criteria for the *H. non-scripta.* All other variations to the native bluebell criterion will be treated as invasive, however, due to the nature of the study, each variant will be confirmed statistically (Results: 3.1).

By accepting these principles of recognition, it is considered that a clearer overview will be gathered in how the variant *hyacinthoide* types (Rich and Woodruff, 1992) are distributed amongst a valley-situated deciduous woodland; clarifying how each variant is distributed from the potential point of source (Marquardt, 2016).

A preliminary review and count in 2017 of the village of Cranham, Gloucestershire and the adjacent woodland of the eastern side of Buckholt Wood (Figure 9), clearly indicated a change in genetic variations (reduction in inter-specific polymorphism) as the two invasive variants established themselves further away from the original point of source (Cranham village) in to the adjacent woodland (Marquardt, 2016 and Grundman *et al.,* 2010). By assessing the observations taken, it was the aim of the research to understand how these changes were affected by distance and elevation and woodland dynamics (Allum, 2016) or whether the initial pattern observed was not consistent throughout the whole of Buckholt Woods.

1.7 Aim of Study

It is the aim of the study to establish quantitatively the following: (1) Can the natural environment protect the increased hybridisation of the H. non-scripta through woodland topography and flora density? (2) Does the local planting of the H. hispanica and/or the H ‘x’ massartiana residentially, impact the United Kingdom’s natural stock of H. non-scripta? (3) Does hybridisation increase or decrease through changes in the natural organic structure of the woodland? (4) How widespread and abundant are is the native bluebell compared to the invasive H. hispanica and hybridised H. ‘x’ massartiana? (5) To what extent do the variants co-occur?

Chapter 2: Materials and Methods

2.1 Study Area

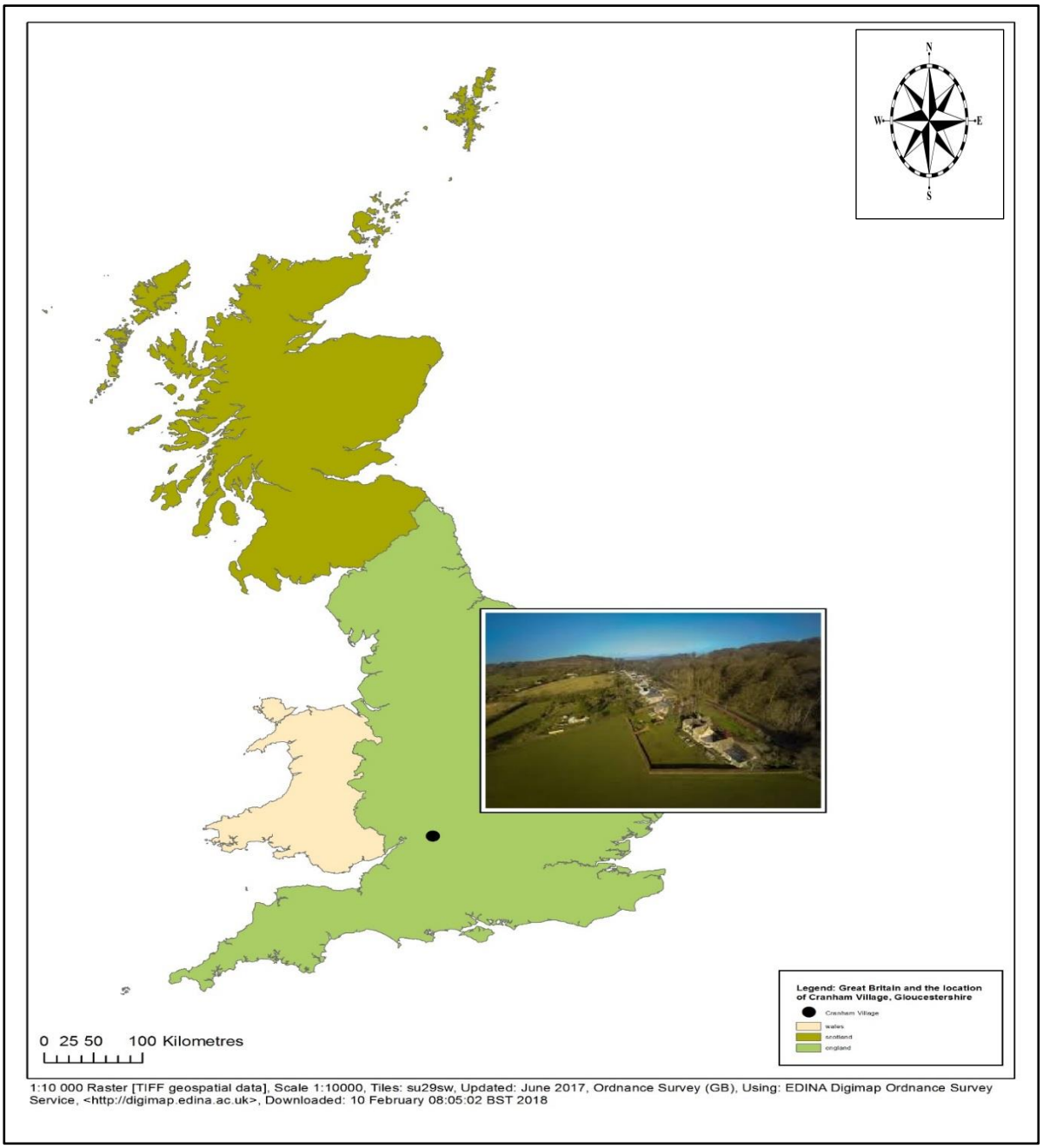

Figure 2. Map indicating the location of Cranham village and Buckholt woods, Gloucestershire: Village - SO 89667 12968, Buckholt Woods - SO 88944 13317. Maps created using QGIS 2.16.1 Nødebo.

The village of Cranham along with Buckholt Woods were chosen due to their proximity to each other. To understand all the variables associated in the hybridisation of bluebells, it was necessary to use a site where human mediated

planting of invasive bluebells (residential plus gardens) were situated close enough to a healthy woodland of geographical variance in topography so that data collected could indicate whether any of these factors impeded or increased hybridisation.

With recent surveys suggesting a reduction in the population of native bluebells, in combination with a possible increase in the population of invasive bluebells, it was an additional aim to understand if the natural environment, with its complexities of geography and floral dynamics, could protect native bluebells from silent extinction.

Buckholt woods forms part of Gloucestershire's Cotswold Commons and Beechwood National Nature Reserves (NNR). This NNR is currently the largest nature reserve in the Cotswolds and is protected as a European Special Area of Conservation due to its rare Beechwood, limestone grasslands and wildlife (Figure 3).

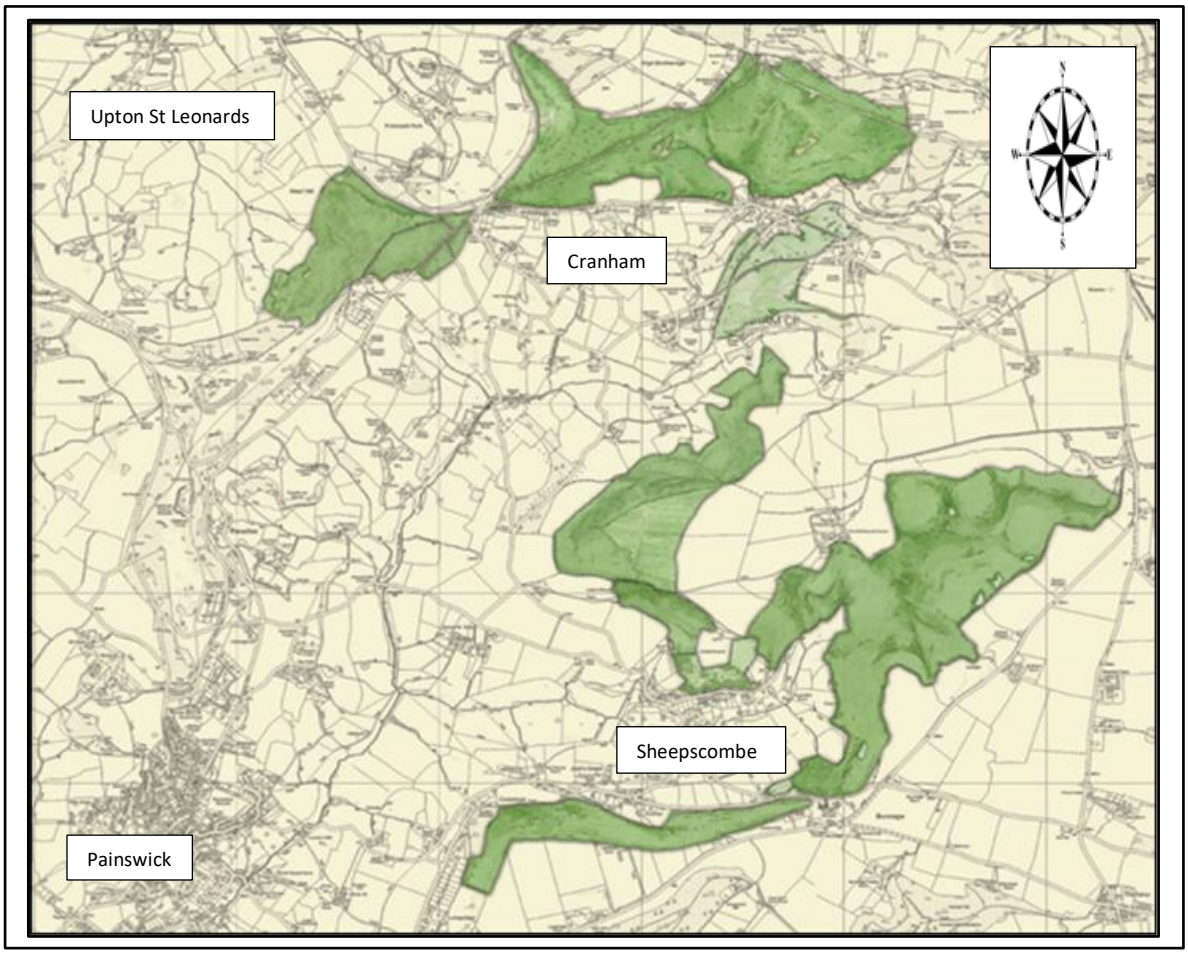

Figure 3. Overview map of the Gloucestershire's Cotswold Commons and Beechwoods National Nature Reserves (NNR) (Map from: NE335: Buckholt Wood: Cotswold Commons and Beechwoods National Nature Reserve., PDF: No scale) (Last accessed: 24/03/2019).

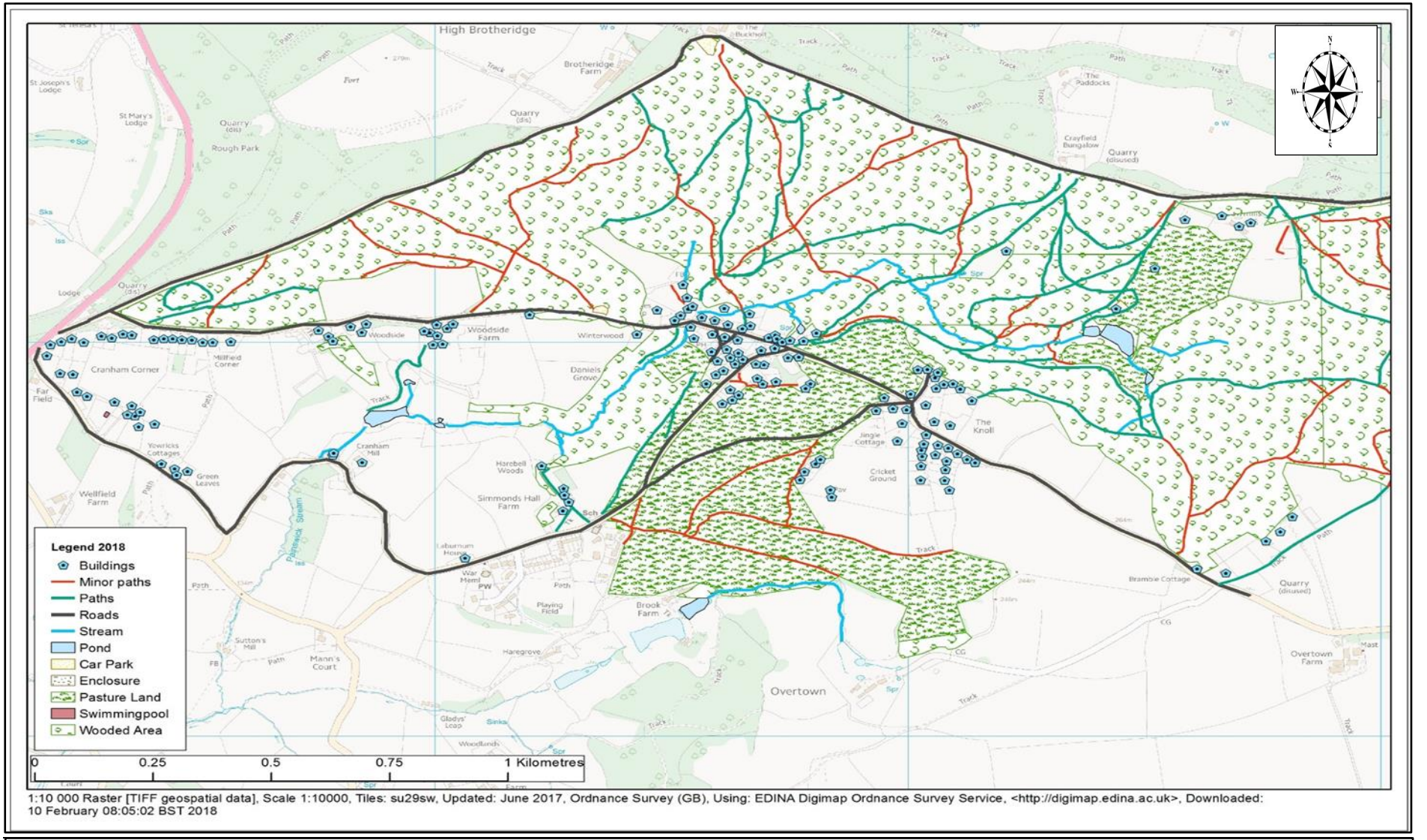

Figure 4. Digitised map of Buckholt Wood and the surrounding area including residential dwellings that make up Cranham Village. Maps created using QGIS 2.16.1 Nødebo. Map created from Edina Digimap, OS Historical Maps (Last accessed: 24/03/2019).

2.2 Bluebell Taxa and Identification

All variants of bluebell in the United Kingdom flower in spring. They are a bulbous perennial which prefer slightly acidic soil, primarily in deciduous woodland. During spring, the bluebells are seen in high densities occupying areas without competition from foliage such as Holly (*Ilex aquifolium*), Ivy (*Hedra helix*) and Wild Blackberry (*Rubus ulmifolius*), however, they will share areas that are occupied by plants such as Ransom also known as wild Garlic (*Allium ursinum*), Sweet Woodruff (Galium ordoratum) and Wood Anemone (Anemone nemerosa).

Pollination is known to be mediated by both the *Bombus* and *Syriphidae* species with the bulb being renewed annually. Positioning with the woodland is important as all variants of bluebells are prone to draught with the *H. non-scripta* being more sensitive than the invasive hyacinthoides (Blackman and Rutter, 1954; Littlemore and Barker, 2001). Seeds are not adapted to dispersal (Knight, 1964) and have no known dormancy apart from surviving the first winter (Thompson and Cox, 1978; Thompson and Grime, 1979). Germination is in late autumn due to seed conditioning induced by high temperatures followed by a drop in temperature to 11 ºC or less (Thompson and Cox, 1978). Seed survivability in the earth and their establishment is facilitated by mycorrhizal association (Merryweather and Fitter, 1995).

Invasive bluebells within the United Kingdom are often regarded as *H. hispanica* but no definitive confirmation exists genetically, to actually clarify that all invasive and or hybridised bluebells are from this phenotype; this also relates to the plants ecological requirements based on phenotype (Turrill, 1952). It is however, considered that commercial cultivators may represent the greatest source of hybridisation across an unknown number of *hyacinthoide* variants which in turn leads to high density of *H. hispanica* and *H. ‘x’ massartiana* in the wild. Recent surveys generally classify the bluebell variants as either native or invasive/alien without actual genotypic or phenotypic certainty.

2.2(a) *Hyacinthoide non-scripta*

H. non-scripta is a vigorous perennial with linear leaves (3 to 6) growing from the base of the plant, each 7 to 16 mm wide. The stem is erect bearing arching racemes (inflorescence of 5 to 12 flowers) of fragrant (Sweet pea / honey fragrance), narrowly tubular, violet-blue, or occasionally white, that are arranged in a drooped nodding pedicle construct.

Figure 5. Images of *H. non-scripta* (sourced from Google Images; Marquardt, 2016; Wetheral, 2017). Petal samples show pale cream anthers and pollen (Wetheral, 27 April 2017).

Each flower is 14-20mm with two bracts at the base. Tepals are strongly recurved at the tips and of a dark violet-blue colour. The stamens in the outer whorl are light in colour and are fused up to 75% of the perianth. Anthers are 2 to 3 mm and are covered in light cream / light golden coloured pollen.

Bulbs produce contractile roots that draw the bulb deeper into the soil horizons, seeking greater moisture. Bulbs can reach depths of 10 to 12cm but struggle with substrates that are difficult to penetrate – such as chalk. Enjoys inclement climates and reproduces well in wet and / or warm weather, although frost and colder weather can have a negative impact on dormant bulbs (Rose, 1981; Stace, 2010).

2.2(b) *Hyacinthoide hispanica*

A perennial adapted to warmer climates with linear leaves (5 to 20) growing from the base of the plant, each 10 to 35 mm wide. The stem is erect bearing arching to rigid racemes (inflorescence of 10 to 35 flowers joining in a spiral construct) with little to no fragrance supporting widely shaped perianth of a violet to light blue colour. Also, commonly occurring are plants with white and pink tepals.

Figure 6. Images of *H. hispanica* (sourced from Google Images; Marquardt, 2016; Wetheral, 2017). Petal samples showing dull blue anthers and pollen (Wetheral, 27 April 2017).

Each flower is 14-18mm with one to three bracts at the base. Tepals are slightly curled back at the tips and of a light violet, with a darker violet stripe along the outside of the petal, mirroring the line of the internal stamen.

The stamens in the outer whorl are light in colour and are fused up to 25% of the perianth. Anthers are 2 to 3mm and are covered in dark violet / blue pollen.
Bulbs also produce contractile roots that draw the bulb deeper into the soil horizons, seeking greater moisture to depths of 10 to 12cm. Enjoys higher temperatures but is not so well suited to wetter climates. During periods of excessive rain, can impede reproduction (Rose. 1981).

2.2(c) *Hyacinthoide 'x' massartiana*

H. 'x' massartiana (also known as *H. 'x' variabillis* (Sell and Murrell, 1996). Now native to the United Kingdom, however has in the last twenty years been noted in all countries with primary source of *H. non-scripta* and a human introduced source of *H. hispanica*. *H. hispanica* first established in Great Britain cca 1683 with hydride variations first recorded cca 1963 and recognised in 1987 (Preston *et al.,* 2002).

A perennial with linear leaves (5 to 20) growing from the base of the plant, each 7 to 35 mm wide. The stem is erect bearing arching to rigid racemes (inflorescence of 10 to 35 flowers joining in a spiral construct) with little to no fragrance supporting widely shaped perianth of a darker violet colour than H. Hispanica. Some plants do support a lighter blue colour. Also, commonly occurring are plants with white tepals.

Figure 7. Images of H. 'x' massartiana (sourced from Google Images; Marquardt, 2016; Wetheral, 2017). Petal samples show a variety of dull greyish undehisced anthers, but pale brown pollen (Wetheral, 27 April 2017).

Each flower is 14-18mm with one to three bracts at the base. Tepals are slightly recurved at the tips and of a darker violet-blue colour than the *H. Hispanica* - but lighter than the *H. non scripta.* The stamens in the outer whorl are light in colour and are fused up to 50% of the perianth. Anthers are 2 to 3mm and are covered in light yellow coloured pollen. Bulbs produce contractile roots to same and the conditions as *H. hispanica* (Rose. 1981).

2.3 Sampling Strategy and Independent variables

2.3(a) Overview of Site and Geography

It was planned that over a three year period (2017- 2019) the whole selected woodland area would be counted within the bluebell's flowering time frame. To ensure accuracy of counts and that all areas were visited, 1:1000 Master Maps were created using the most recent available data (2016) from Digimap (https://digimap.edina.ac.uk/) (Example – Figure 31) supported by a Garmin GPSMAP 62 hand held device.

The terrain undulated considerably which made count areas difficult to access from an incline perspective. Based on this factor and the size of the areas, it was decided to use the main cycle and horse paths (Rides) to differentiate between count areas. The areas ran horizontally from left to right, with paths dividing the count locations into blocks (north to south). Further detail about count locations explained at 2.3(b).

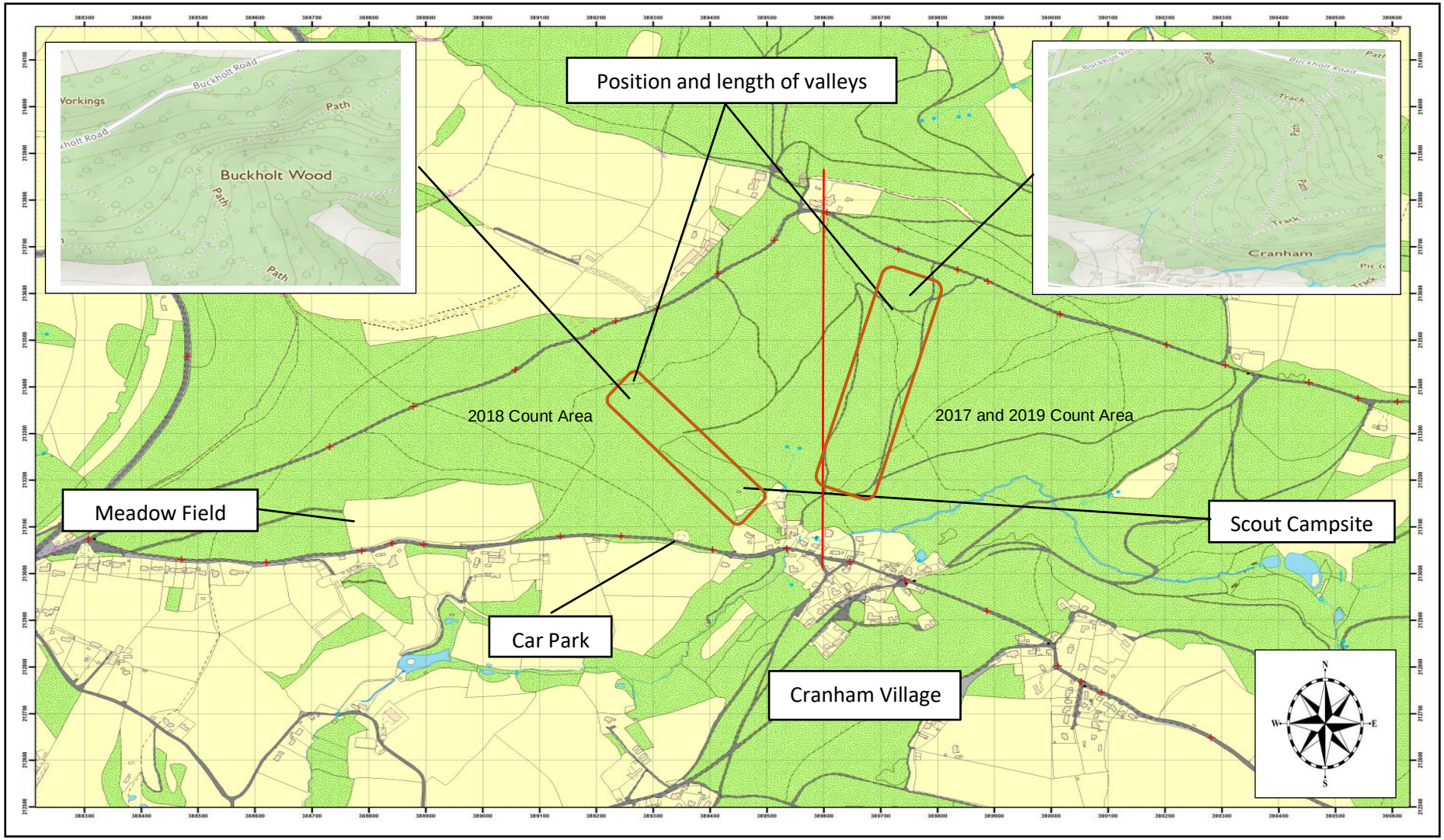

Figure 8. Overview of count area (red dots indicate circumference). (1:1,000 – Master Map: each Square =100m). Maps created using QGIS 2.16.1 Nødebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 24/03/2019)

At higher levels it proved more difficult to segment the counts due to topography. A ravine of significant depth runs from the northern edge of the Scout camp site up to the local minor road (Buckholt Road). Once the furthest edge of the camp site had been reached, counting was forced to the outer edge of the northwest area, rather than continuing the counting practice of moving from west to east horizontally; progressing up the rising incline from south to north (Figure 9 and 29).

Topography to the north western edge undulated to a greater degree with a higher density of trees. This made counting in smaller segments problematic and larger swathes were mapped to counter this.

Work maps (Example - Figure 30) of the area were produced prior to the counts taking place. The hope was to support movement and plan count areas prior to going to the woods. Once the counts started it was clear that human mediated change (horse and cycle) had introduced and degraded pathways that to this date have still not been mapped.

2.3(b) Survey Techniques

Areas were chosen based on their location and how their position fell within the remit of the thesis question (height and flora density affecting hybridisation). Counts were started at the southern south west corner and conducted horizontally (left to right) between mapped footpaths. This area is referred to as a ‘region’.

Regions were further defined into smaller ‘count areas’ based on bluebell density and ‘herb rich zone’ division. Once a count area had been selected, a numerical random generator would provide the grid reference to be surveyed (Figure 10). Dependent on area suitability one of two survey method types were chosen.

Two forms of survey adopted were;

Survey Type One (In-depth survey): involved a stratified random sampling format to fully survey the abundance of native, alien and hybridised bluebells. Additional information was gathered to see how those variables affected the growth of hybrids within each in-depth surveyed location. Additional information gathered included; elevation (m/above sea level (asl)), atmospheric temperature (ºC), wind speed (m/s), light measurement (Lux), soil moisture content (%), soil temperature (ºC), soil

construct (depth = 12.7cm), pH and incline (degrees and ratio) (Table 3 and Figures 25 to 28). Up to 30m x 30m measured transect ('X' and 'Y' axis), dependent on topography, was mapped out and five numerical random generated grid references were chosen for review (n=5 counts per location) (https://www.random.org). Each grid reference was surveyed using a 1m x 1m (100 square) quadrat. All bluebells within the positioned quadrat were counted by taxon and plant and soil samples were collected for 'keying' (Table 31 to 32).

Survey Type Two (Bluebell area mapping and bluebell count): This was a variation on the planned sampling technique (Survey sample one), and was adjusted by the removal of the in depth method used to ascertain the true non-bias of taxonomic variation based on probable variables. The full count method proved very time consuming and was not conducive to surveying a full woodland of approximately 4.8km². To counter this, it was decided to try and plot all bluebells and count a selection of bluebells (n=5 counts per count area) using a cluster sampling method.

Where an area of bluebells was intersected by other foliage ('margin') and formed a discernible count location (Figure 10), a single start point was established near to the path (Ride). The area of bluebells was calculated by walking the circumference from the start point using the 'Track Manager' option on the Garmin GPSMAP 62 (GPS) and entering the track as a waypoint (Figures 34, page 50).

During this exercise, clusters of plants were counted to a maximum quantity of 100 plants. Depending on the size (m²) this count quantity was altered, as some groups of bluebells did not have a maximum quantity of 100 plants. Each count location was marked by date and time, height above sea level and GPS position.

Soil Sample Locations: Three random areas were selected based on the lack of vegetation. The aim of this was to compare the soil samples taken against the soil samples taken from the 'full count' areas where other vegetation was often present. Along with the soil samples additional readings were taken such as: elevation (m/asl), atmospheric temperature (ºC), wind speed (m/s), light measurement (Lux), soil moisture content (%), soil temperature (ºC), soil construct (depth = 12.7cm), pH and incline (degrees and ratio) (Figures 24 to 26).

Locations	2017	count 1	count 2	count 3	count 4	count 5	Totals		
5	Quadrats	6	3	3	3	3	18		
	Total plants counted	100	64	58	344	105	671		
Locations	2018	count 1	count 2	count 3	count 4	count 5	count 6	Totals	
64	Quadrats	64	32	30	19	17	12	174	
	Total plants counted	5000	2939	2589	1435	1382	1037	14382	
Locations	2019	count 1	count 2	count 3	count 4	count 5	count 6	count 7	Totals
64	Quadrats	79	34	24	17	13	7	3	177
	Total plants counted	6250	3231	2035	1398	917	541	277	14649

Table 1. Number of locations and quadrats per location counted within Buckholt Woods, Cranham, Gloucestershire, during the years 2017, 2018 and 2019.

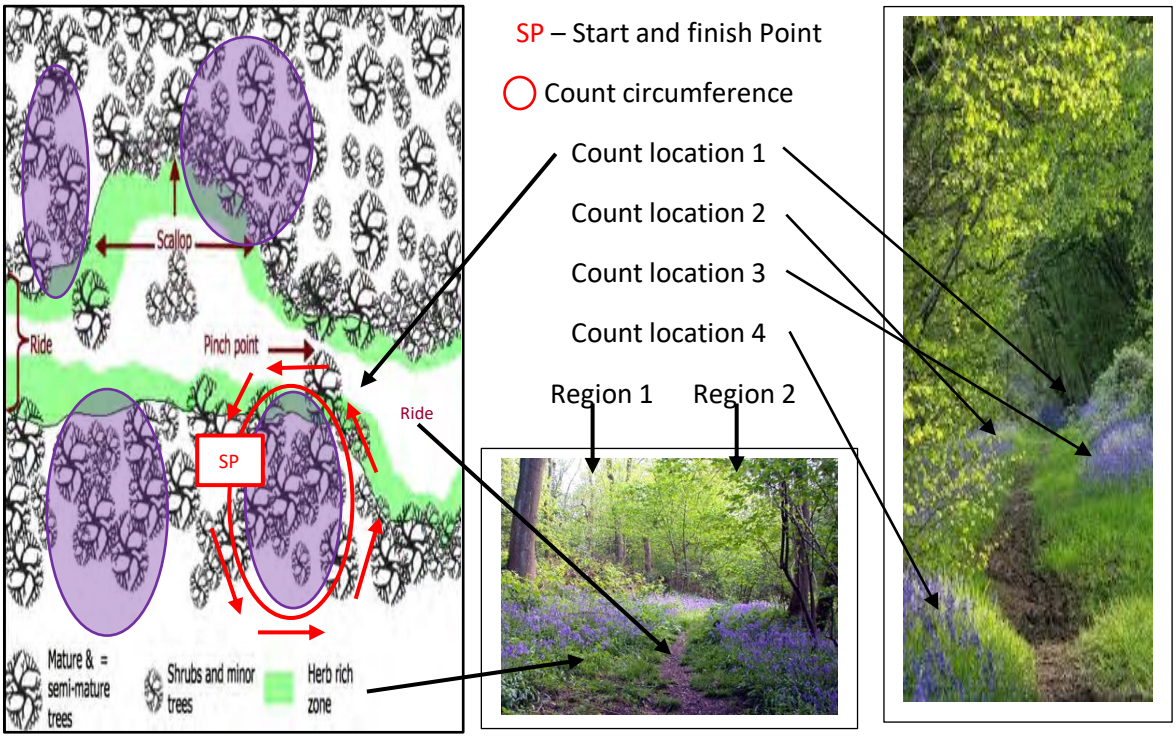

Figure 9. Survey example by Region and Count Location (Photographs: Google Images; diagram: English Woodland Grant Scheme, 2005)

Each taxon group by elevation were surveyed and counted to establish the population percentage by count location. Chi Squared Test of Independence and Bivariate Correlation (Spearman's Rho) in SPSS were used to confirm whether there was an association between each taxonomic group and an increase in elevation using frequency data converted to percentage by population (Kohn *et al.,* 2009). Co-occurrence was assessed graphically and descriptively based on elevation and also as an overall population indicator for the entire surveyed woodland. Further analysis considered habitat specificity evaluating the three taxa according to Levin's B theory (Hulbert, 1978) to quantify observed deviation against expected habitat occupancy (based on information presented by the Cotswold Commons and Beechwood National Nature Reserves (NNR)).

2.3(d) Climate Conditions

Climatic influences were analysed using Time Series Modelling Analysis as a 'Moving Average Computation' and Bivariate Correlation (Spearman's Rho) in IBM SPSS based on data provided by the Metrological Office for the South West of England and Wales 2019. The aim of these analysis was to see if there were a significant change in meteorological differences over time that would support the bluebell frequency data found within Buckholt Woods.

2.3(e) Woodland Flora by Taxon and Soil Samples

As part of the 'Survey Type Two' process, data collected were analysed using a Multi-nominal Regression analysis model in IBM SPSS. The aim of these analysis was to see if there were an association between each count year (west of woodland 2018 and east of woodland 2019), based on other topographical features rather than that of elevation.

Survey Type One gathered biotic samples for comparison and abiotic readings were collated to consider association to the frequency of bluebells noted in each specific sample location (Table 3). A Simple Linear Regression in SPSS was used to ascertain the association between frequency counts by taxa and pH levels in

conjunction with graphical models to determine clarification between each abiotic reading, taxa frequency and location.

Chapter 3: Results

Survey data from both type one and type two surveys were consolidated by year into tables (2018; 2017 and 2019 combined) and standardised in the following formats for statistical testing:

3.1 Testing of taxa by elevation

Elevation range (165m to 174m) was condensed into five equal groups of metres above sea level. To complement this standardisation, frequency count data were also condensed under each elevation group (Table 2). Frequencies were averaged by taxa within in each elevation group. A Chi Squared Test of Independence was used with a weighted frequency conversion (Waite, 2000).

When elevation was tested against taxa to see whether increases in elevation would alter the percentage of hybridised bluebells found at the woodland, results demonstrated a non-significant correlation (X^2(12)>=15.00, p=.241) (Table 2 and Figure 11) between the four variables (dependent – elevation; independent – 3 x taxa*: H.'x' massartiana, H. non-scripta* and *H. hispanica*).

However, a Chi Square analysis using a weighted frequency NPAR test, whereby, each taxon was tested against elevation individually, proved significant; indicating the probability of a relationship between the elevations observed and the frequency of each of the two main species of *Hyacinthoide; H. non-scripta* - X^2(12)>=45.00, p=<0.001 and *H. 'x' massartiana* - X^2(12)>=45.00, p=<0.001 (Figure 10).

Elevation	XM	NS
180-209	82	18
210-229	36	64
230-249	33	67
240-259	82	18
260-279	13	87

Table 2. Taxa by mean average totals consolidated into five grouped ranges of elevation.

Both sets of results indicate further consideration outside of the initial hypothesis. By testing each taxon individually against elevation, has pointed the research towards an alternative variable for consideration. The data at this point indicated a perceivable association of bluebells based on an east/west geographical and topographical divide rather than a comparison towards elevation only (Figure 18).

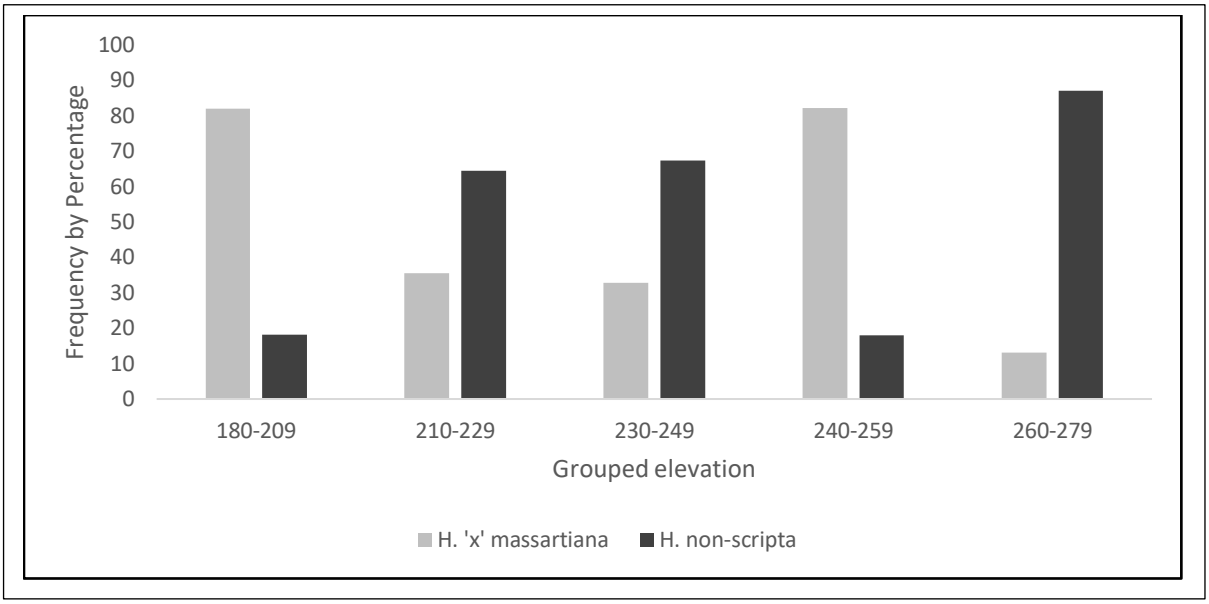

Figure 10. Consolidated yearly frequency counts by grouped elevation range.

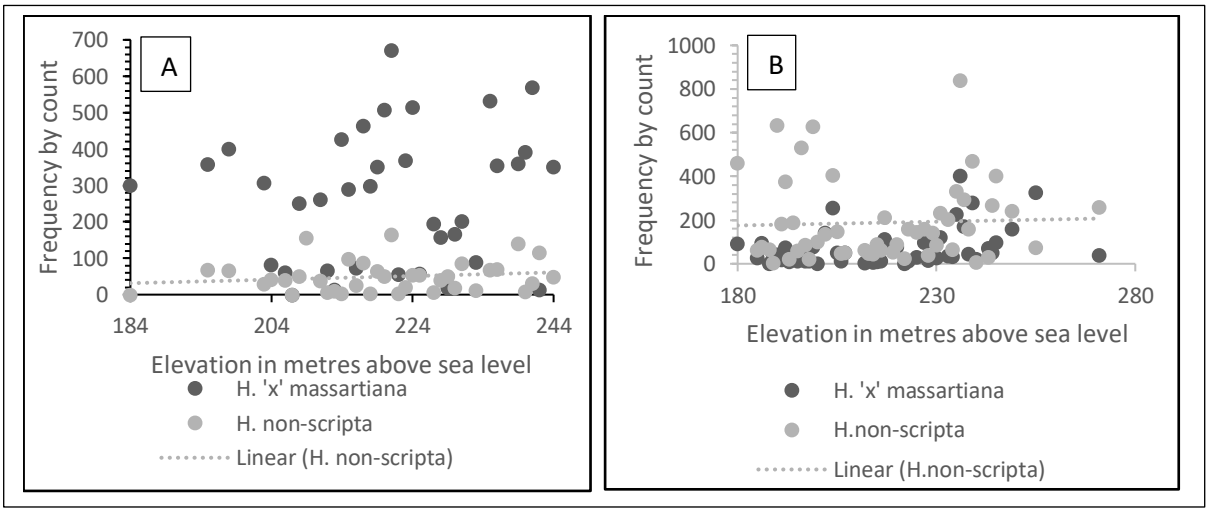

Figure 11. Graphs show non-transformed frequency count data distribution when analysed against non-transformed elevation range. Graph A – 2018 counts (west). Graph B – 2017 and 2019 counts (east).

3.1(a) Inter and intra species testing

Comparisons of intra-specific and inter-specific taxa was carried out using a Bivariate Correlation (Spearman's Rho) analysis and Chi Squared test of

Independence using the full range of frequency data. Through a number of tests, a pattern occurred between the data collected in the west of the woodland (2018) and of that collected in east (2017 and 2019). Initial test was to ascertain whether a correlation could be seen between intra-species from across both years, looking for a frequency split that would highlight an association through numerical quantities. This graphed frequencies as expected (Figure 12).

A significant result between both sets of *H. 'x' massartiana* data (Figure 12) was seen, with a non-significant result noted for the combined sets of the *H.non-scripta* data. It is proposed that this result is due to the frequency of *H. non-scripta* data collected in 2018 (west) being of a much lower percentage than that collected in the east (2017 and 2019) (Table 3).

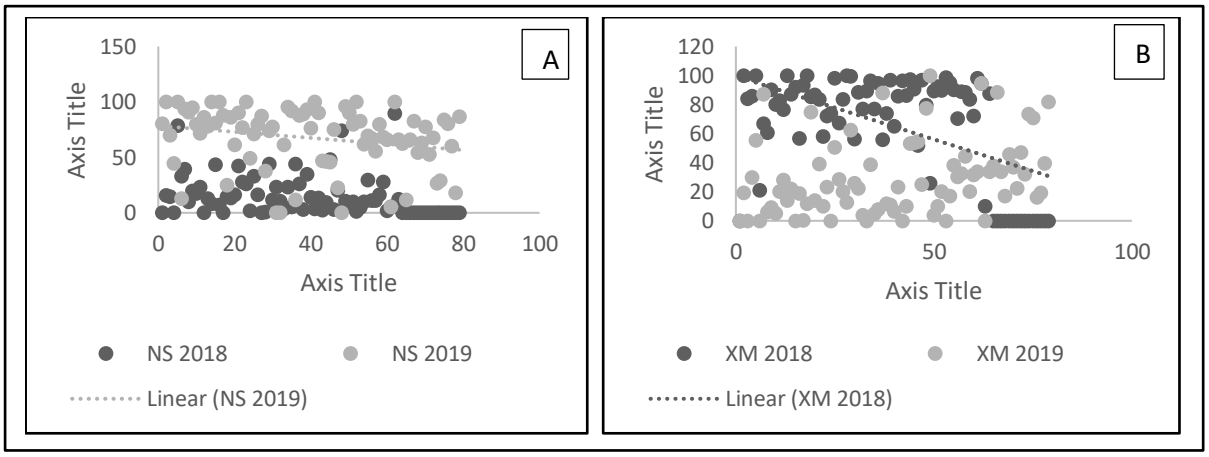

Figure 12. Correlation of intra-species data collected in 2018 (west) and 2019 (east). Correlation for both graphs registers as weak. *H. non-scripta* graph A indicates a non-significant result; *Rho* = -0.028 (F1, 77 = 0.05918, *p*=>0.81). *H.'x' massartiana* graph B demonstrates a high probability of difference between those samples collected 2018 compared to sample collected in 2019 (*Rho* = -0.06 (F1, 77 = 4.840, *p*=<0.03)).

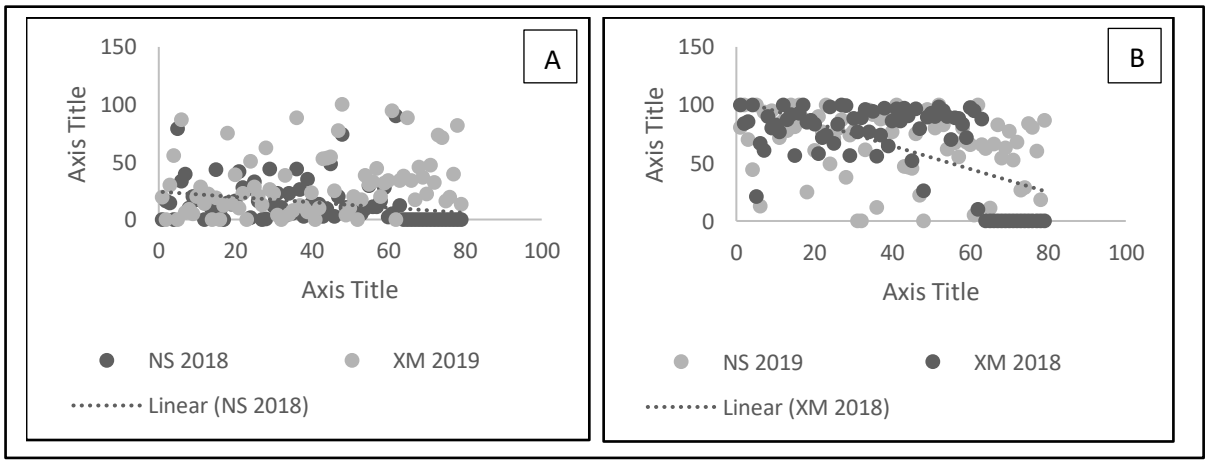

Figure 13. Correlation of Inter-species data collected in 2018 (west) and 2019 (east).Correlation for both graphs registers as weak. A significant result for both data sets indicates a strong association between *H. non-scripta* vs *H. 'x' massartiana* by alternative years. This suggests that the difference between the frequency counts are clear alternatives and once plotted have an association through percentage total by topographical division: (A) NS 2018 vs XM 2019 = *Rho* = -0.081 (F1, 78 = 0.05159, *p*=<0.001), (B) NS 2019 vs XM 2018 = *Rho* = -0.38 (F1, 78 = 0.05634, *p*=<0.001).

2018		2017 & 2019	
Total XM %	86.20	Total XM %	29.09
Total NS %	13.80	Total NS %	69.53
Total H %	0.01	Total H %	1.37
Grand total %	100.00	Grand total %	100.00

Table 3. Overall percentage cover of bluebells in Buckholt Woods, Cranham, Gloucestershire. 2018 = west side of Buckholt Woods; 2017 and 2019 east side of Buckholt Woods. Key: XM – H. 'x' massartiana, NS – H. non-scripta and H – H. hispanica.

Analysing the inter-specific data, based on the same parameters of west and east, highlighted a significant result across both tests (Figure 13). These results show a correlation between each data set over the two years and how, as a percentage of the total count, the alternative values for each taxa, demonstrates a swap in percentage totals by taxa across the west/east geographical divide (Table 3).

3.2 Climate Conditions

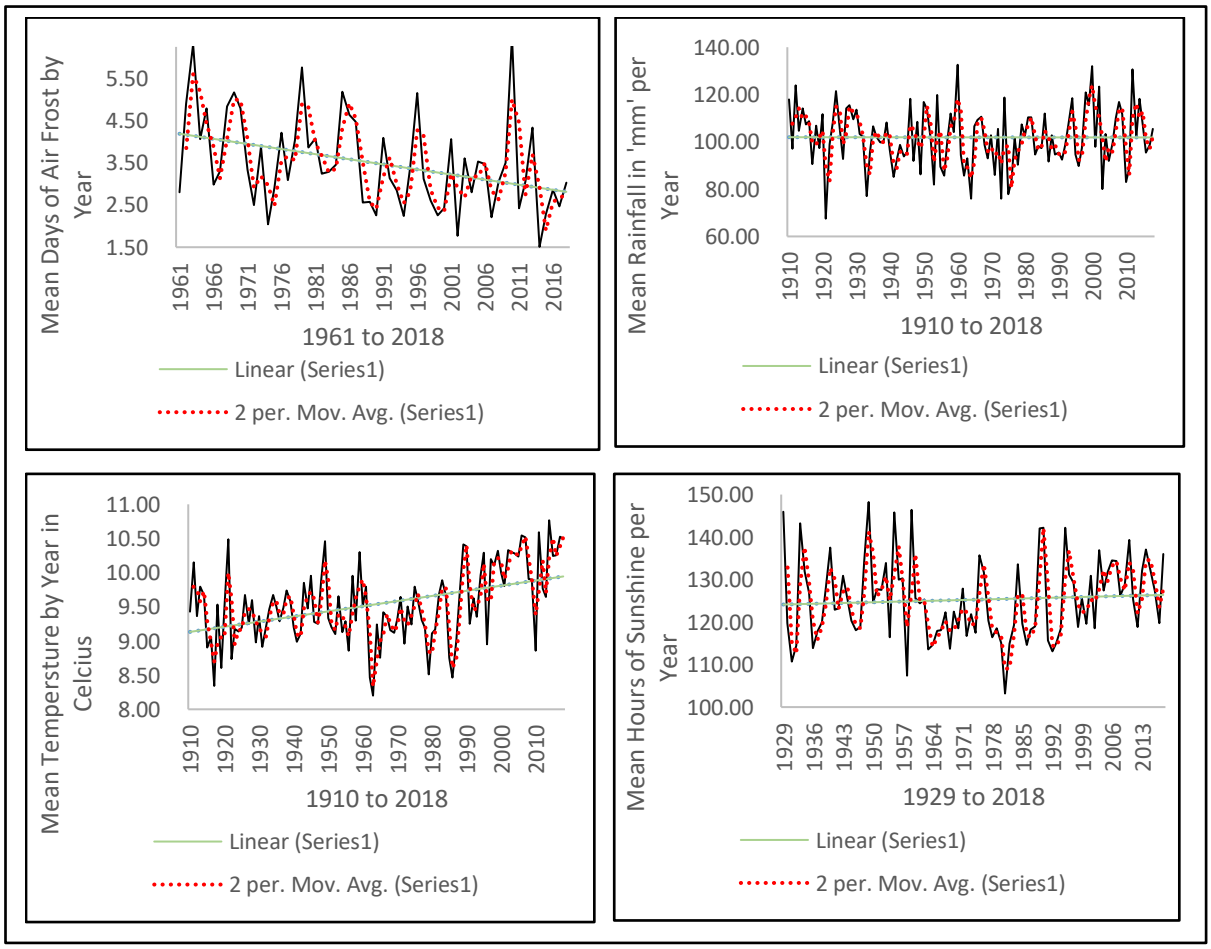

Figure 14. Time Series Analysis for prime abiotic factors that may contribute to inter-species competition and an increased level of hybridisation of *H. 'x' massartiana* over *H. non-scripta.*

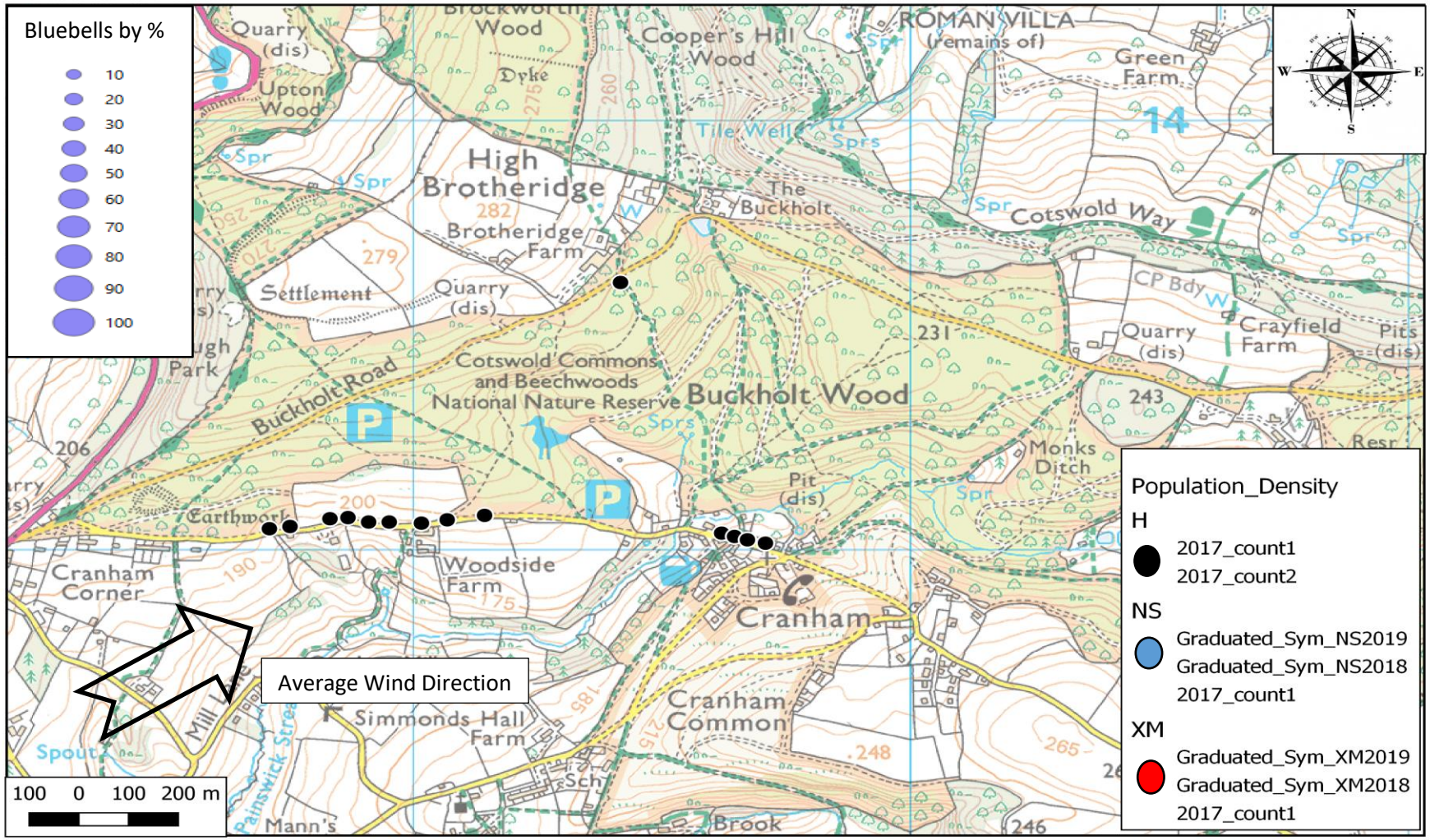

Figure 15. Overview of *H. hispanica* frequency counts using 3D spatial analysis. (1:50,000 – Explorer Map). Maps created using QGIS 2.16.1 NØdebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 14/05/2019)

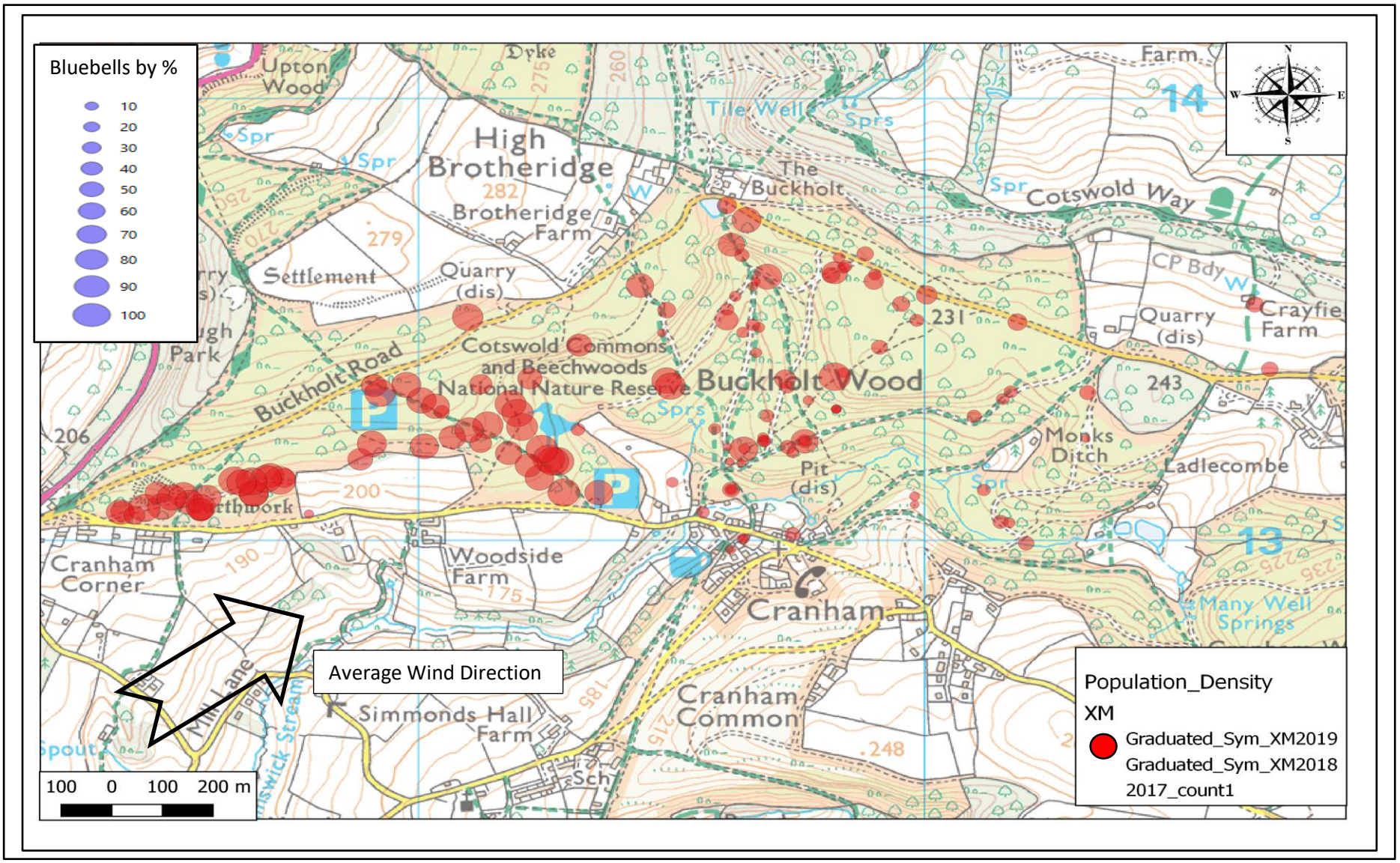

Figure 16. Overview of *H. 'x' massartiana* frequency counts using 3D spatial analysis. (1:50,000 – Explorer Map). Maps created using QGIS 2.16.1 NØdebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 14/05/2019)

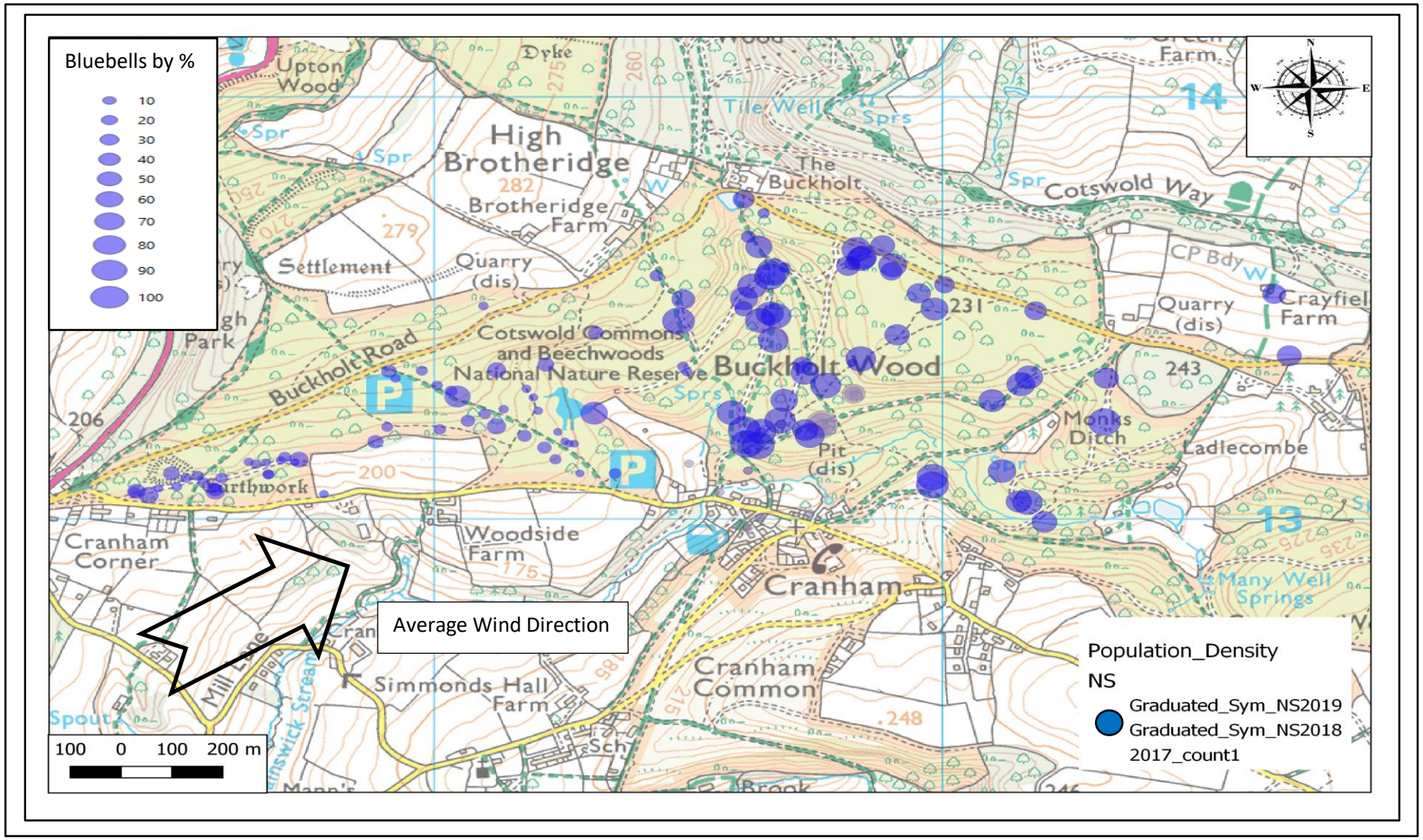

Figure 17. Overview of *H. non-scripta* frequency counts using 3D spatial analysis. (1:50,000 – Explorer Map). Maps created using QGIS 2.16.1 NØdebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 14/05/2019)

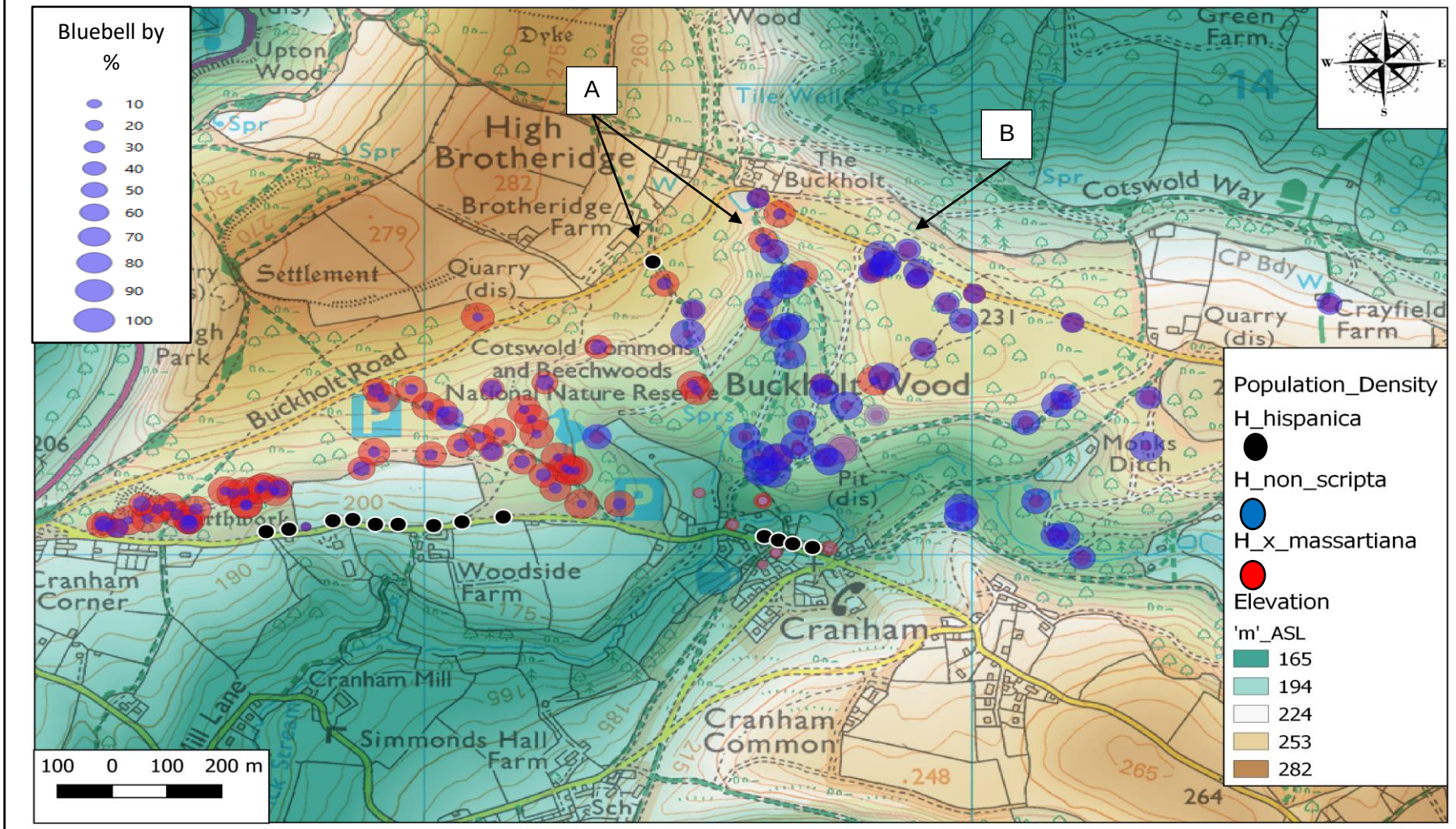

Figure 18. Overview of *hyacinthoide* frequency counts by taxa using 3D spatial analysis including colour coded elevation. (1:50,000 – Explorer Map). Maps created using QGIS 2.16.1 NØdebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 14/05/2019)

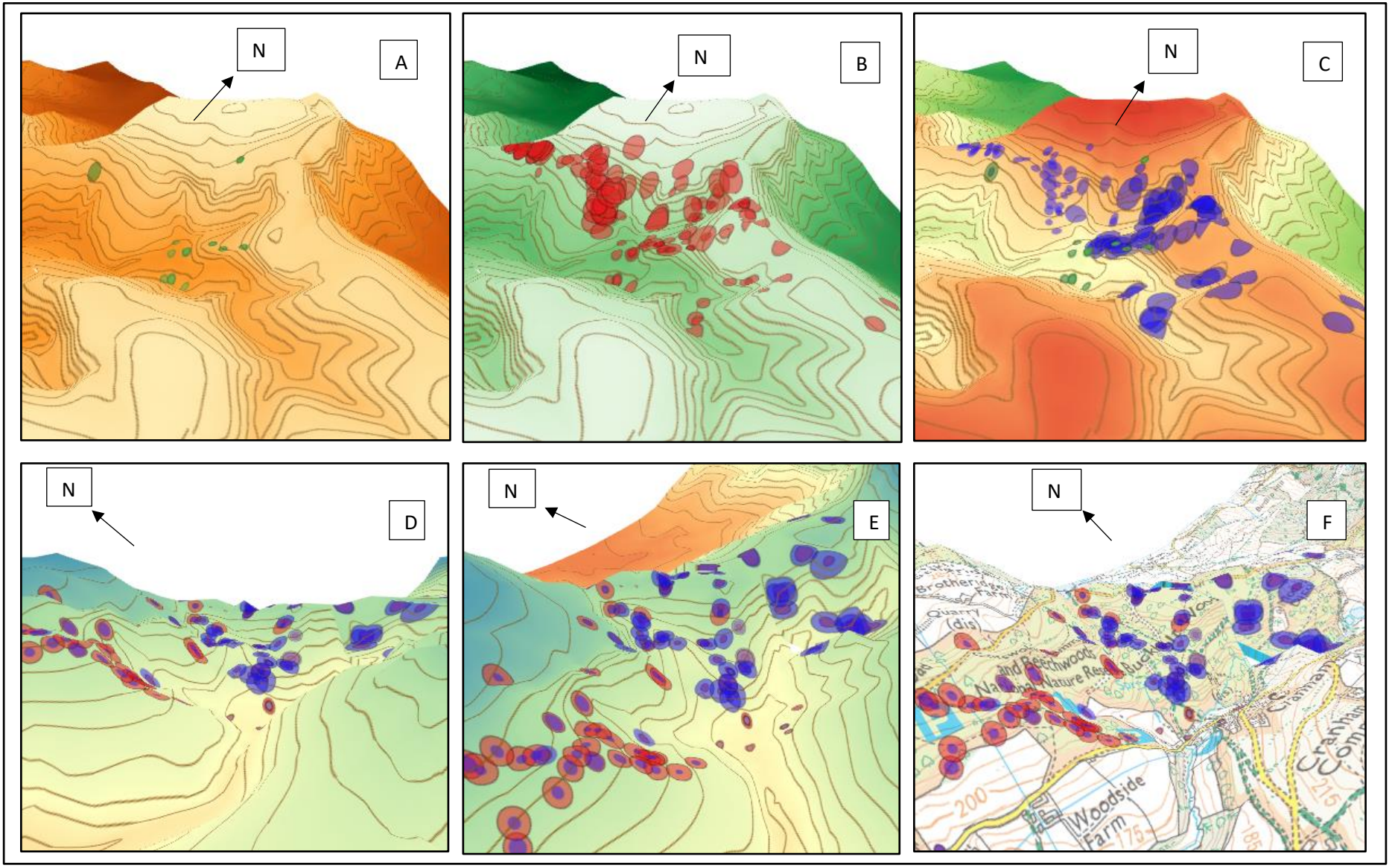

Figure 19. 3D topographical maps with hyacinthoide distribution using spatial analysis. A – Sites of *H.hispanica* frequency collection. B – Sites of *H.'x' massartiana* frequency collection. C – Sites of *H. non-scripta* frequency collection. D – Spatial analysis of taxon distribution from west to east. E – Main area of taxon division as seen from 3D spatial analysis. F- Taxon spatial analysis plotted to 1:50,000 OS map showing all localised infrastructure. (1:50,000 – Explorer Map). Maps created using QGIS 2.16.1 NØdebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 14/05/2019)

Data indicates a move to warmer mean temperatures by year (r = 0.255, n = 110, p = <0.001) with a reduction in mean days of air frost (r = -0.367, n = 58, p = 0.005), whilst mean average rainfall in 'mm' per year (r = -0.32, n = 109, p = <0.62) and mean hours of sunshine per year (r = 0.73, n = 90, p = 0.30) show little change over the data period.

Data indicates a general warming for the localised area with a reduction in air frost days which in turn favours the invasive *H. hispanica* and *H. 'x' massartiana* (Marquardt, 2016). *H. non-scripta* habitually is more reliant on ground cooling during the autumn and winter periods and more tolerant of wetter rather than dryer spring conditions (Marquardt, 2016).

3.3 Flora by Taxon and Soil Condition

3.3(a) Flora distribution by taxa

Flora distribution across the woodland is not fully uniform and data collected at this juncture is not able to definitively conclude as to why this should be the case (See 3.4(b)). However, a variety of trees can be seen throughout all levels with the Beech (*Fagus sylvatica*) being the most dominant. Most notable plant is Ivy (*Hedra helix*) (Figures 20 to 23)

Data collection was limited to twelve sites (Survey Type One), one of which included the village gardens of Cranham; of which no woodland plants were found. Associating flora with other abiotic and biotic factors in graph and table form ($X^2(25)>=21.50$, $p=.664$), shows no correlation to each other with the exception of the level of pH compared to the density of bluebells sampled by taxa at those locations (Figures 24 to 26).

A vicariance can be defined by the change of percentage in taxa between where 2018 counts finish and 2017 and 2019 counts started. Topographically the terrain changes, moving from a relative flat plateau in the west (2018 count) in to a steep valley in the east (2017 and 2019 counts), culminating in a semi-flat plateau. Centrally this area is partitioned by a thin strip of calciferous grassland of approximately 6 to 8 meters wide.

High percentages of *H. 'x' massartiana* can be seen in the northern area of the east (2017 and 2019 counts) (Figure 18 – position A) weakening the overall percentage of *H. non-scripta* in the eastern count area. It is suggested that this may be correlated to the adjacent woodland and placement of residential dwellings at this location. The contrary can be seen at the north-eastern side (Figure 18 – position B) where with the lack of residential dwellings, the maintenance of native bluebells is held at a higher percentage overall (69.53% - Table 3).

Survey Type One conducted at sample sites 1 to 6 shows a probability that the level of pH may have an effect on the type of bluebell that out competes the other at certain levels.

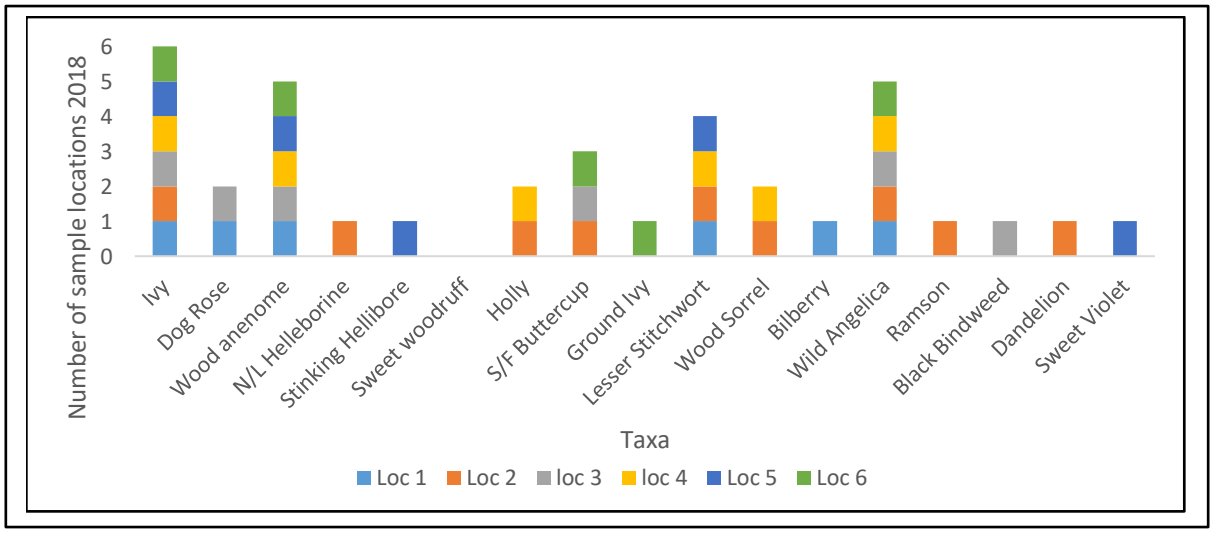

Figure 20. Survey Type One conducted on the western side of the woodland in 2018 and the plants sampled at each full sample site.

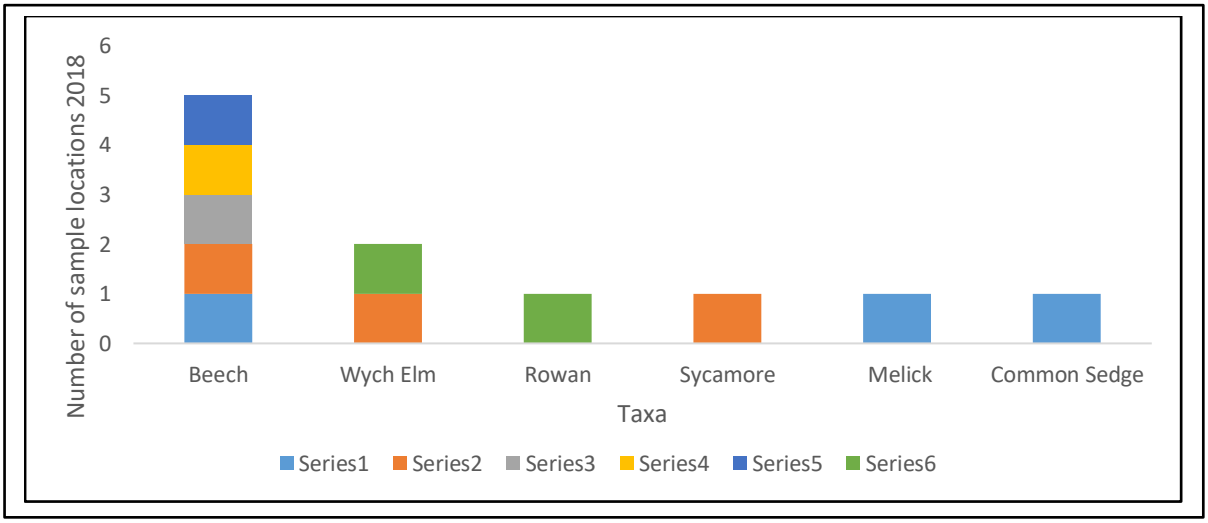

Figure 21. Survey Type One conducted on the western side of the woodland in 2018 and the trees sampled at each full sample site.

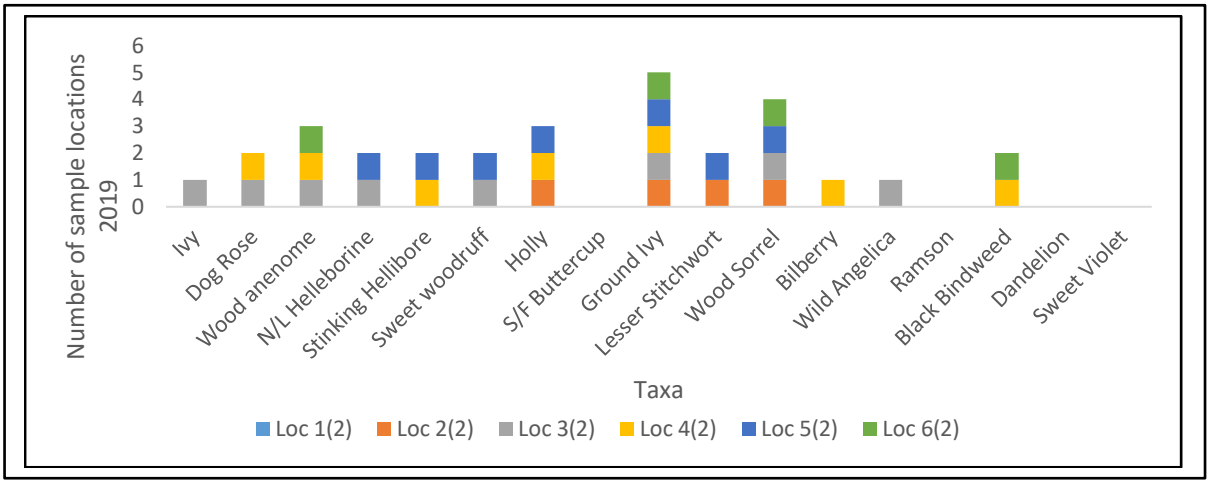

Figure 22. Survey Type One conducted on the eastern side of the woodland in 2017/2019 and the flora sampled at each full sample site.

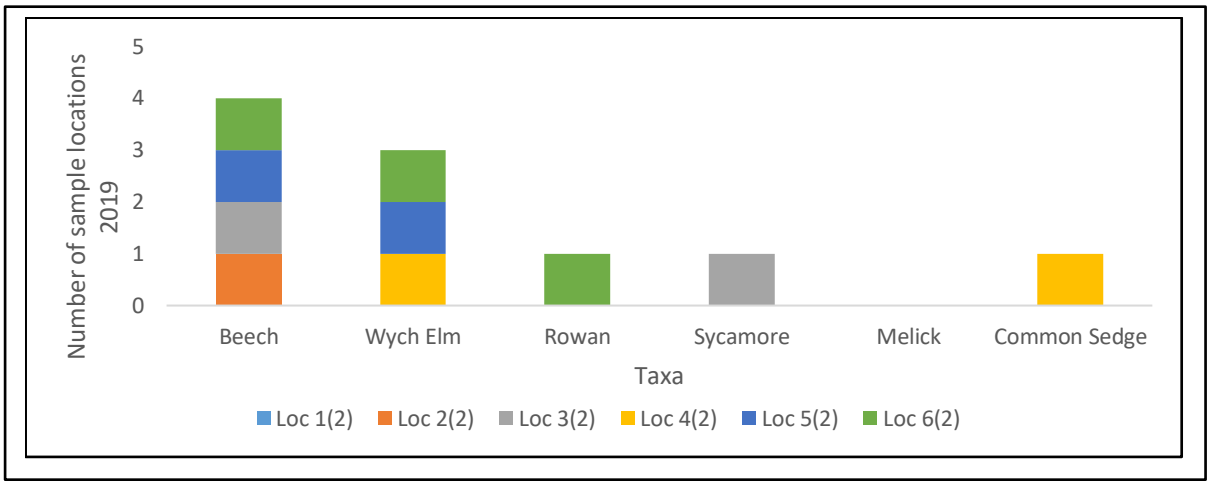

Figure 23. Survey Type One conducted on the eastern side of the woodland in 2017/2019 and the trees sampled at each full sample site.

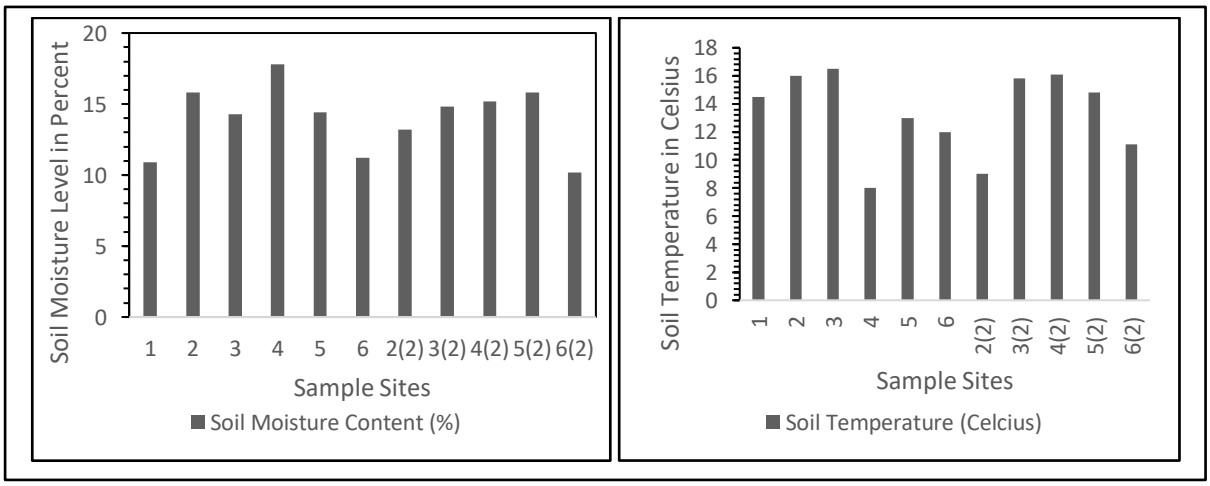

Figure 24. Survey Type One abiotic and biotic data. All locations sampled with the exception of 1(2), gardens within Cranham village.

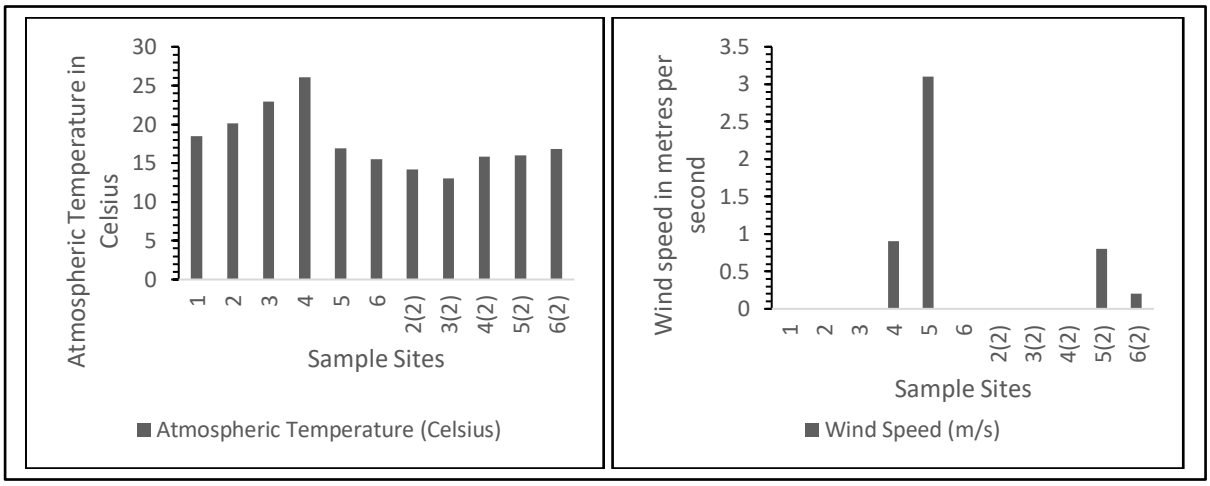

Figure 25. Survey Type One abiotic and biotic data. All locations sampled with the exception of 1(2), gardens within Cranham village.

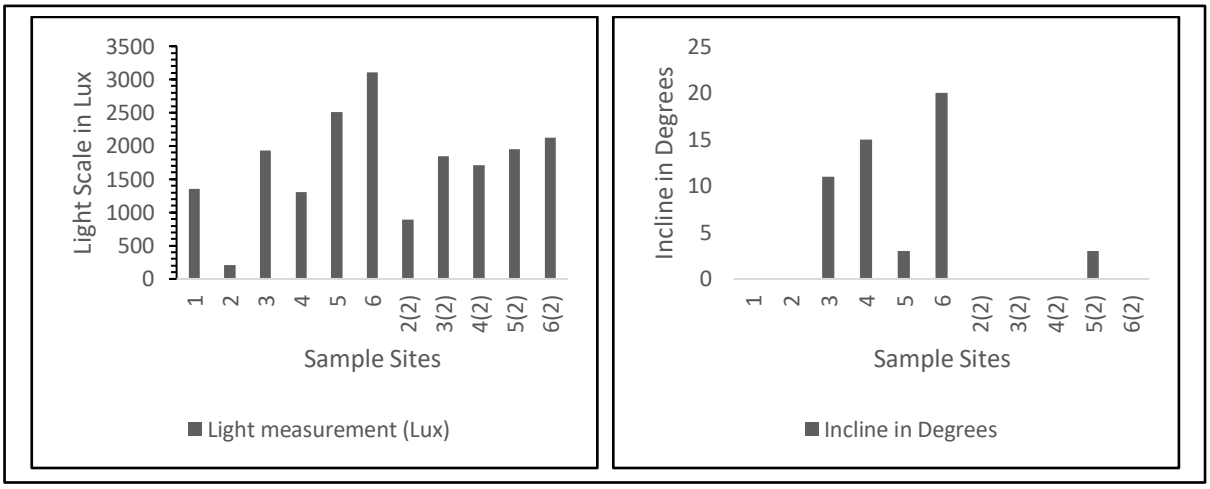

Figure 26. Survey Type One abiotic and biotic data. All locations sampled with the exception of 1(2), gardens within Cranham village.

3.3(b) Soil Condition

Soil pH graphically indicates a discernible trend in the level of pH and the type of bluebell that grow predominantly in that location, however, whilst there is a strong correlation, the overall result indicates a non-significant result (R = .998, $F_{1,3}$ = 156.986, p = <.06). Further research by G. H. Knight (1964) noted that bluebells can be found in dryer soils with a pH range of pH 4.5 to 5.0 as described by Blackman and Ritter (1954), but indicated that most bluebells were physically weaker and in minimal abundance. Bluebells found in moist, loamy soils with a pH range of

between 7.5 and 8, were recorded in greater abundance with a strong phenotypic presence (Knight, 1964).

Location	H. 'x' massartiana Total	H. non scripta Total	Total Bluebell Count	pH
1	13	116	129	8.0
2	57	53	110	7.0
3	167	18	185	5.0
4	91	10	101	6.0
5	9	7	16	6.0
6	82	41	123	5.0

Table 4. Data corresponding the quantity of bluebells by taxa as a percentage compared to soil pH.

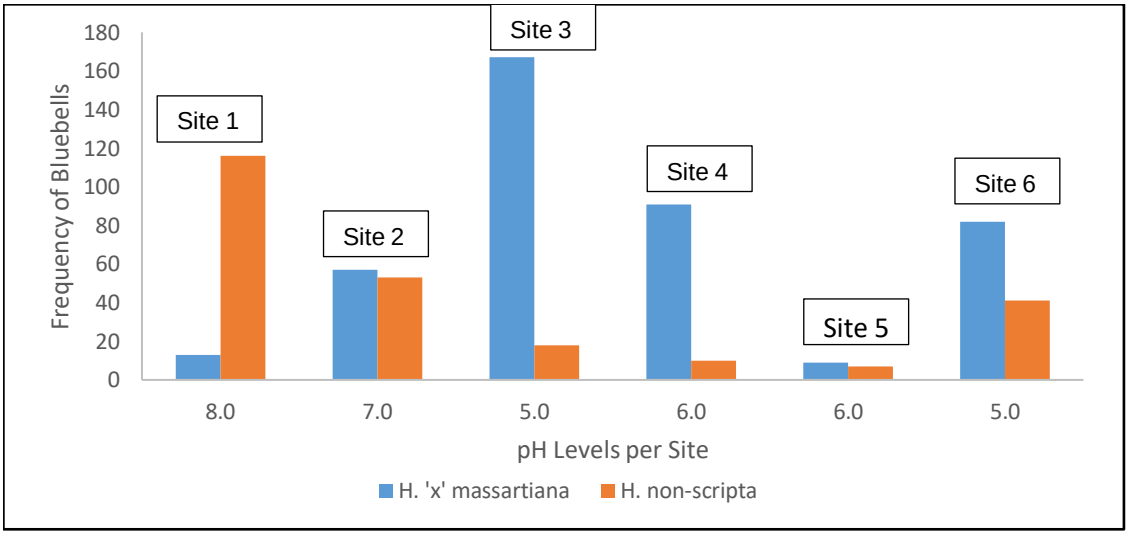

Figure 27. Graph demonstrating the correspondence of bluebells by taxa as a percentage in relation to soil pH.

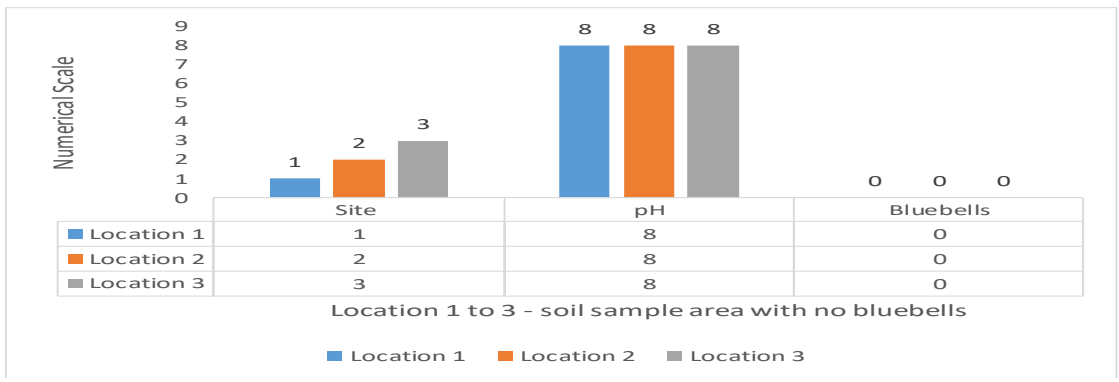

Figure 27(a). Sample test areas with no flora growth x 3.

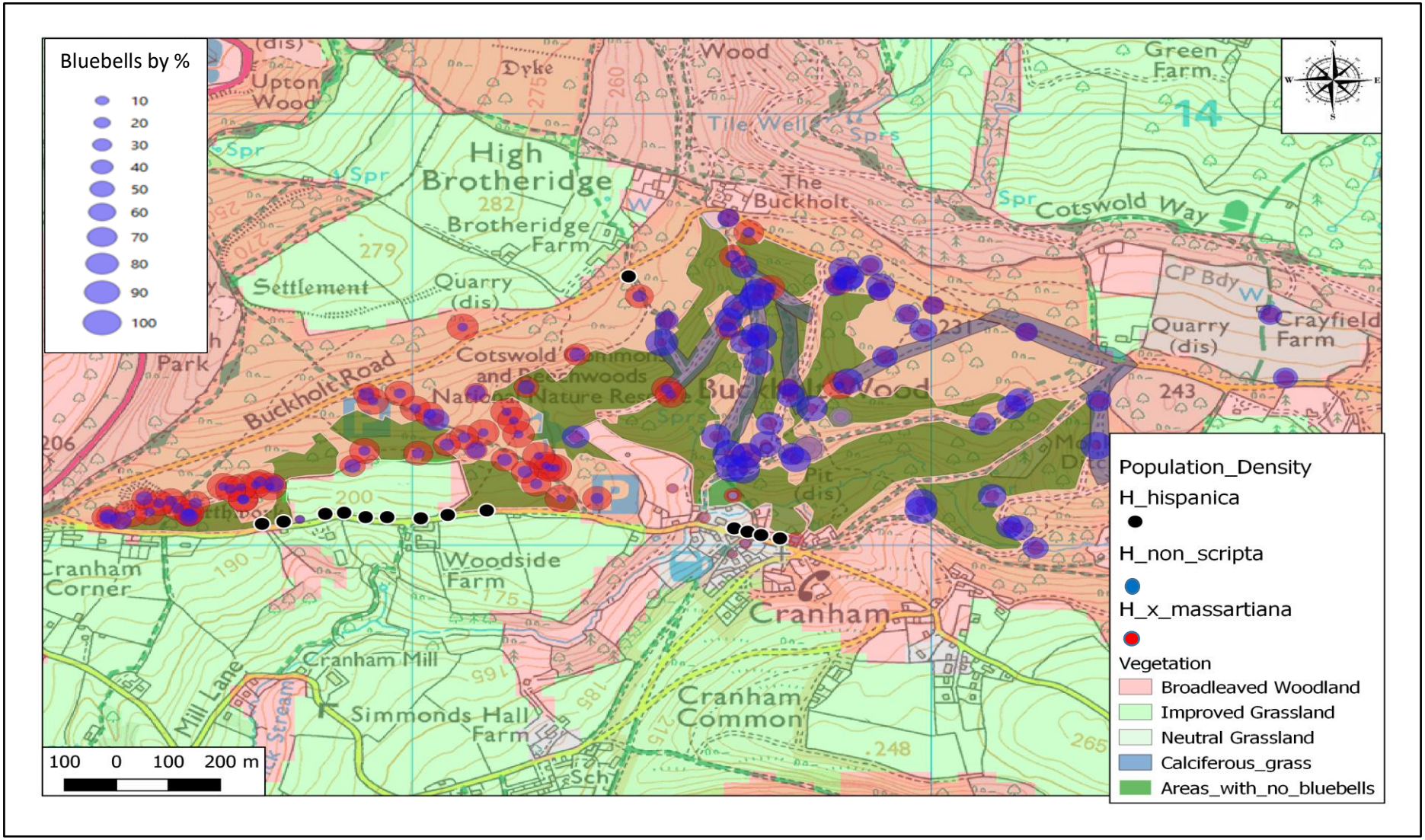

Figure 28. Bluebell distribution as a percentage by taxa compared to vegetation by type and areas where no bluebells were located due to varying biotic factors (1:50,000 – Explorer Map). Maps created using QGIS 2.16.1 Nødebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 14/05/2019)

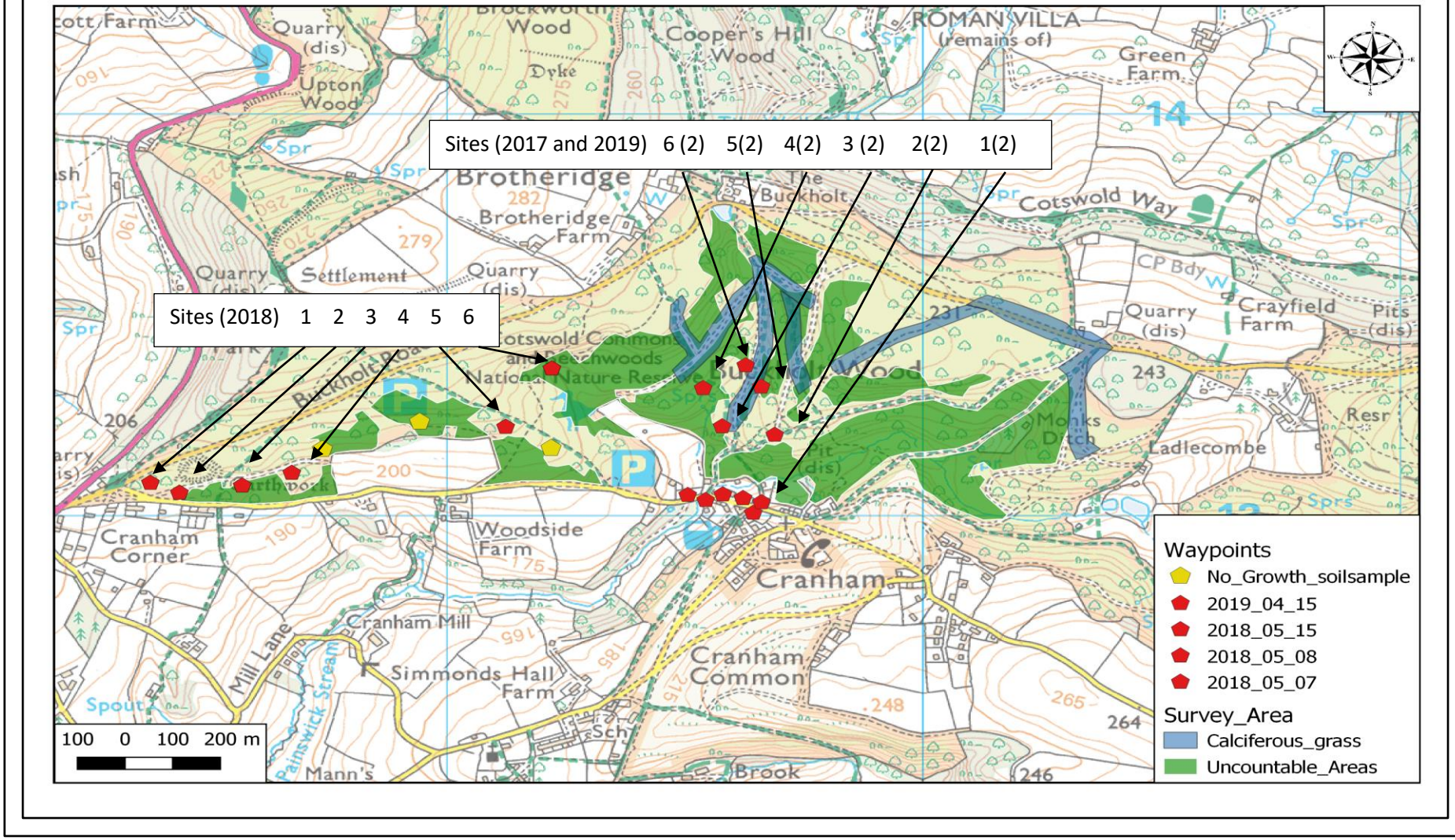

Figure 29. Survey Type One sample sites. (1:50,000 – Explorer Map). Maps created using QGIS 2.16.1 NØdebo. Map Ordnance Survey from https://osmaps.ordnancesurvey.co.uk/51.81960,-2.15116,16 (Last accessed: 14/05/2019)

Chapter 4: Conclusion

The initial test survey in 2017 to understand co-occurrence and how it manifested itself within Buckholt Woods indicated a transition from invasive *H.hispanica* through to *H. 'x' massartiana* and finally concluding in higher intraspecific densities of *H. non-scripta.* This prompted further research to quantify the hypothesis that hybridisation could be reduced through woodland topography and/or flora density (le Roux et al., 2012).

The positioning of Cranham village in close proximity to the woodland, in conjunction with a significant point of source (gardens and hedge-ways) that mirrored the base width of the woodland, seemed ideal. This hypothetically, allowed for cross pollination from a south-westerly direction for wind mediated pollination and a close enough distribution of *H. hispanica* to support insect pollinators moving from the point of origin into the woodland (Myers and Bazely, 2003).

Survey data in 2018 started to change the original hypothesis with a shift inter-specifically between what was noted in 2017 on the eastern side of the woodland compared to the new data gathered on the western side of the woodland (Adams *et al.,* 2014). In 2019 data collected substantiated the original survey data in 2017 but to a lesser percentage frequency.

A valley divides each count area, running from north to south. The data suggests that this acts as a natural transition zone between inter-specific densities, although questions still remain as to whether this change in frequency percentage is topographical alone or acts in-conjunction with the calciferous grasses that also run from north to south close to the division zone (Myers and Bazely, 2003).

It was also noted that the percentage frequency on the eastern side of the valley reduced once a larger area had been surveyed. It is suggested the results for 2017 compared to 2019 are supplemented due to the survey sites in 2017 not including areas near to the Buckholt road, a feature that circumvents the woodland. It is observed that higher percentages of *H. 'x' massartiana* can be seen to the western and northern edge of the woodland, which lay close to other sources of hybridised bluebells, such as adjacent woodland and residential dwellings.

Results show spatial and temporal variability which moves towards genetic drift at this current point. Increases in weather patterns, especially those that increase ambient temperature, ground temperature and dryness, may not suit the ecological needs of the British Isles' habitual *H. non-scripta* but lend itself to the habitual requirements of the *H. hispanica* and *H. 'x' massartiana.* Ranta *et al.,* (2006) highlights the debate between genetic drift and natural selection through deterministic and directional forces (Fisher, 1930; Wright, 1932 and 1948) whereby spatial structures are necessary for the creation of the heterogeneity necessary for evolutionary change.

It is suggested that increases in abiotic factors, and the native bluebells slow adaptation in genetic resilience, will constitute a probable move towards a slow silent extinction that will be observed in the years to come as sympatric speciation continues (Kramer and Havens, 2009). Alternatively, it may well be that the polymorphic phenotypic variants noted from 2017 to 2019 are the heterogenetic adaptation due to global warming.

Although not conclusive and requiring further, long term research, it is hoped that the pure physiology of the native bluebell can be maintained by topography, flora density and/or a combination of both (Kramer and Havens, 2009).

It is clear that generalised ground dwelling plants such as Ramson (*Allium ursinum*) do not interfere or alter the density/polymorphic variations of bluebells. Although, plants of hardier nature such as bramble (*Rosaceae rubus*) do inhibit dispersal.

Notable was that no one count area of *H. 'x' massartiana*, maintained a 100% count of one species, whereas this was achieved by the *H. non-scripta.*

4.1 Other Considerations

General consensus questions as to what constitutes the full range of variation in the country's population of bluebells, and Kohn *et al.,* (2009) refers to the fact that no discernible proof through an in-depth study, at present, can quantify the actual level of hybridisation within the whole of the British Isles (Kohn *et al.,* 2009). Marquardt (2016), has quantified two variants in the Iberian Peninsula (Figure 35, page 52) which may be treated as a baseline for understanding what constitutes certain morphological variations, however, morphological variations experienced within the

woodland of Cranham do not restrictively meet those variations noted by Marquardt (2016) (Figure 30, page 46).

As described in the methods section, the basis of data collection used the parameters laid down by Rose (1999) and Stace (2010) and any plant that did not meet those accepted parameters were regarded as a hybrid. Since the release of Kohn *et al.,* (2019) who suggests that the demise of the native bluebell is unlikely due to weakening pollen from invasive bluebells, those parameters have to be reconsidered at this juncture.

However, as for the case of this study, the data stands and so do the parameters that the data was surveyed against. Moreover, to class all recorded variants as *H. 'x' masssartiana* would be un-scientifically sound based on Marquardt (2016) and Kohn *et al.,* (2019) findings. Variants need to be quantified with genetic testing and until the true origin can be substantiated, all variants recorded in Buckholt woodland will be referred to as 'subtle variations'.

4.2 Limitations in Data Collection

Future counts or surveys could be enhanced with support. Geographically, whilst surveying full counts to ascertain area², larger areas made the process difficult to manage due to the density of the woodland. Flora density on occasions, limited the ability to easily find the original start point. Although data collected will be viable for future research, accuracy would have been enhanced with the addition of equipment that would have aided the start and finish of a GPS track survey.

4.3 Future Research

In Kohn *et al.,* (2009) the paper summarises it case with the following statement, 'it is evident that alien *Hyacinthoides* taxa pose a significant potential risk to native *H. non-scripta*.' Although a number of papers exist focusing and highlighting genetic variation, it is none-the-less, important to substantiate those findings fully in the field. Additionally, this form of research will support those wild native species affected by anthropogenically introduced flora. The following research is proposed:

- Expand research locations in 2020 to repeat and verify Buckholt Wood findings.
- Test phenotypic variation with the use of DNA testing for those areas and plants already surveyed.
- Research seed dispersal based on species type and test for differences in dispersal capability.
- Research differences in natural vegetative reproduction to test for potential variation in 'Hardiness Rating'.
- Research whether inter-species root competition increases the likelihood of uneven dispersal and quantify if density dependence plays an active part in successful hybridisation.
- Quantify variations in hydrological and pH requirements between species and establish any differences in hardiness.
- Research hexapod invertebrates to understand what contribution insects have on *hyacinthoide* reproduction and distribution. Understand how wind pollination compares with hexapod mediated pollination.
- Establish whether natural barriers such as calciferous grassland zones inhibit hybridisation.
- To understand whether long term limited exposure to low densities of invasive species limits and/or slows down mutation compared to high density exposure as noted in the inter-competitive zones experienced in the Iberian Peninsula.

(Word Count: 6544)

References

Adams, H. R; Barnard, h. R; Loomis, A. K. (2014). Topography alters tree growth–climate relationships in a semi-arid forested catchment. . Ecosphere. University of Colorado, Institute of Arctic and Alpine Research, Department of Geography. http://dx.doi.org/10.1890/ES14-00296.1

Ali, S. S; Pfosser, M; Wetschnig, W; Martinez-Azorin, M; Crespo, M. B; Yu, Y. (2013). Out of Africa: Miocene dispersal, vicariance, and extinction within Hyacinthaceae subfamily Urgineoideae. Journal of Integrative Plant Biology, 55(10):950–64, 2013.

Allum, N. L. (2016) A Population Study of Hyacinthoides non-scripta: Density dependence, Phenology and Environment, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/11610/

Blackman, G.E; Rutter, A.J. (1954). Endymion nonscriptus (L.) Garcke. Journal of Ecology 42, 629–638.

Brocklebank,K. J; Hendry, G. A. F. (1989). Characteristics of plant species which store different types of reserve carbohydrates. New Phytologist, 112(2):255–260, 1989.

Buerki, S; Jose, S; Yadav, S. R; Goldblatt, P; Manning, J. C; Forest, F. (2012). Contrasting biogeographic and diversification patterns in two Mediterranean-type ecosystems. PLoS One, 7(6):e39377, 2012.

Chase, M. W; Reveal, J. L; Fay, M. F. (2009). A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. Botanical Journal of the Linnean Society, 161(2):132–136, 2009.

Cooke, A. S. (1997). Effects of grazing by muntjac (Muntiucus reevesi) on bluebells (Hyacinthoides non-scripta) and a field technique for assessing feeding activity. Journal of Zoology, 242 (2):365–369, 1997.

Fisher, R. A. (1930). The Genetical Theory of Natural Selection. Oxford: Oxford University Press, Chp 12.

Grime, J. P; Hodgson, J. G; Hunt, R. (1988). Comparative plant ecology: a functional approach to common British species. Springer, 1988.

Grundmann, M; Rumsey, F. J; Ansell, S. W; Russell, S. J; Darwin, S. C; Vogel, J. C; Spencer, M; Squirrell, J; Hollingsworth, P. M; Ortiz, S; Schneider, H. (2010). Phylogeny and taxonomy of the bluebell genus Hyacinthoides, Asparagaceae [Hyacinthaceae]. Taxon, 59(1):68–82, 2010.

Hewitt, G. M. (1988). Hybrid zones-natural laboratories for evolutionary studies. Trends in Ecology and Evolution, 3(7):158–167, 1988.

Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society, 58(3):247–276, 1996.

Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society, 68(1-2):87–112, 1999.

Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences, 359 (1442):183–95, 2004.

Hewitt, G. M. (1999). Quaternary phylogeography: the roots of hybrid zones. Genetica, 139(5): 617–638, 2011.

Hodkinson, D.J; Thompson, K. (1997). Plant dispersal: the role of man. Journal of Applied Ecology 34, 1484–1496.

Hurlbert, S.H. (1978). The measurement of niche overlap and some relatives. Ecology 59, 67–77.

Huxel, G.R. (1999). Rapid displacement of native species by invasive species: effects of hybridization. Biological Conservation 89, 143–152.

Ingrouille, M. (1995). Historical Ecology of the British Flora. Chapman & Hall, London.

Knight, G. H. (1964). Some factors affecting the distribution of *Endymion non-criptus* (L.) Garcke in Warwickshire woods. J. Ecol. 52:405-421.

Kohn, D. D; Hulme, P. E; Hollingsworth, P. M; Butler, A. (2009). Are native bluebells (Hyacinthoides non-scripta) at risk from alien congenerics? Evidence from distributions and co-occurrence in Scotland. Biological Conservation, 142(1):61–74.

Kramer, A. T; Havens, K. (2009), Plant conservation genetics in a changing world. Cell Press, Special Issue: Plant science research in botanic gardens. Volume 14, Issue 11, November 2009, Pages 599-607. https://doi.org/10.1016/j.tplants.2009.08.005.

le Roux, P. C; Virtanen, R; Heikkinen, R. K; Luoto, M. (2012). Biotic interactions affect the elevational ranges of high-latitude plant species. Ecography. A Journal of Space and Time Ecology. First published: 13 March 2012. P. C. le Roux, Dept of Geosciences and Geography, Univ. of Helsinki, FI-00014 Helsinki, Finland. E-mail: peter.leroux@helsinki.fi. https://doi.org/10.1111/j.1600-0587.2012.07534.x

Littlemore, J; Barker, S. (2001). The ecological response of forest ground flora and soils to experimental trampling in British urban woodlands. Urban Ecosystems 5, 257–276.

Marquardt, J. (2016). Hybirdisation in Bluebells (*Hyacinthoides* spec.). Using next-generation sequencing to reconstruct a natural hybrid zone in Spain. Submitted in partial fulfilment of the requirements of the Degree of Doctor of Philosophy. Queen Mary University of London. file:///E:/NS6222%20Bioscience%20Dissertation/Research%20on%20Bluebells/MARQUARDT_Jeannine_SBCS_final_110517%20highlighted%20for%20use.pdf (Last Accessed: 17/05/19).

Marques, I; Rossell´o-Graell, A; Draper, D; Iriondo, J. M. (2007). Pollination patterns limit hybridization between two sympatric species of Narcissus (Amaryllidaceae). American Journal of Botany, 94(8):1352–1359, 2007.

Merryweather, J; Fitter, A. (1995). Arbuscular mycorrhiza and phosphorus as controlling factors in the life history of Hyacinthoides non-scripta (L.) Chouard ex Rothm. New Phytologist 129, 629–636.

Meteorological Office, 2005. Crown copyright. Gridded data sets: http://www.metoffice.gov.uk/research/hadleycentre/obsdata/ ukcip/index.html. Maps: <http://www.metoffice.gov.uk/ climate/uk/averages/index.html>.

Mulholland, D.A; Schwikkard, S. L; Crouch, N. R. (2013). The chemistry and biological activity of the hyacinthaceae. Natural Product Reports, 30(9):1165–1210, 2013.

Myers, J. H; Bazely, D. R. (2003). Ecology and Control of Introduced Plants. Chp 5, pp 120-146. ISBN: 0-521-35778-0.

Ortiz, S; Rodr´ıguez-Oubin˜a, J. (1996). Taxonomic characterization of populations of Hyacinthoides sect. Somera (Hyacinthaceae) in the northwestern Iberian Peninsula. Plant Systematics and Evolution, 202(1-2):111–119, 1996.

Ortiz, S; Buj´an, m; Rodr´ıguez-Oubin˜a, J. (1999). A revision of European taxa of Hyacinthoides section Somera (Hyacinthaceae) on the basis of multivariate analysis. Plant Systematics and Evolution, 217(1-2):163–175, 1999.

J. R. Packham (1992). "Soils, climate and zonation". Functional Ecology of Woodlands and Forests. Springer. pp. 97–140. ISBN 978-0-412-43950-6.

Packham, J. R; Harding, D. J;Hilton, G. M; Stuttard, R. A. (1992). Functional Ecology of Woodlands and Forests. Springer. ISBN-10: 0412439506.

Pfosser, M; Speta, F. (1999). Phylogenetics of Hyacinthaceae based on plastid DNA sequences. Annals of the Missouri Botanical Garden, 86(4):852–875, 1999.

Pfosser, m; Wetschnig, W; Ungar, S; Prenner. G. (2003). Phylogenetic relationships among genera of Massonieae (Hyacinthaceae) inferred from plastid DNA and seed morphology. Journal of Plant Research, 116(2):115–132, 2003.

Pigott, C. D. (1984). The flora and vegetation of Britain - ecology and conservation. New Phytologist, 98(1):119–128, 1984.

Pilgrim, E; Hutchinson, N. (2004). Bluebells for Britain – the report of the 2003 Bluebells for Britain Survey. In: Vines, G. (Ed.). Plantlife, Salisbury, UK.

Preston, C.D; Pearman, D.A. (2002). New Atlas of the British and Irish Flora. Dines, T.D. (Eds.), Oxford University Press, Oxford.

Ranta, E; Lunberg, P; Kaitala, V. (2006). Ecology of Populations. EBC, Cambridge University Press. Chp 12, pp. 330. ISBN: 0-521-67033-0.

Rich, T. C. G; Woodruff, E. R. (1992). Rcording Bias in BotanicalSurveys. Watsonia, 19, 73-95 (1992).

Rix, M. (2004). Hyacinthoides non-scripta Hyacinthaceae. Curtis’s Botanical Magazine 21, 20–25.

Rose, F. (1999). Indicators of ancient woodland-the use of vascular plants in evaluating ancient woods for nature conservation. British Wildlife, 10:241–251, 1999.

Rumsey, D.J. (2006). Introduction to Baysian Statistics. William M. Bolstad. The American Statistician, 2006, Vol., 60,98-99.

Sell, P; Murrell, G. (1996). In: Flora of Great Britain and Ireland, vol. 5. Cambridge University Press, Cambridge.

Simmonds, M; Sims, N. K. (2004). DNA and phytochemistry of bluebells. Curtis's Botanical Magazine, 21 (1):103–104, 2004monds, 2004

Sims, N. K; John, E. A; Stewart, A. J. A. (2014). Short-term response and recovery of bluebells (Hyacinthoides non-scripta) after rooting by wild boar (Sus scrofa). Plant Ecology, 215(12):1409–1416, 2014.

Stace, C. A. (2010). "Hyacinthoides Heist. ex Fabr. (Endymion Dumort.) – bluebells". New Flora of the British Isles (3rd ed.). Cambridge: Cambridge University Press. pp. 920–921. ISBN 978-0-521-70772-5.

Thompson, P. A; Cox, S. A. (1978). Germination of bluebell (Hyacinthoides non-scripta (L.) Chouard) in relation to its distribution and habitat. Annals of Botany, 42(177), 1978.

Thompson, K; Grime, J.P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 67, 893–921.

Turrill, W.B. (1951). Some problems of plant range and distribution. Journal of Ecology 39, 205–227.

Vandelook, F; van Assche, J. A. (2008). Temperature requirements for seed germination and seedling development determine timing of seedling emergence of three monocotyledonous temperate forest spring geophytes. Annals of Botany, 102(5):865–875, 2008.

Waite, S. (2000). Statistical Ecology in Practice, A guide to Analysing Environmental and Ecological field Data. Pearson's Educational Limited. Chapters 4, 7, 8 and 9. ISBN: 0-582-23634-7.

Watson, A. A; Nash, R. J; Wormald, M. R; Harvey, J.D; Dealler, S; Lees, E; Asano, N; Kizu, H; Kato, A; Griffiths, R. C; Cairns, R. J; Fleet, G. W. J. (1997).

Glycosidaseinhibiting pyrrolidine alkaloids from Hyacinthoides non-scripta. Phytochemistry, 46(2): 255–259, 1997.

Wetheral. (2017). Bluebells ~ Hyacinthoides, Bluebells in Cumbria, Wetheral, 27 April 2017). http://www.cumbriabotany.co.uk/look-out-for/bluebells/

Wright, S. N. (1932). The Roles of Mutation, Inbreeding, Crossbreeding and Selection in Evolution. Proceedings from the 6th International Congress of Genetics, 1:356-366, Chp 12.

Wright, S. N. (1948). On the Role of Directed and Random Changes in Gene Frequency in the Genetics of Populations. Evolution 2: 279-294. Chp 12.

Supplementary Information

Figure 30. Polymorphic bluebell variants photographed from 2017 to 2019.

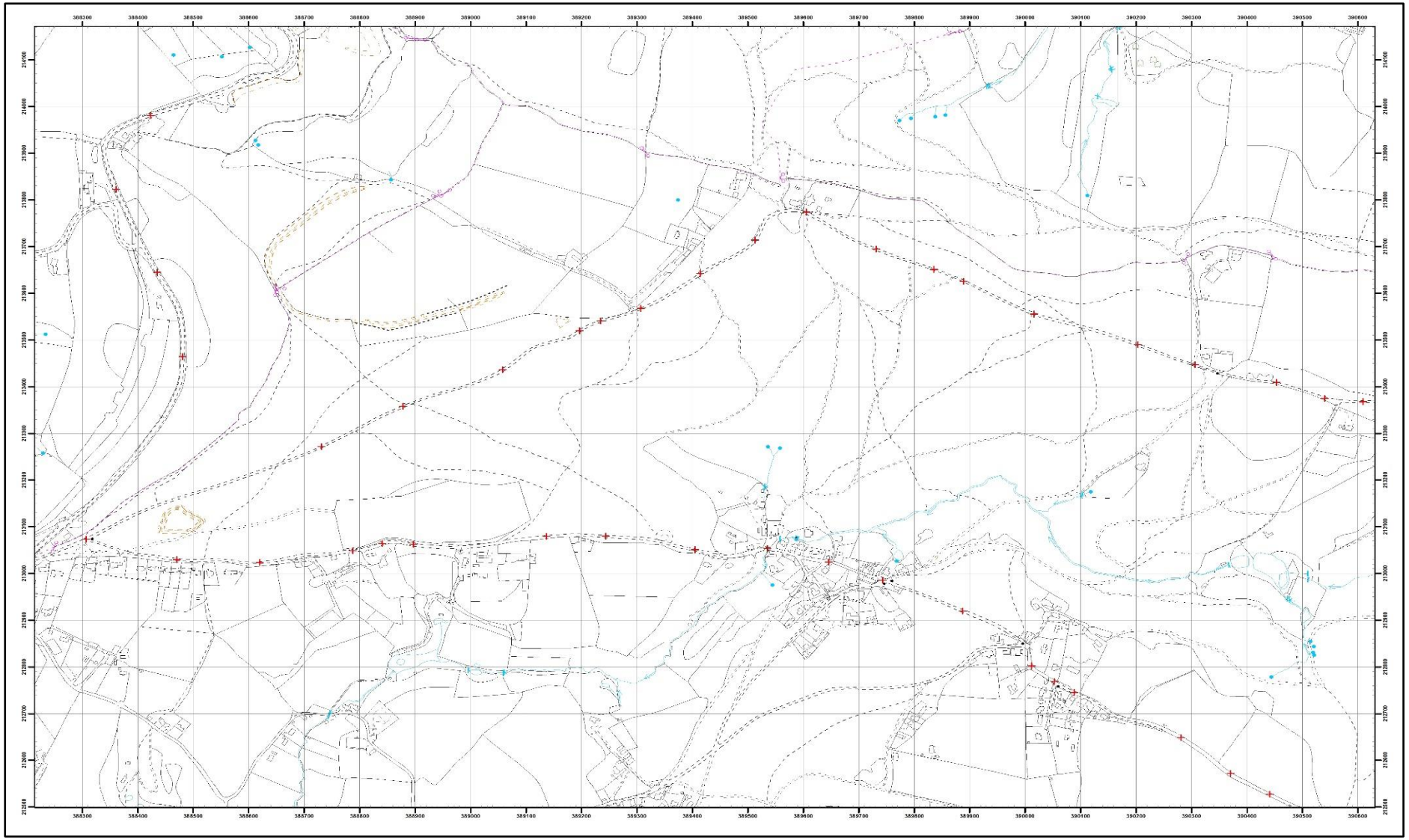

Figure 31. 1:1000 Master Maps were created using the most recent available data (2016) from Digimap (https://digimap.edina.ac.uk/) to support ease of surveying

Loation	Date Count carried out (dd/mm/yryr)	Time of Track	GPS Co-Ordinates	QGIS Track and Way point reference	Elevation (m) above Sea Level	Atmospheric Temperature (Celsius)	Area Size (m²)	Wind Speed (m/s)	Light measurement (Lux)	Soil Moisture Content (%)	Soil Temperature (Celcius)	Incline (Degrees and ratio)	Photographs numbers to support survey	Random Selector
1	07/05/2018		51.48977 -2.10209	2018_05_07	242	18.5	900.00	0	1350	10.9	14.5	0	50-56, 262	8x3, 9x9, 8x7, 5x2, 2x3
2	07/05/2018		51. 48961 -2. 10146	2018_05_07	225	20.1	900.00	0	204	15.8	16	0	73-77, 263	4x0, 2x2, 5x8, 7x6, 4x12
3	07/05/2018		51. 48971 -2.10032	2018_05_07	230	22.9	900.00	0	1930	14.3	16.5	11 / 1:567	105-108, 264	12x3, 10x8, 13x5, 14x5, 14x8, 14x9
4	08/05/2018		51. 48988 -2° 09941	2018_05_08	208	26.1	900.00	0.9	1310	17.8	8	15 / 1:401	118-121, 265	14x1, 12x1, 16x3, 16x9, 8x8
5	08/05/2018		51. 49050 -2. 09551	2018_05_08	219	16.9	900.00	3.1	2510	14.4	13	3 / 1:19.08	266,	10x1, 2x9, 4x2, 12x4, 11x5
6	11/05/2018		51. 49129 -2. 09467	2018_05_08	204	15.5	900.00	0	3110	11.2	12	20 / 1:2.15	267,	Stratified due to slope - 1, 2, 6, 9,
1	08/05/2018		51. 49021 -2. 09886	2018_05_08	217	15.4		0	1630	8.5	13	10 / 1:567	24, 258	
2	08/05/2018		51. 49058 -2. 09708	2018_05_08	223	16		0	1840	12.2	14	10 / 1:567	51, 259	
3	08/05/2018		51. 49023 -2. 09469	2018_05_15	207	15.8		0.8	1820	15	15	0	88, 260	
1	07/05/2018	11:07:16	N 51° 48.966' W 002° 10.032'		221		14.04						57-59	
2	07/05/2018	11:12:13	N 51° 48.968' W 002° 10.032'		221		34.25						60-62	
3	07/05/2018	11:17:16	N 51° 48.976' W 002° 10.469'		216		93.98						63-67	
4	07/05/2018	11:28:39	N 51° 4.965' W 002° 10.170'		215		2.68						68-72	
5	07/05/2018	12:23:38	N 51° 48.966' W 002° 10.167'		206		22.78						77-81	
6	07/05/2018	12:34:29	N 51° 48.958' W 002° 10.469'		214		7.24						84-86	
7	07/05/2018	12:41:25	N 51° 48.970' W 002° 10.129'		217		61.05						89-96	
8	07/05/2018	12:52:05	N 51° 48.972' W 002° 10.098'		212		14.83						97-98	
9	07/05/2018	13:07:09	N 51° 48.989' W 002° 10.107'		217		73.16						99-104	
10	07/05/2018	13:10:45	N 51° 48.983' W 002° 10.082'		220		33.20						Did not take	
11	07/05/2018	13:17:55	N 51° 48.986' W 002° 10.062'		215		3.26						Did not take	
12	07/05/2018	13:26:27	N 51° 48.977' W 002° 10.051'		217		2.55						Did not take	
13	07/05/2018	14:38:22	N 51° 48.983' W 002° 10.021'		215		0.04						Did not take	
14	07/05/2018	14:46:46	N 51° 49.004' W 002° 09.975'		220		12.43						109-113	
15	07/05/2018	14:58:04	N 51° 49.001' W 002° 09.962'		224		933.98						114-116	
16	07/05/2018	15:05:29	N 51° 49.003' W 002° 09.945'		222		15.75						117	
17	07/05/2018	15:09:19	N 51° 49.006' W 002° 09.920'		221		128.16						122-123	
18	07/05/2018	15:56:11	N 51° 48.980' W 002° 09.959'		228		447.49						124-125	
19	08/05/2018	11:17:47	N 51° 49.004' W 002° 09.896'		217		15.93						1 to 2	
20	08/05/2018	11:21:39	N 51° 49.998' W 002° 09.902'		208		146.71						3 to 4	
21	08/05/2018	11:33:53	N 51° 49.010' W 002° 09.913'		209		1,466.10						5 to 16	
22	08/05/2018	11:52:51	N 51° 49.007' W 002° 09.889'		213		104.65						17-22	
23	08/05/2018	12:23:30	N 51° 49.026' W 002° 09.767'		221		403.16						25-37	
24	08/05/2018	12:30:07	N 51° 49.030' W 002° 09.758'		231		404.15						38-46	
25	08/05/2018	12:52:01	N 51° 49.049' W 002° 09.738'		233		32.77						47-50	
26	08/05/2018	13:21:21	N 51° 49.046' W 002° 09.648'		223		15.06						52	
27	08/05/2018	13:31:54	N 51° 49.057' W 002° 09.600'		220		74.52						53-57	
28	08/05/2018	13:49:24	N 51° 49.043' W 002° 09.099'		198		1,005.40						58-62	
29	08/05/2018	14:36:09	N 51° 48.988' W 002° 09.941'		219		3,350.30						63-79	
30	08/05/2018	14:56:34	N 51° 49.038' W 002° 09.503'		209		656.59						80-87	
31	08/05/2018	15:30:54	N 51° 49.008' W 002° 09.450'		211		355.31						89-95	
32	15/05/2018	11:19:17	N 51° 48.990' W 002° 09.408'		184		2,126.60						116-118	
33	15/05/2018	11:27:05	N 51° 49.028' W 002° 09.418'		203		849.89						119-120	
34	15/05/2018	11:32:02	N 51° 49.043' W 002° 09.446'		218		110.01						121-123	
35	15/05/2018	11:39:18	N 51° 49.072' W 002° 09.539'		220		465.36						124-127	
36	15/05/2018	11:55:33	N 51° 49.066' W 002° 09.571'		221		961.85						128-133	
37	15/05/2018	11:59:39	N 51° 49.023' W 002° 09.469'		219		52.39						134-136	
38	15/05/2018	12:08:43	N 51° 49.088' W 002° 09.618'		228		5.10						137-141	
39	15/05/2018	12:19:46	N 51° 49.095' W 002° 09.629'		236		729.77						142-156	
40	15/05/2018	12:40:16	N 51° 49.102' W 002° 09.653'		235		2,000.30						157-168	
41	15/05/2018	13:07:02	N 51° 49.111' W 002° 09.724'		241		1,876.80						169-180	
42	15/05/2018	13:32:19	N 51° 49.120' W 002° 09.735'		239		2,261.00						181-198	
43	15/05/2018	13:42:04	N 51° 49.121' W 002° 09.679'		244		1,502.60						199-201	
44	15/05/2018	13:55:44	N 51° 49.122' W 002° 09.551'		215		9.38						205-209	
45	15/05/2018	14:15:35	N 51° 49.098' W 002° 09.500'		223		31.94						212-216	
46	15/05/2018	14:22;48	N 51° 49.087' W 002° 09.488'		240		1,097.60						217-220	
47	15/05/2018	14:29:11	N 51° 49.070' W 002° 09.480'		227		419.74						221-223	
48	15/05/2018	14:39:11	N 51° 49.028' W 002° 09.427'		214		389.08						224	
49	15/05/2018	14:45:24	N 51° 49.031' W 002° 09.434'		209		25.71						225	
51	15/05/2018	15:10:40	N 51° 48.990' W 002° 09.348'		195		8,979.00						226-238	
52	15/05/2018	15:15:48	N 51° 49.067' W 002° 09.384'		203		41.77						239-240	
53	15/05/2018	15:33:45	N 51° 49.170' W 002° 09.382'		215		78.70						241-242	
54	18/05/2018	10:30:00	GPS Failed		Various		Various						243-257	
55	18/05/2018	10:30:00	GPS Failed		Various		Various						As above	
56	18/05/2018	10:30:00	GPS Failed		Various		Various						As above	
57	18/05/2018	10:30:00	GPS Failed		Various		Various						As above	
58	18/05/2018	10:30:00	GPS Failed		Various		Various						As above	
							39,346.08							

Loation	Count 1 (X Massartiana)	Count 1 (Non Scripta	Count 1 (Hispanica)	Total	% Cover (based on 100 counted plants)	% Cover (based on 100 counted plants)	Count 2 (X Massartiana)	Count 2 (Non Scripta	Count 1 (Hispanica)	Total	% Cover (based on 100 counted plants)	% Cover (based on 100 counted plants)	Count 3 (X Massartiana)	Count 3 (Non Scripta	Count 1 (Hispanica)	Total	% Cover (based on 100 counted plants)	% Cover (based on 100 counted plants)
1	0	8	0	8	0.00	100.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
2	13	17	0	30	43.33	56.67	9	17	0	26	34.62	65.38	7	1	0	8	87.50	12.50
3	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
4	34	9	0	43	79.07	20.93	10	1	0	11	90.91	9.09	0	0	0	0	#DIV/0!	#DIV/0!
5	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	9	6	0	15	60.00	40.00
6	11	2	0	13	84.62	15.38	20	6	0	26	76.92	23.08	31	8	0	39	79.49	20.51
1																		
2																		
3																		
1	56	44	0	100	56.00	44.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
2	74	26	0	100	74.00	26.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
3	74	26	0	100	74.00	26.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
4	29	5	0	34	85.29	14.71	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
5	60	39	0	99	60.61	39.39	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
6	27	2	0	29	93.10	6.90	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
7	100	0	0	100	100.00	0.00	70	3	0	73	95.89	4.11	0	0	0	0	#DIV/0!	#DIV/0!
8	67	6	0	73	91.78	8.22	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
9	64	8	0	72	88.89	11.11	21	48	0	69	30.43	69.57	62	16	0	78	79.49	20.51
10	66	8	0	74	89.19	10.81	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
11	85	13	0	98	86.73	13.27	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
12	67	13	0	80	83.75	16.25	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
13	46	9	0	55	83.64	16.36	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
14	80	3	0	83	96.39	3.61	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
15	76	8	0	84	90.48	9.52	90	10	0	100	90.00	10.00	76	9	0	85	89.41	10.59
16	56	2	0	58	96.55	3.45	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
17	76	2	0	78	97.44	2.56	56	2	0	58	96.55	3.45	0	0	0	0	#DIV/0!	#DIV/0!
18	76	18	0	94	80.85	19.15	58	16	0	74	78.38	21.62	24	6	0	30	80.00	20.00
19	80	0	0	80	100.00	0.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
20	60	40	0	100	60.00	40.00	100	0	0	100	100.00	0.00	0	0	0	0	#DIV/0!	#DIV/0!
21	76	24	0	100	76.00	24.00	88	12	0	100	88.00	12.00	64	9	0	73	87.67	12.33
22	13	10	0	23	56.52	43.48	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
23	65	35	0	100	65.00	35.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
24	70	30	0	100	70.00	30.00	44	19	0	63	69.84	30.16	88	36	0	124	70.97	29.03
25	89	11	0	100	89.00	11.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
26	76	12	0	88	86.36	13.64	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
27	77	23	0	100	77.00	23.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
28	66	12	0	78	84.62	15.38	87	9	0	96	90.63	9.38	24	4	0	28	85.71	14.29
29	92	8	0	100	92.00	8.00	78	22	0	100	78.00	22.00	95	5	0	100	95.00	5.00
30	74	26	0	100	74.00	26.00	75	25	0	100	75.00	25.00	81	19	0	100	81.00	19.00
31	92	8	0	100	92.00	8.00	94	6	0	100	94.00	6.00	76	24	0	100	76.00	24.00
32	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00
33	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00
34	99	1	0	100	99.00	1.00	99	1	0	100	99.00	1.00	100	0	0	100	100.00	0.00
35	88	12	0	100	88.00	12.00	96	4	0	100	96.00	4.00	100	0	0	100	100.00	0.00
36	89	11	0	100	89.00	11.00	93	7	0	100	93.00	7.00	73	27	0	100	73.00	27.00
37	77	23	0	100	77.00	23.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
38	18	51	0	69	26.09	73.91	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
39	0	24	0	24	0.00	100.00	84	16	0	100	84.00	16.00	96	4	0	100	96.00	4.00
40	92	8	0	100	92.00	8.00	80	20	0	100	80.00	20.00	100	0	0	100	100.00	0.00
41	95	5	0	100	95.00	5.00	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00
42	100	0	0	100	100.00	0.00	12	88	0	100	12.00	88.00	70	30	0	100	70.00	30.00
43	93	7	0	100	93.00	7.00	98	2	0	100	98.00	2.00	88	12	0	100	88.00	12.00
44	58	42	0	100	58.00	42.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
45	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00	92	8	0	100	92.00	8.00
46	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00	92	8	0	100	92.00	8.00
47	98	2	0	100	98.00	2.00	96	4	0	100	96.00	4.00	0	0	0	0	#DIV/0!	#DIV/0!
48	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00
49	100	0	0	100	100.00	0.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
51	95	5	0	100	95.00	5.00	81	19	0	100	81.00	19.00	100	0	0	100	100.00	0.00
52	8	30	0	38	21.05	78.95	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
53	72	28	0	100	72.00	28.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
54	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00	55	54	0	109	50.46	49.54
55	44	11	0	55	80.00	20.00	89	11	0	100	89.00	11.00	95	5	0	100	95.00	5.00
56	75	25	0	100	75.00	25.00	85	15	0	100	85.00	15.00	88	12	0	100	88.00	12.00
57	100	0	0	100	100.00	0.00	40	3	0	43	93.02	6.98	100	0	0	100	100.00	0.00
58	38	2	0	40	95.00	5.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
	4206	794	0	5000	84.12	15.88	2553	386	0	2939	86.87	13.13	2286	303	0	2589	88.30	11.70

Loation	Count 4 (X Massartiana)	Count 4 (Non Scripta	Count 1 (Hispanica)	Total	% Cover (based on 100 counted plants)	% Cover (based on 100 counted plants)	Count 5 (X Massartiana)	Count 5 (Non Scripta	Count 1 (Hispanica)	Total	% Cover (based on 100 counted plants)	% Cover (based on 100 counted plants)	Count 6 (X Massartiana)	Count 6 (Non Scripta	Count 1 (Hispanica)	Total	% Cover (based on 100 counted plants)	% Cover (based on 100 counted plants)
1	1	14	0	15	6.67	93.33	6	47	0	53	11.32	88.68	6	47	0	53	11.32	88.68
2	28	18	0	46	60.87	39.13	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
3	0	0	0	0	#DIV/0!	#DIV/0!	90	1	0	91	98.90	1.10	77	17	0	94	81.91	18.09
4	2	0	0	2	100.00	0.00	45	0	0	45	100.00	0.00	0	0	0	0	#DIV/0!	#DIV/0!
5	0	0	0	0	#DIV/0!	#DIV/0!	0	1	0	1	0.00	100.00	0	0	0	0	#DIV/0!	#DIV/0!
6	16	3	0	19	84.21	15.79	4	22	0	26	15.38	84.62	0	0	0	0	#DIV/0!	#DIV/0!
1																		
2																		
3																		
1	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
2	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
3	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
4	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
5	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
6	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
7	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
8	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
9	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
10	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
11	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
12	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
13	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
14	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
15	88	12	0	100	88.00	12.00	96	4	0	100	96.00	4.00	89	11	0	100	89.00	11.00
16	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
17	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
18	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
19	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
20	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
21	55	8	0	63	87.30	12.70	48	18	0	66	72.73	27.27	75	14	0	89	84.27	15.73
22	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
23	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
24	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
25	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
26	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
27	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
28	48	17	0	65	73.85	26.15	94	6	0	100	94.00	6.00	81	19	0	100	81.00	19.00
29		0	0	0	#DIV/0!	#DIV/0!		0	0	0	#DIV/0!	#DIV/0!		0	0	0	#DIV/0!	#DIV/0!
30	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
31	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
32	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
33	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
34	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
35	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
36	89	11	0	100	89.00	11.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
37	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
38	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
39	85	15	0	100	85.00	15.00	90	10	0	100	90.00	10.00	0	0	0	0	#DIV/0!	#DIV/0!
40	98	2	0	100	98.00	2.00	94	6	0	100	94.00	6.00	68	32	0	100	68.00	32.00
41	93	7	0	100	93.00	7.00	95	5	0	100	95.00	5.00	86	14	0	100	86.00	14.00
42	80	20	0	100	80.00	20.00	98	2	0	100	98.00	2.00	0	0	0	0	#DIV/0!	#DIV/0!
43	72	28	0	100	72.00	28.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
44	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
45	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
46	100	0	0	100	100.00	0.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
47	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
48	100	0	0	100	100.00	0.00	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
49	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
51	14	12	0	26	53.85	46.15	68	32	0	100	68.00	32.00	0	0	0	0	#DIV/0!	#DIV/0!
52	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
53	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!	0	0	0	0	#DIV/0!	#DIV/0!
54	0	0	0	0	#DIV/0!	#DIV/0!	100	0	0	100	100.00	0.00	100	0	0	100	100.00	0.00
55	98	2	0	100	98.00	2.00	88	12	0	100	88.00	12.00	100	0	0	100	100.00	0.00
56	94	5	1	100	94.00	5.00	100	0	0	100	100.00	0.00	98	2	0	100	98.00	2.00
57	100	0	0	100	100.00	0.00	95	5	0	100	95.00	5.00	100	0	0	100	100.00	0.00
58	0	0	0	0	#DIV/0!	#DIV/0!	0		0	0	#DIV/0!	#DIV/0!	0		0	0	#DIV/0!	#DIV/0!
	1261	174	1	1435	87.87	12.13	1211	171	0	1382	87.63	12.37	880	156	0	1036	84.94	15.06

Figure 32. Count data for 2018

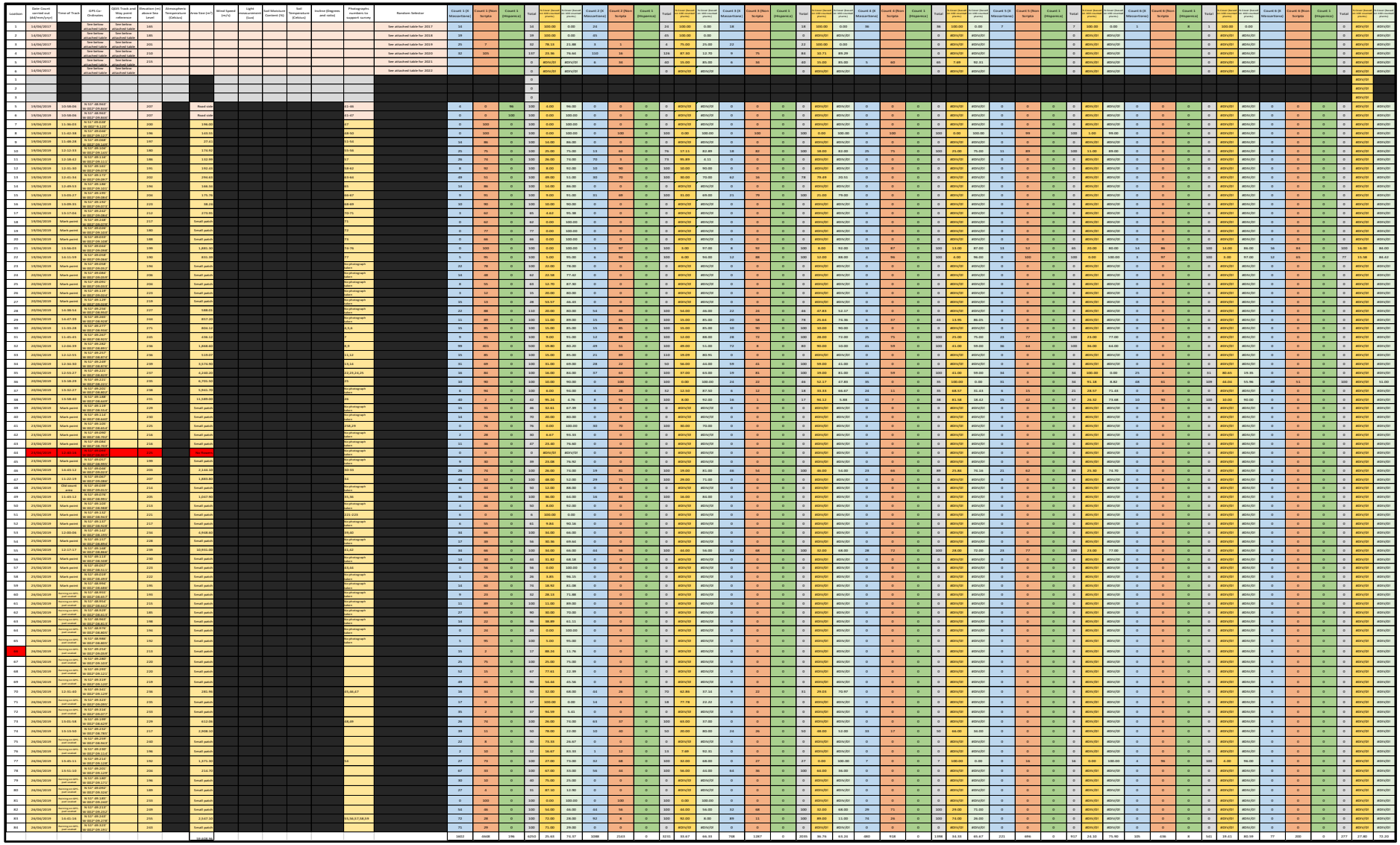

Figure 33. Count data collected 2017 and 2019.

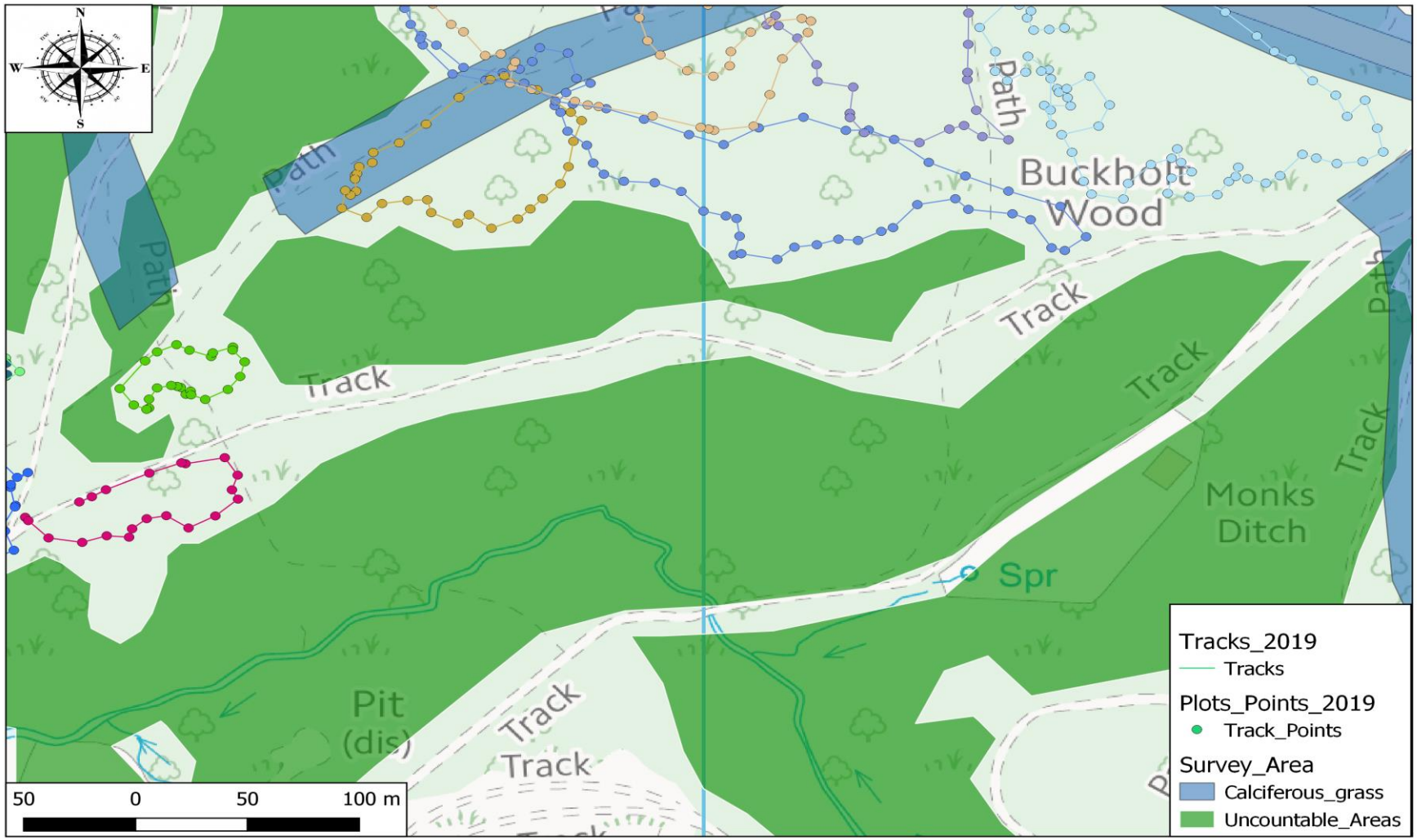

Figure 34. Count locations and how the information was tracked using the 'Track Manager' option on the Garmin GPSMAP 62 (GPS) and entering the track as a waypoint.

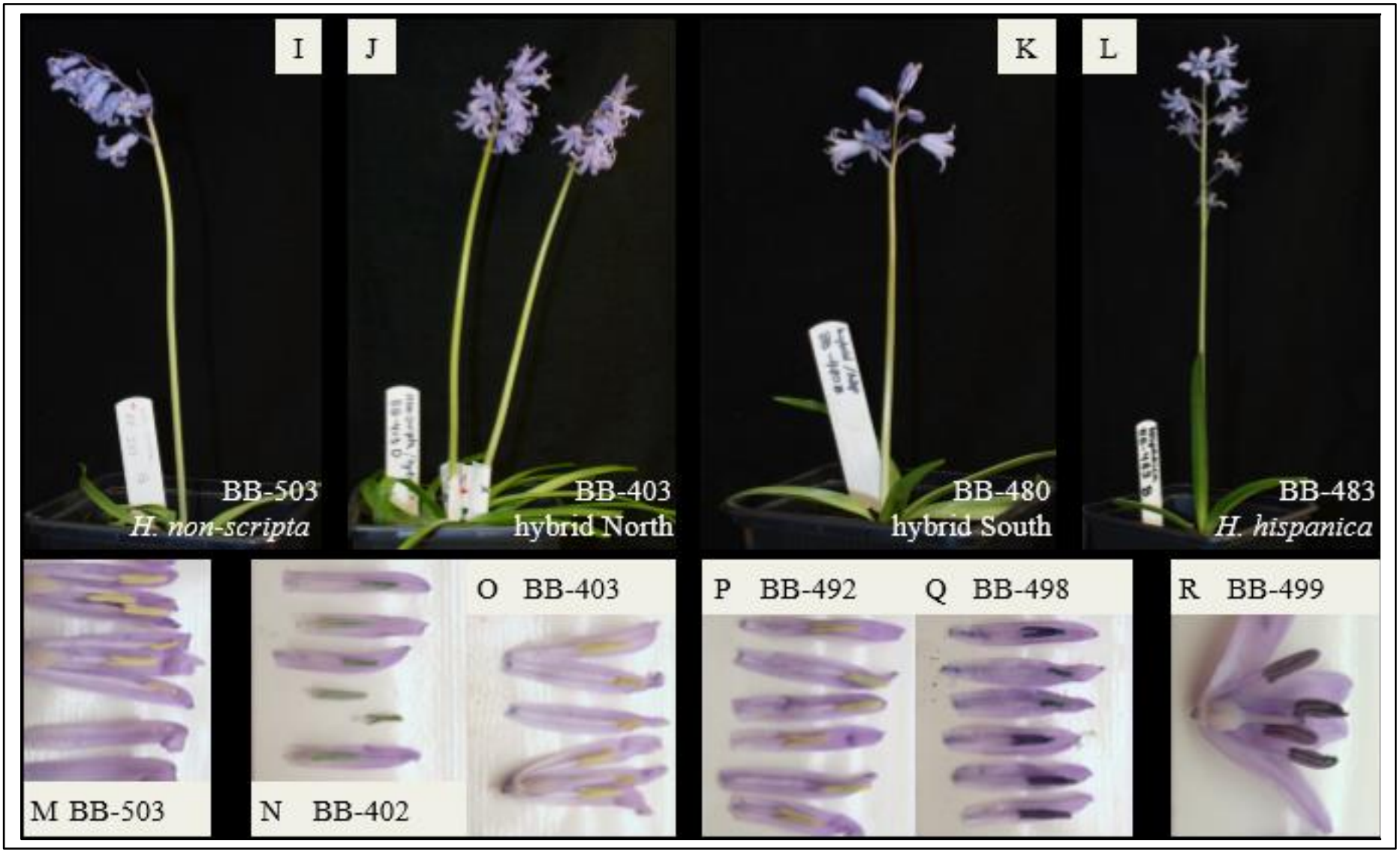

Figure 35. Images from Marquardt (2016) showing the hybridised phenotypic variations created in hybrid zone of Spain.