Universidade de Évora - Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Biologia

Tese de Doutoramento

Use and Effects of aromatic plants in Blue Tit nests

Bárbara Afonso Pires

Orientador(es) | Anabela Dias Ferreira Belo
João Eduardo Morais Gomes Rabaça
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Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.
Al andar se hace camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante, no hay camino,
sino estelas en la mar.

António Machado

Ao meu melhor do Mundo, o meu Pai

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POPH
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Abstract

Use and Effects of aromatic plants in Blue Tit nests

Some bird species also incorporate aromatic plants in their nests. To explain this behaviour several hypotheses have been proposed, mainly related to hiding/shading of the nest, but also with regulation of nest water loss and heat rates. In secondary cavity-nesting species like Blue Tits, hypotheses related to reduction of parasite populations and improvement of nestlings' condition are considered more appropriate to explain this behaviour. These species, because their nests are hidden and because they often reuse the same cavities year after year, are more exposed to higher abundances of parasites, arising mainly from overwintering parasites.

This study aims to understand **i)** how nestlings of secondary cavity-nesting species are affected by parasites present in nests, **ii)** how aromatic plants are used in Blue Tit (our model species) nests and **iii)** how that use influences nest features, reproductive parameters and nestling condition.

Nestlings of cavity-nesting species are affected by parasites present in nests through close contact with parasites and through parental condition. Parents and nestlings adopt different defence mechanisms to limit and/or alleviate deleterious effects of parasites.

Incorporation of specific aromatic plants in Blue Tit nests produced nestlings with longer tarsi but only in large broods. Even when aromatic plants were experimentally incorporated in nests, female Blue Tits found the need to incorporate aromatic plants of their choice, with a very diverse pattern of use, and not always directly related to the availability of these plants in the nests' vicinities. Significant associations between aromatic plants incorporated in nests by females, nest weight, laying date and reproductive parameters were observed.

These results provide new insights on how female Blue Tits use aromatic plants in their nests and how this use is related to nest construction features, reproductive parameters and nestling condition.

Resumo

Uso e Efeitos de plantas aromáticas em ninhos de Chapim-azul

Algumas aves, além do material que utilizam para construção do ninho, também adicionam plantas aromáticas. Para explicar este comportamento foram propostas várias hipóteses relacionadas com dissimulação/sombra dos ninhos, mas também com regulação da perda de água e níveis de calor. Em aves cavernícolas secundárias, como o Chapim-azul, as hipóteses relacionadas com redução das populações de parasitas e melhoria da condição das crias têm sido valorizadas para explicar este comportamento. Estas espécies, cujos ninhos são abrigados e muitas vezes reutilizados ano após ano, estão mais expostas a maiores abundâncias de parasitas, principalmente dos que fazem a hibernação de inverno nas cavidades.

Este estudo pretende compreender **i)** como as crias de aves cavernícolas são afetadas por parasitas presentes nos ninhos, **ii)** como as plantas aromáticas são utilizadas em ninhos de Chapim-azul e **iii)** como essa utilização influencia características dos ninhos, parâmetros reprodutivos e condição das crias.

As crias de aves cavernícolas são afetadas por parasitas presentes nos ninhos por contacto direto com esses parasitas, mas também através da condição dos progenitores. Progenitores e crias adotam diferentes mecanismos de defesa para limitar e/ou aliviar os efeitos prejudiciais desses parasitas.

A incorporação de plantas aromáticas específicas em ninhos de Chapim-azul produziu crias com maior tarso, mas apenas em ninhadas grandes. Mesmo com suplementação de plantas aromáticas nos ninhos, as fêmeas de Chapim-azul adicionaram plantas escolhidas por si, com um padrão de uso muito diverso, e nem sempre diretamente relacionado com a disponibilidade dessas plantas na área circundante dos ninhos. Foram observadas relações significativas entre plantas aromáticas adicionadas aos ninhos pelas fêmeas, peso dos ninhos, data de postura e parâmetros reprodutivos.

Estes resultados fornecem novas perspectivas de como as fêmeas de Chapim-azul utilizam plantas aromáticas nos seus ninhos e de como este uso está

relacionado com características dos ninhos, parâmetros reprodutivos e condição das crias.

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Chapter 1

General Introduction

Bird nests are structures with a major function: provide warm and safe conditions for the development of eggs and nestlings (Collias & Collias, 1984; Hansell, 2000). Each bird nest is a result of evolution and mirrors the morphology, physiology, behaviour and ecology of the builder species (*e.g.* Collias & Collias, 1984; Hansell, 2000). To some extent, building of a nest is limited by the availability of nesting sites and nesting materials (Briggs & Deeming, 2016; Harrison & Castell, 2002), because nesting-site and nest structure are species-specific traits (*e.g.* Biddle *et al.*, 2018a; Harrison & Castell, 2002). Nests can go from simple platforms to lift eggs and nestlings from the ground (*e.g.* scrapes), to open structures that hide the eggs (*e.g.* open-cup nests) or insulated cavities that enclose eggs and nestlings (*e.g.* cavity-nests) (Harrison & Castell, 2002).

Nests in cavities are used by cavity-nesting bird species, which are among the most specialized bird species of forest ecosystems (Devictor *et al.*, 2010). This group of cavity-nesting birds can be divided in primary cavity-nesters, which excavate holes in trees (*e.g.* woodpeckers) and secondary cavity-nesters that breed in the tree holes, previously excavated by the first group (*e.g.* tits) (Zangari *et al.*, 2013). Due to this level of specialization, many of these species are considered of high ecological and conservation interest to forest management studies and may be used as indicators of different impacts on the landscape (Zangari *et al.*, 2013). Nests in cavities ensure protection of eggs and nestlings from weather conditions and predators (Collias & Collias, 1984) and altricial species like most passerines strongly benefit from being raised in this type of enclosed nest, since their developing period is longer than that of other bird species (Collias & Collias, 1984).

Blue Tits *Cyanistes caeruleus*, the model species used in this study, are cavity-nesters and build their nests in natural tree cavities readily adopting man-made nest-boxes (Figure 1; Harrison & Castell, 2002; Mainwaring, 2017). As other secondary cavity-nesters, Blue Tits are among the most intensively studied bird species, because nest-boxes allow routine monitoring and experimental manipulation of nests, adults, eggs and nestlings (Lambrechts *et al.*, 2010). Additionally, Blue Tits show a high tolerance for regular inspections to their nests and to being manipulated (Flegg, 1987). All these features explain why the Blue Tit is an excellent model species for demographic, ecological and behavioural studies (Flegg, 1987; Lambrechts *et al.*, 2010).

Figure 1 - Female Blue Tit in the nest-box incubating the eggs. Aromatic plants can be seen in the bottom right corner (photo by B. Pires).

Blue Tits build their nests mostly with moss, grass, dead leaves and small twigs, using feathers and mammal hair as lining material (Harrison & Castell, 2002; Mainwaring, 2017). Apart from these basic materials, Blue Tit females also incorporate in their nests fragments of fresh aromatic plants (see Dubiec *et al.*, 2013;

Scott-Baumann & Morgan, 2015). This behaviour has also been observed and/or studied in other bird species - European Starlings *Sturnus vulgaris* (*e.g.* Gwinner *et al.*, 2018), Spotless Starlings *Sturnus unicolor* (*e.g.* Polo *et al.*, 2015), Bonelli's Eagle *Hieraaetus fasciatus* (*e.g.* Ontiveros *et al.*, 2008), Red-shouldered Hawks *Buteo lineatus* (*e.g.* Dykstra *et al.*, 2009), Tree Swallows *Tachycineta bicolor* (*e.g.* Dawson, 2004) and Cape Sparrows *Passer melanurus* (*e.g.* Milton & Dean, 1999) - and many hypotheses have been considered to explain it. The crypsis hypothesis (the plants hide the nest), the water loss hypothesis (the plants reduce water loss), the shading hypothesis (the plants provide shade to eggs and nestlings), the insulation hypothesis (the plants reduce rates of heat) and the aesthetic hypothesis (the plants cover nest debris) (reviewed in Dubiec *et al.*, 2013 and Scott-Baumann & Morgan, 2015). However, as this behaviour is mostly seen in cavity-nesting species, these hypotheses are not considered since this type of nest is, by definition, hidden, shaded and, due to the use of moss in its construction, have high water content (Biddle *et al.*, 2019).

In cavity-nesting species, three other hypotheses are the most accepted to explain the incorporation of aromatic plants: the nest protection hypothesis (the plants decrease nest parasites and pathogens; see Scott-Baumann & Morgan, 2015), the drug hypothesis (the plants benefit chicks' development through increased immune function; see Gwinner *et al.*, 2000) and the courtship hypothesis (the plants are incorporated in nests by males as a way of attracting females; see Brouwer & Komdeur, 2004). The last hypothesis has been mostly considered in European and Spotless Starlings, since aromatic plants are brought to nests only by males (Brouwer & Komdeur, 2004). In Blue Tits, however, the most considered hypotheses to explain the behaviour are the nest protection hypothesis and the drug hypothesis for three reasons. First, nest construction and incorporation of aromatic plants are behaviours displayed only by females (Petit *et al.*, 2002). Second, Blue Tits often reuse nests year after year, like other secondary cavity-nesters, thus being exposed to higher abundances of parasites and pathogens, due to nest-dwelling overwintering parasites (Dubiec *et al.*, 2013; Møller, 1989). Third, female Blue Tits incorporate aromatic plants in their nests throughout the breeding season, after laying the first eggs and until nestlings leave the nest (Figure 2; Lambrechts & Dos Santos, 2000), suggesting a continued protection against parasites.

Figure 2 - Blue Tit nestlings in the nest with aromatic plants in the nest cup (yellow arrow; photo by B. Pires).

Three assumptions were proposed by Clark and Mason (1985) and reviewed by Scott-Baumann and Morgan (2015) to support the Nest Protection Hypothesis. First, aromatic plants must be actively incorporated in nests by birds, which has been often observed in the case of Blue Tits (see Mennerat *et al.*, 2009b; Petit *et al.*, 2002). These plants are placed in the nest cup and represent a small fraction of the plants available in the nests' surroundings (Petit *et al.*, 2002; Pires *et al.*, 2012). In fact, Mennerat *et al.* (2009b), observed that the aromatic composition of a nest partially results from the availability of those plants in the territory, but mostly it results from female individual preference. Second, plants selected must be rich in volatile chemical compounds. Aromatic plants found in Blue Tit nests are rich in essential oils, which are complex mixtures of organic compounds, such as monoterpenes, phenols and sesquiterpenes (Koul *et al.*, 2008; Upadhyay, 2010); these essential oils show diversified biological activity, such as antimicrobial, antibacterial, anti-inflammatory and anti-parasitic, among others (*e.g.* Aissa *et al.*, 2019; Cavanagh & Wilkinson, 2005; Marongiu *et al.*, 2010). Third, plants must have a negative effect on parasite and/or pathogen abundances. In Blue Tits, Mennerat *et al.* (2008) found that the presence of aromatic plants in nests did not decrease the level of parasite infestation, but bacterial growth was negatively affected (Mennerat *et al.*, 2009c); in European Starlings, the presence of aromatic plants in nests had no significant effect on mite numbers, but inhibited bacterial growth (Gwinner & Berger, 2006); in Bonelli's Eagle nests, branches of *Pinus pinaster* reduced parasitic loads of *Protocalliphora* larvae (Ontiveros *et al.*, 2008).

The Drug Hypothesis is strengthened by any of the assumptions proposed for the Nest Protection Hypothesis, but a fourth assumption must be observed: parasites must represent a threat to nestling development. Independently of its type, bird nests may host other biotic communities due to debris produced during the breeding period, remains of prey and, most importantly, sessile nestlings that can provide parasites with blood, skin and feathers (Moyer & Clayton, 2004). This biotic community is mainly formed by arthropods such as insects and mites (ectoparasites) that benefit from the nest environment across all nesting stages (Bouslama *et al.*, 2001; Collias & Collias, 1984; Møller, 1990).

Several authors have pointed out that the presence of ectoparasites in nests may have detrimental effects on hosts' condition and reproductive success by reducing parental care (Hurtrez-Boussès *et al.*, 1998) and/or by reducing fledging condition or survival (Hurtrez-Boussès *et al.*, 1997; Merino & Potti, 1995a). Aromatic plants, however, may play an important role in this relationship: Mennerat *et al.* (2009a) observed that the presence of aromatic plants in nests improved Blue Tit nestlings' condition, but Pires *et al.* (2012) observed that it did not increase nestlings' survival rate; in Bonelli's Eagle nests, branches of *Pinus pinaster* had a positive influence on fledglings' survival (Ontiveros *et al.*, 2008). Ectoparasites present in nests are also vectors of many blood parasites: several dipterans are responsible for the transmission of *Leucocytozoon*, *Haemoproteus* and *Plasmodium* (Atkinson *et al.*, 2008; Valkiūnas, 2005) and mites are responsible for the transmission of *Trypanosoma* (Votýpka & Svobodová, 2004). These parasites are known to affect adult condition (Fargallo & Merino, 1999; Tomás *et al.*, 2007b) and reproductive success (Dufva, 1996; Sanz *et al.*, 2001), but studies on nestling condition are scarce. Under a parasite attack, adult birds can not direct most energy resources for maintenance and/or reproduction, because energy is a limited resource and parasites are draining those resources (Atkinson *et al.*, 2008; Møller, 1997). If reproduction is negatively affected by parasitism, then a trade-off might occur between current and future reproduction, due to increased reproductive costs imposed by parasites (Møller, 1997). On the other hand, as nestlings' immune system is still developing (Fargallo & Merino, 2004), under a parasite attack the limited resources used for growth are allocated to fight parasite infections, reducing nestling quality and eventually affecting population recruitment.

It is now clear that the behaviour of incorporating aromatic plants in nests, as well as other defence mechanisms (see Simon *et al.*, 2005b), evolved from the presence of parasites in nests (Merino, 2010). This tight relation is even more noticeable in cavity-nesting species, since they often reuse the same cavities in successive reproductive seasons (Clark & Mason, 1985; Møller *et al.*, 2009). This knowledge revealed the triangular relationship among these three identities - nests, aromatic plants and parasites. The study of these associations is important at a biological, ecological and evolutionary scale, since it allows us to understand (1) how host life history traits are affected and evolve, since parasites become an important component of the hosts' trade-off structure (Møller *et al.*, 2009), (2) how aromatic plants are used and how they can lessen parasites' harmful effects and (3) how the parent-offspring relation is affected by parasitism, especially under poor environmental conditions (habitat, weather, food availability).

Objectives and structure of the thesis

This thesis aims to (1) understand how nestlings of cavity-nesting passerines are affected by ectoparasites and vector-borne blood parasites and (2) how aromatic plants present in Blue Tit nests (see Appendix) relate to several traits regarding reproductive performance and nestling condition.

To achieve the first objective, we conducted an exhaustive bibliographic revision on how nestlings of cavity-nesting passerines are affected by both ectoparasites present in nests and vector-borne blood parasites, and on defence mechanisms adopted by both parents and nestlings. Additionally, we also reviewed how nestlings of cavity-nesting passerines are affected by parents' condition (Chapter 2).

For the second objective we conceived, implemented and carried out a project at the Portuguese Air Force Base N. 6, in Montijo (Figure 3), where a total of 200 nest-boxes were placed ca. 25 m apart from each other. The area has extended stands of *Pinus pinea* L. with smaller stands of *Quercus suber* L. and *Eucalyptus globulus* Labill.

Figure 3 - Air Force Base N. 6 in Montijo (38º41'40.45'' N, 9º02'43.55'' W), with study area outlined in yellow. Image provided by Google Earth Pro (viewing altitude: 3.49 km) on February 10th.

This study included four breeding seasons (from 2015 to 2018) and nests were monitored from March to July on a yearly basis, in order to follow nest construction, breeding events and nestling development. Nest-boxes were made in pine wood, following the British Trust for Ornithology guidelines in respect to Blue Tit nest box orientations (Figure 4; Du Feu, 2002).

Figure 4 - Nest-box for Blue Tits (width = 12 cm, depth = 15 cm, height = 17.5 cm, floor to entrance hole = 11 cm, entrance hole diameter = 2.6 cm) (photo by F. Gomes).

This objective included several specific targets designed to answer the following questions:

a. How specific aromatic plants incorporated in Blue Tit nests would affect reproductive parameters (number of fledglings and nestling survival rate) and nestling condition (weight and tarsus length), taking clutch size into consideration (Chapter 3);

b. How will Blue Tit females react to the incorporation of specific aromatic plants in their nests and how plants naturally added by females relate to availability of those plants in the study area; also, how females use these plants in the different breeding stages (Chapter 4);

c. How Blue Tit nest weight relates to several aspects of breeding performance, brood mass and aromatic plant species used by females in the nests (Chapter 5).

Chapter 2

How are nestlings of cavity-nesting birds affected by parasites?

In preparation for submission as:

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Abstract

Nests are structures built by birds to ensure a warm and protected environment for eggs and nestlings. These conditions and the presence of easy-targeted nestlings welcome a multitude of nest-dwelling ectoparasites (*e.g.* mites, ticks, fleas and flies) that exploit hosts' resources, reducing their survival and condition. Nestlings are particularly vulnerable to these highly mobile ectoparasites, since they remain in nests for periods of several weeks.

Evaluation of nestlings' condition includes body weight, tarsus length and hematocrit measures, although alternative parameters, such as wing and primary feather length and mass/tarsus index, are considered in some studies. Body weight is the most common and easily accepted parameter since it is known to affect nestling survival.

Adult birds adopt behaviours that will reduce the probability of arrival and persistence of parasites in nests, control the development of parasite populations and limit negative impacts on both themselves and nestlings. Moreover, nestlings tend to adjust their behaviours to decrease or alleviate contact with parasites present in the nest. Parents' condition or infection status affects reproductive effort and breeding parameters. Females are the parental sex more frequently studied in this respect since they spend longer periods of time in nests during incubation and brooding.

Ectoparasites present in nests can also act as vectors of blood parasites but studies considering the effect of blood parasites on nestlings' condition are scarce and not consensual.

In this paper we review the current knowledge on (1) the presence and abundance of parasites in nests of hole-nesting birds, (2) how parasites influence nestlings' physical condition and survival and (3) which defence mechanisms are adopted by adults and nestlings.

Keywords

Cavity-nesters, ectoparasites, condition parameters, nestlings, defence mechanisms.

Introduction

The basic and general function of a bird nest is to provide warm and safe conditions for the development of eggs and nestlings (Collias & Collias, 1984). Nests in cavities represent a welcoming spot to a multitude of parasites (Fargallo & Merino, 2004; Martínez-de la Puente *et al.*, 2013) due to the presence of easy-targeted food supply (nestlings) and a relatively protected environment from predators and weather conditions (Cantarero *et al.*, 2013; Collias & Collias, 1984). Ectoparasites often found in bird nests include arthropods such as mites, ticks, fleas and flies (see Mazgajski *et al.*, 1997; Møller, 1990; Roby *et al.*, 1992). They act without the need of an intermediate host (Richner *et al.*, 1993) and exploit a variety of resources, such as skin, feathers or blood (Moyer & Clayton, 2004).

Nestlings are especially vulnerable to these highly mobile ectoparasites, because they are bounded to nests for periods of several weeks (Buechler *et al.*, 2002; Fargallo & Merino, 2004; Mazgajski & Kedra, 1997). Burkett-Cadena *et al.* (2010) tested ectoparasites' preference of nestlings over adults and found no preference for hosts although ectoparasites were more successful attacking immobile and sparse-feathering nestlings (Blackmore & Dow, 1958, as cited in Burkett-Cadena *et al.*, 2010, p.395). According to the Tasty Chick Hypothesis (Christe *et al.*, 1998), the last hatched chick would be preferred by ectoparasites, since it presents a naïve immune system and a poorer condition compared to older siblings that are heavier, in better condition and more immunocompetent (Christe *et al.*, 1998; Roulin *et al.*, 2003).

Nest-dwelling ectoparasites have detrimental effects on host's fitness and health, reducing their survival (Fauth *et al.*, 1991; Richner *et al.*, 1993) and condition (Bouslama *et al.*, 2001; Hurtrez-Boussès *et al.*, 1997; Merino & Potti, 1995a). Both parents and nestlings develop anti-parasite mechanisms to minimize detrimental effects of parasites, such as nest-site choice (Mazgajski, 2007a; Orell *et al.*, 1993), cleanness of nests (see Dubiec *et al.*, 2013 and references therein) or changes in behaviour (Christe *et al.*, 1996; Simon *et al.*, 2005b).

To understand the interactions between hosts and parasites, it is necessary to know how parasites influence hosts' reproductive success and survival (Sanz *et al.*, 2001). The aim of this review is to understand how presence of parasites in nests of cavity-nesters, either as ectoparasites or blood parasites, influence nestlings' survival and condition and which defence mechanisms are adopted by both parents and nestlings to mitigate parasites' detrimental effects.

Effects of parasites on nestlings

In a study with Blue Tits *Cyanistes caeruleus*, Bouslama *et al.* (2001) observed mites, ticks, blow flies and adult fleas in these birds' nests with a prevalence above 80%. These ectoparasites may have detrimental effects on their hosts, mainly nestlings, including tissue damage (skin and feathers), microparasite transmission, blood consumption and anaemia (Allander, 1998; Hurtrez-Boussès *et al.*, 1997; Johnson & Clayton, 2003; Tomás *et al.*, 2007c). Blood consumption alone can cause the loss of more than 55% of the chick's blood volume (Hurtrez-Boussès *et al.*, 1997).

Effects of ectoparasites on host reproductive success and nestlings' condition have been extensively studied in many bird species (Allander, 1998; Richner *et al.*, 1993; Tompkins *et al.*, 1996). Regarding host reproductive success, two main factors should be considered. First, most studies include ectoparasites that attack both nestlings and their parents, such as mites, ticks and fleas (Hurtrez-Boussès *et al.*, 1997) and parental care may be reduced due to parasite attacks. In these cases, results on nestlings' condition should be viewed with caution since nestlings' condition may be influenced not only by parasite attacks, but also by a low parental provisioning (Hurtrez-Boussès *et al.*, 1998). Second, ectoparasite attacks on nestlings may reduce reproductive success by direct effects on survival or by indirect effects on fledgling condition (Hurtrez-Boussès *et al.*, 1997; Merino & Potti, 1995a).

Evaluation of nestlings' condition, in most of the studies, includes body weight, tarsus length and hematocrit measures, although alternative parameters have been considered in some studies (Table 1).

Nestling weight, a parameter known to affect nestling survival (Merino *et al.*, 1998), is the parameter most frequently reported and it is either negatively related to parasite presence or no effect of parasites is observed. We found only one study where nestling weight was positively affected by the presence of ectoparasites in nests (Allander, 1998) and the author argued that this may be due to differences in parasite load, food provisioning, environmental reasons or the interaction between these factors. For instance, Martínez-de la Puente *et al.* (2009) observed that parasite load in nests (blackflies and biting midges) may vary with weather conditions, such as wind speed and temperature. Merino and Potti (1996) observed that mite and blowfly prevalence decreased in a cold and wet year, but flea prevalence did not.

Table 1 - Effect of ectoparasites found in bird nests on several parameters of nestling condition: (-) negatively affected; (+) positively affected; (0) no effect detected.

Host species	**Nestlings' Age (days)**	**Ectoparasites**	**Weight**	**Tarsus length**	**Hematocrit**	**Other parameters**	**References**
Parus major	14	*Ceratophyllus gallinae*	-	-	-	Wing length - 0	Richner *et al.*, 1993
Parus major	14	*Ceratophyllus gallinae*	-	-			Christe *et al.*, 1996
Parus major	13	*Ceratophyllus gallinae* 1990 1991 1992	 0 + -				Allander, 1998
Cyanistes caeruleus	15	*Protocalliphora azurea*	-	-	-		Hurtrez-Boussès *et al.*, 1997
Cyanistes caeruleus	14	*Ceratophyllus gallinae*	0	0		Feather length - 0	Tripet & Richner, 1997
Cyanistes caeruleus	15	*Protocalliphora* sp.	-	-	-		Hurtrez-Boussès *et al.*, 1998
Cyanistes caeruleus	15	Various ectoparasites	0	+	-		Bouslama *et al.*, 2001
Cyanistes caeruleus	15	Various ectoparasites	0	-			Bouslama *et al.*, 2002
Cyanistes caeruleus	15	*Protocalliphora* sp.			-		Simon *et al.*, 2005a
Cyanistes caeruleus	13	Various ectoparasites				Mass/tarsus index - -	Tomás *et al.*, 2008
Ficedula hypoleuca	9	*Ceratophyllus gallinae*	0				Mappes *et al.*, 1994

Table 1 - Effect of ectoparasites found in bird nests on several parameters of nestling condition: (-) negatively affected; (+) positively affected; (0) no effect detected (continued).

Host species	Nestlings' Age (days)	Ectoparasites	Weight	Tarsus length	Hematocrit	Other parameters	References
Ficedula hypoleuca	13	*Dermanyssus gallinoides*	-	-			Merino & Potti, 1995a
		Protocalliphora azurea	-	-			
Ficedula hypoleuca	13	*Dermanyssus gallinoides*					Merino & Potti, 1996
		1991	-	-			
		1992	0	0			
		1993	-	0			
		Ceratophyllus gallinae					
		1991	0	0			
		1992	0	-			
		1993	0	0			
		Protocalliphora azurea					
		1991	0	0			
		1992	0	0			
		1993	0	0			
Ficedula hypoleuca	12	*Dermanyssus hirundinis*	0	0			Bauchau, 1997

Table 1 - Effect of ectoparasites found in bird nests on several parameters of nestling condition: (-) negatively affected; (+) positively affected; (0) no effect detected (continued).

Host species	Nestlings' Age (days)	Ectoparasites	Weight	Tarsus length	Hematocrit	Other parameters	References
Ficedula hypoleuca	13	*Dermanyssus gallinoides*	-	0			Merino *et al.*, 1998
		Protocalliphora azurea	0	0			
		Ceratophyllus gallinae	0	0			
Ficedula hypoleuca	13	*Protocalliphora azurea*	-	-	-		Merino & Potti, 1998
Troglodythes aedon	12	*Protocalliphora braueri*		0			Eastman *et al.*, 1989
Troglodythes aedon	16	*Protocalliphora parorum*	-		0		Johnson & Albrecht, 1993
Troglodythes aedon	11	*Protocalliphora* sp.	-	0			Morrison & Johnson, 2002
Hirundo rustica	15	*Ornithonyssus bursa*	-	0			Møller, 1990
Sturnus vulgaris	17	Flea larvae	-	0		Wing length - 0	Mazgajski *et al.*, 1997
		Flea imagines	-	0		Wing length - 0	

Tarsus length measured when nestlings are close to fledging has been considered a valid parameter to assess body condition in birds (Bouslama *et al.*, 2002; Hurtrez-Boussès *et al.*, 1997; Merino & Potti, 1996). However, Mazgajski and Kedra (1997) argued that tarsus length is not a good parameter to estimate nestling development under a parasite load, since it is partially inherited (*e.g.* Garnett, 1981) and the period in which it develops more rapidly is in the beginning of nestlings' life, when none or few parasites are present in the nest (Alatalo & Lundberg, 1986). Nonetheless it is a parameter often used to assess nestling condition and it is either negatively related to parasite presence or no effect of parasites is observed. A single study (Bouslama *et al.*, 2001) reported longer tarsi in parasitised nests as compared to heat-treated ones. The authors of the study compared this result with others in which nestlings were heavily affected by parasites in higher latitudes (*e.g.* Hurtrez-Boussès *et al.*, 1997; Hurtrez-Boussès *et al.*, 1998; Merino & Potti, 1995a; Richner *et al.*, 1993) and offered two explanations for this: either i) nestlings are not necessarily affected by higher parasite intensities or ii) at lower latitudes, parasites are less harmful or nestlings are more resistant to parasite attacks.

Hematocrit is a physiological variable that reflects the oxygen-carrying capacity of blood, metabolic rate and thermoregulatory ability of birds (Markowski *et al.*, 2015). It can vary with several factors such as age, sex, reproductive status, nutritional status and season (Fair *et al.*, 2007). Some authors recommend its study in association with other erythrocyte measures, such as total white blood cells count, haemoglobin or heterophil counts (Fair *et al.*, 2007; Lill *et al.*, 2013). To our knowledge, those studies are scarce, especially concerning presence of ectoparasites in nests or infection by blood parasites, and they tend to regard adult birds, but not nestlings. In fact, Nadolski *et al.* (2006), studying nestling Great Tits *Parus major*, provided a fair explanation for this, in which blood characteristics develop with age and thus significant differences are observed in blood parameters between adults and nestlings. The same study reported that, as white blood cells counts are reliable indicators of pathogenic and parasitic infections, in broods where fledging survival is lower and heterophils values are higher, a bacterial infection could be present. Dubiec and Cichoñ (2001) also studying nestling Great Tits, observed that some blood parameters (*e.g.* leucocyte levels) decrease as the season progresses, but not hematocrit. A few studies related effects of parasites and blood parameters. Chapman and George (1991) studying Cliff Swallows *Petrochelidon pyrrhonota*, observed that birds from treated nests with reduced parasite loads had lower leucocyte, lymphocyte, heterophil and eosinophil counts when compared to birds from untreated nests. Valera *et al.* (2006) studying both adults and nestlings of Lesser Grey Shrikes *Lanius minor* observed that hematocrit values did not differ between parasitised and non-parasitised individuals, but adults parasitised with blood parasites had significantly lower sedimentation rates than unparasitised ones. The same study reported very low prevalence of blood parasites and ectoparasites in nestlings. Krams *et al.* (2010) studying adult Siberian Tits *Poecile cinctus*, observed no effects of parasites on blood parameters. Granthon and Williams (2017) in a study that included four species of songbirds, observed that parasites infection had no effect on either hematocrit values or on heterophil/leucocyte ratio.

Other parameters, such as wing length, feather length or mass/tarsus index, are used to assess nestlings' condition. The mass/tarsus index was negatively related to presence of ectoparasites in nests (Tomás *et al.*, 2008), but no effect was observed in the other parameters. As these parameters are used in very few studies, it is difficult to assess their validity.

The influence of parasites on nestlings' condition depends partially on brood size (Mazgajski *et al.*, 1997). Infected nestlings from smaller broods are heavier and in better condition than infected nestlings from larger broods (Mazgajski *et al.*, 1997; Richner *et al.*, 1993), mainly because smaller broods can be provisioned more properly with food (Richner & Heeb, 1995).

In addition, the effects of ectoparasites may go beyond body condition, as most ectoparasites often found in nests are vectors of blood parasites (Atkinson *et al.*, 2008; Bennett *et al.*, 1995; Merino *et al.*, 1997; Merino *et al.*, 2000; Scheuerlein & Ricklefs, 2004). Blood parasites are usually considered as low or non-pathogenic probably because they produce chronic infections in birds (Bennett *et al.*, 1993). However, detrimental effects of these diseases on several bird fitness variables and survival have been documented (Martínez-de la Puente *et al.*, 2010; Merino *et al.*, 2000).

To our knowledge, studies of blood parasites carried out on nestlings are scarce and their results are not consensual (see Dunn *et al.*, 2017). Cosgrove *et al.* (2006) made a single positive observation of infection by *Leucocytozoon*. Fargallo and Merino (2004) and Martínez-de la Puente *et al.* (2013) studied the same population of Blue Tits and found *Leucocytozoon* and *Trypanosoma* in nestlings' blood, but differences in age of nestling sampling resulted in different prevalence results. Merino and Potti (1995c) observed that intensity of infection in offspring is four times higher than in adults. The study conducted by Merino *et al.* (1996) was the only that related infection by blood parasites with nestlings' condition, observing that prevalence of *Trypanosoma* in Pied Flycatcher *Ficedula hypoleuca* nestlings negatively affected their weight and tarsus length.

Despite the scarcity of these studies, it is possible to understand that infection of nestlings by blood parasites is affected by several factors, such as prepatent periods of parasites (Martínez-de la Puente *et al.*, 2013; Merino & Potti, 1995c), annual differences in vector abundance (Tomás *et al.*, 2008) and differences in immunological condition of nestlings (Martínez-de la Puente *et al.*, 2013).

Defence mechanisms

Defence mechanisms can be viewed from either adults or offspring perspective. Parents adopt behaviours that will reduce the probability of arrival and/or persistence of parasites in nests and limit negative impacts on both themselves and nestlings (Simon *et al.*, 2005b). Nest site choice has been considered a conditioned activity due to the presence of over-wintering parasites in nest-boxes (Mazgajski, 2007a). In several Passerine species females prefer to nest in clean and empty nest-boxes (see Oppliger *et al.*, 1994; Merino & Potti, 1995b; Rendell & Verbeek, 1996a) since nest-boxes with old nest material have higher parasite loads (Mazgajski, 2007b; Rendell & Verbeek, 1996b). Clutches are smaller (Mazgajski, 2007b) in boxes with old nests and egg-laying starts later in infested nests (Oppliger *et al.*, 1994). Yet, presence of old nest material is important at least for some birds (Mappes *et al.*, 1994; Orell *et al.*, 1993), mainly migrants, since the existence of an already built nest saves time and energy that will be useful at later stages of the breeding season (Mazgajski, 2007a). The use of aromatic plants as anti-parasite defence in nests has been documented in several studies (*e.g.* Dubiec *et al.*, 2013). Among other explanations, plants are placed in nests to avoid parasites or decrease their numbers (Nest Protection Hypothesis; *e.g.* Pires *et al.*, 2012; Scott-Baumann & Morgan, 2015) or to protect nestlings from detrimental effects of parasites (Drug Hypothesis; *e.g.* Mennerat *et al.*, 2009a). Selected plants rich in volatile secondary chemical compounds are incorporated in nests throughout the breeding season and females chose them based on their individual preference, that is, aromatic plants present in nests vary significantly with female identity, but only lightly with availability of these plants in the nests' territories (Mennerat *et al.*, 2009b). Parental effort (nest cleanness, food delivery, etc.) is increased under certain circumstances to meet the needs of parasitised nestlings and counterbalance deleterious effects of parasites (Nilsson, 2003; Tomás *et al.*, 2008). In parasitised nests, parents tend to spend more time in activities such as nest cleaning (Buechler *et al.*, 2002; Hurtrez-Boussès *et al.*, 1998). Similarly, under high infestations parents may increase food deliverance to nestlings (Bańbura *et al.*, 2001; Bańbura *et al.*, 2004; Hurtrez-Boussès *et al.*, 1998; Tripet & Richner, 1997), although Morrison and Johnson (2002) reported no differences in parental provisioning between parasitised and unparasitised nests. This depends directly on food availability at the time of reproduction, which means that when food is scarce or less profitable, nestling survival might be at risk, and an increase in mortality is expected after fledging (Simon *et al.*, 2004).

Defence mechanisms adopted by nestlings aim to decrease or alleviate contact with parasites present in nests (Simon *et al.*, 2005b), leading to changes in their behaviour or physiological responses. Begging behaviour increased by 140% in infected Great Tit broods compared to parasite-free ones (Christe *et al.*, 1996) and authors concluded that sibling competition for food brought by parents is higher in infested nests. Heeb *et al.* (2000) observed that Great Tit nests experimentally-infested with fleas had higher prevalence but lower intensities of *Protocalliphora*. The authors explained that due to flea infestation, a change in nestling metabolism may have produced stronger olfactory cues and therefore nests were easily located by *Protocalliphora* females. A secondary explanation considered by the authors resides on results of two other studies in which males (Christe *et al.*, 1996) or both parents (Tripet & Richner, 1997) increase food delivery rates to nests with higher infestation levels. In these cases, *Protocalliphora* females may be attracted by nestlings' calls and parents' food delivery rates, as hypothesized by Tomás and Soler (2016). Nestlings from parasitised nests spent more time repositioning, preening and scratching to avoid and/or remove parasites compared to nestlings from unparasitised nests (Hurtrez-Boussès *et al.*, 1997; Simon *et al.*, 2005b). As a consequence, comfort and resting were behaviours adopted for longer periods of time in unparasitised nests (Simon *et al.*, 2005b).

Nestling immune response to parasites may also be considered a defence mechanism, physiologically produced. The development of the immune system takes several weeks after hatching (Arriero, 2009) and it may be activated to fight pathogen and parasite infection, which is costly and functions as a trade-off with other activities (Merino, 2010; Sheldon & Verhulst, 1996). For example, nestlings with increased immunocompetence showed lower weight gain and a reduced expected size at 12 days of age (Brommer, 2004). In a case of nest mite infestation, Moreno *et al.* (2008) observed that nestling immune function decreased considerably when mite infestation increased from low to moderate. Even more, the same study reported an irregular trend of nestling immune function, considering four increasing levels of mite infestation. The authors suggested that at the onset of mite infestation, the immune system increases its action in response to the infestation, but this action decreases after a certain level of infestation. Nestling immune capacity may also depend on raising conditions (Arriero, 2009), maternal condition during the nestling period (Tomás *et al.*, 2007b) and parental (mostly maternal) condition in the pre-laying period (Grindstaff *et al.*, 2006; Moreno *et al.*, 2008; Reid *et al.*, 2006; Tschirren *et al.*, 2009).

Parents' condition or infection status

Parents' condition or infection status affects reproductive effort and breeding parameters. As females spend longer periods of time in nests during incubation and brooding (*e.g.* Tomás *et al.*, 2008), they have been mostly considered in studies on the effect of parasites at different stages of the breeding cycle. However, in some studies both parents (Marzal *et al.*, 2005; Merilä & Andersson, 1999; Podmokla *et al.*, 2014) or males alone (Norris *et al.*, 1994; Ruiz-de-Castañeda *et al.*, 2009) were also considered.

Male involvement in nests is related to delivery of food to females and nest defence (Fargallo & Merino, 2004). Early breeding males have higher levels of infection by *Haemoproteus* and as they increase their reproductive effort producing more fledglings, they become more susceptible to infection (Norris *et al.*, 1994; Ruiz-de-Castañeda *et al.*, 2009).

In terms of ectoparasite abundance, Tomás *et al.* (2007a, 2008) observed that nests attended by parasitised females have higher abundances of ectoparasites than nests of unparasitised females. Moreover, Merino *et al.* (2000) and Knowles *et al.* (2010) reported that fledging success was higher for parasite-free females.

Parasite effects on laying date have been also studied. In Blue Tits different results have been found: Fargallo and Merino (1999) found no differences between parasitised and unparasitised females, while Merilä and Andersson (1999) recorded that infected females started laying earlier than uninfected birds and Podmokla *et al.* (2014) found that when both parents are infected, laying was delayed.

Clutch size is smaller in nests of parasitised females (Dufva, 1996; Marzal *et al.*, 2005; Merilä & Andersson, 1999), although Fargallo and Merino (1999) observed no difference between nests of infected and non-infected females. Dufva (1996) observed that infected females lay smaller eggs. Infection level is increased by larger clutches (Fargallo & Merino, 2004; Merilä & Andersson, 1999) and clutch desertion is higher in infected females (Sanz *et al.*, 2001). Infected females hatched fewer eggs (Dufva, 1996; Knowles *et al.*, 2010; Marzal *et al.*, 2005; Sanz *et al.*, 2001) and produced nestlings with smaller tarsus length (Tomás *et al.*, 2005) and in poorer condition (Merilä & Andersson, 1999). Some studies report that nestlings from infected females were less affected by parasites and were in better condition than nestlings from non-infected females (Buechler *et al.*, 2002; Heeb *et al.*, 1998; Podmokla *et al.*, 2014). Protection transferred via the egg from females to nestlings

(Buechler *et al.*, 2002) and an increase in reproductive effort by parents (Buechler *et al.*, 2002; Podmokla *et al.*, 2014) are possible explanations for these results.

Contrary to the assumption reported above that parasitaemia affects reproductive effort and breeding parameters, Norris *et al.* (1994), Sanz *et al.* (2001) and Fargallo and Merino (2004) warned that it may be the reproductive effort that affects parasitaemia, since a higher investment in reproduction will expose parents to higher numbers of ectoparasites and higher stress of immune functions, increasing probabilities of developing chronic infections and diseases.

Final remarks

The presence of parasites in nests affects nestlings in many ways producing detrimental effects on survival and condition. Nestling mass and tarsus length are the most frequently reported parameters to evaluate nestling condition in relation to ectoparasites in nests and most studies report no effect or a negative effect on these parameters. However, in order to reach firm conclusions on the effect of parasites on birds, several factors should be controlled. For example, age of sampling, geographical location, breeding habitat or bird densities.

Ectoparasites also act as vectors of a multitude of diseases transmitted by blood parasites but their effects on nestling condition and survival is not completely known. The small number of studies carried out so far on different passerine species and differences in age of sampling preclude consistent comparisons.

Studies considered in this review revealed important knowledge about how nestlings are affected by parasites, although many more rigorous studies are needed. So far, information gathered is sparse, fragmented and, sometimes, contradictory. The implementation of standardized methods (age of sampling, parameters considered, etc.) will allow comparisons between studies to be more effective.

Chapter 3

Condition of nestling Blue Tits (*Cyanistes caeruleus*) is affected by experimental addition of aromatic plants in large broods.

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Abstract

Some passerines incorporate aromatic plants in their nest cups. These plants are hypothesized to i) reduce parasite loads, mitigating deleterious effects on nestlings (Nest Protection Hypothesis), ii) have positive effects on nestlings' growth and body condition (Drug Hypothesis) and/or iii) play a role in mate attraction, reflecting a quality that may be beneficial to nestling rearing (Courtship Hypothesis). In this study we aimed to examine if experimental addition of aromatic plants had positive effects in reproductive performance (number of fledglings produced and nestling survival rate) and nestling body condition (weight and tarsus length). In addition, we study if those potential effects were more noticeable in different brood sizes - small and large - through an observational approach. No effect of treatment was observed in either reproductive performance or nestling condition parameters. However, a significant effect of year and brood size was observed regarding nestling weight while nestling tarsus length was improved in large broods of nests supplemented with aromatic plants.

Keywords

Nests, aromatic plants, Drug Hypothesis, Nest Protection Hypothesis.

Introduction

Some passerines actively incorporate fragments of aromatic plants rich in volatile chemical compounds in the cup of their nests (Deeming & Reynolds, 2015; Wimberger, 1984). Plants used represent a small fraction of the plants available in the nest surrounding areas and a small part of the nest material (Clark & Mason, 1988; Dubiec *et al.*, 2013; Mennerat *et al.*, 2009a; Pires *et al.*, 2012). Additionally, they also reflect the female's individual preference (Mennerat *et al.*, 2009b).

These plants are hypothesized to (1) reduce parasite loads, mitigating deleterious effects on nestlings (Nest Protection Hypothesis), (2) have positive effects on nestlings' growth and body condition (Drug Hypothesis) and (3) play a role in mate attraction, reflecting a quality that may be beneficial to nestlings' rearing (Courtship Hypothesis) (Brouwer & Komdeur, 2004; Dubiec *et al.*, 2013; Gwinner *et al.*, 2000; Wimberger, 1984).

In the case of Blue Tits *Cyanistes caeruleus* the Nest Protection Hypothesis and the Drug Hypothesis are considered to explain this behaviour. Female Blue Tits start placing aromatic plants in their nests at the onset of egg-laying and continue to do so during the incubation and nestling stages (Lambrechts & Dos Santos, 2000), contrary to European Starlings *Sturnus vulgaris* and Spotless Starlings *Sturnus unicolor* in which males only bring aromatic plants to nests until egg-laying starts (Gwinner *et al.*, 2000; Polo *et al.*, 2015). This behaviour suggests that these plants may have a fitness-related effect during these stages (Mennerat *et al.*, 2008, 2009a).

Studies regarding the relationship of aromatic plants in nests with breeding parameters and nestlings' morphometric indicators of condition (*e.g.* body weight and tarsus length) are scarce and the few results available are far from conclusive. Regarding breeding parameters, a previous study showed that the number of fledglings produced is not affected by presence of aromatic plants in nests of European Starlings (Brouwer & Komdeur, 2004), contrary to what was found in

Spotless Starlings and Tree Swallows *Tachycineta bicolor* (Dawson, 2004; Shutler & Campbell, 2007). In Blue Tits no effect of treatment on nestlings' survival was observed in a study carried out with 3 Corsican populations (Mennerat *et al.*, 2008).

Studies focusing on nestling weight found no influence of aromatic plants in nests of European Starlings (Brouwer & Komdeur, 2004; Clark & Mason, 1988; Fauth *et al.*, 1991), Blue Tits (Mennerat *et al.*, 2008; Mennerat *et al.*, 2009a, Tomás *et al.*, 2012), Spotless Starlings (Polo *et al.*, 2015), and Tree Swallows (Dawson, 2004; Shutler & Campbell, 2007). However, an increase in nestlings' weight was observed in European Starlings (Gwinner *et al.*, 2000; Gwinner & Berger, 2006; Gwinner *et al.*, 2018), but no influence of aromatic plants on nestlings' tarsus length was observed in another study, on the same species (Fauth *et al.*, 1991), nor in Spotless Starlings (Polo *et al.*, 2015).

When brood size is considered to compare Blue Tit nestlings' condition between nests supplemented or not with aromatic plants, it was observed that nestlings are heavier in smaller broods, but nestling weight was not affected by the presence of aromatic plants (Mennerat *et al.*, 2008). Yet, another study documented that nestling mass gain is positively affected by aromatic plants in enlarged broods (Mennerat *et al.*, 2009a).

In this study, we aim to examine if specific aromatic plants supplemented to nests have positive effects on reproductive performance (number of fledglings and nestling survival rate), a key feature of organisms in evolutionary ecology, behavioural studies and conservation biology (Labocha & Hayes, 2012), and nestling body condition (weight and tarsus length). We predict that nests supplemented with aromatic plants would produce more fledglings, show higher survival rates and nestlings would be in better condition as compared to control nests. An additional objective was set to examine the interaction effect of treatment and brood size on reproductive parameters and nestling condition, considering brood size as a proxy for parental effort. Differences in nestling condition should be more noticeable in large broods rather than in small ones, due to increased resource competition. For the same reasons, a positive effect of aromatic plants should also be more noticeable in large broods.

Methods

Study site and field protocols

The study was conducted in four consecutive breeding seasons, from 2015 to 2018, at the Portuguese Air Force Base No. 6 - Montijo located in the left margin of the Tagus Estuary (38º41’40.45’’ N, 9º02’43.55’’ W). The area is mostly covered with stands of *Pinus pinea* L. and a few stands of *Quercus suber* L. and *Eucalyptus globulus* Labill. Two-hundred nest-boxes were installed in pine stands 25 m apart from each other, facing south-east, with the Tagus Estuary in the northwest side. Nest-boxes were monitored weekly throughout each breeding season to register laying date (March 1st = day 1), hatching date (day 0 of nestling life), brood size at hatch (number of hatchlings), brood size at fledging date (number of nestlings that reached 15-days old) and nestlings’ survival rate (percent of fledglings per hatchlings). Biometric measurements were carried out when nestlings were 15 days-old. Tarsus length was measured using a digital calliper to the nearest 0.1 mm and weight was determined with a PESOLA Spring scale 30 g (PESOLA Präzisionswaagen AG, Switzerland) to the nearest 0.1 g and precision of ± 0.3%.

Aromatic plant treatment

From the day the first eggs were observed in nests until nestlings were 15 days-old, an aromatic plant treatment was conducted in the occupied nest-boxes (N = 51): every 3 days, 1 fragment of each of the 3 aromatic plants used (*Lavandula dentata* L., *Calamintha nepeta* L. (Savi) and *Dittrichia viscosa* (L.) Greuter) were deposited in 24 nests (aromatic group) and a non-aromatic herb (grass) was placed in 27 nests (control group). These 3 plant species were used in the aromatic group because in a previous study we have found that they were naturally placed in nests by female Blue Tits and were used more than expected considering their availability in the nests’ surrounding area (Pires *et al.*, 2012). When placing each treatment in each nest, aromatic plants observed in the nest cup, either placed by us or by the female, were removed, to keep all nests under similar conditions.

Statistical analysis

We studied the effects of aromatic plants and brood size on the reproductive performance and nestling condition using a factorial ANOVA. Two classes of brood size were established, based on the average number of hatchlings observed in the studied population: i) small broods (below the population average number of hatchlings); ii) large broods (above the population average number of hatchlings).

Nest treatment (aromatic/non-aromatic) and brood size were included as fixed factors, whereas multiple parameters regarding reproductive performance (number of fledglings and survival rate) and nestling condition (weight and tarsus length) were included as dependent variables. Since reproductive performance and nestling condition may change over the reproductive period and under different environmental conditions, the sampling year (2015, 2016, 2017 and 2018) was also included as a fixed factor and laying date as a covariable (continuous variable). A factorial ANOVA was conducted, to determine both the main and the interaction effects of the factors on the dependent variables. When statistically significant results were observed, pairwise comparisons were also computed.

All quantitative results were presented as mean ± SD. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, N.Y., USA). A significant level was set at 0.05.

Results

During our 4-year study, reproductive and nestling condition parameters (Table 2) were studied in 51 Blue Tit nests (24 aromatic nests and 27 control nests). The average number of hatchlings in the studied population was 7.41 (N = 51); therefore, we considered as small broods those with 7 hatchlings or less and as large broods those with more than 7 hatchlings.

Table 2 - Reproductive and nestling condition parameters in Blue Tit nests for both treatments.

	Aromatic nests (N = 24)	**Control nests (N = 27)**
	Reproductive parameters	
Number of fledglings	7.38 ± 1.79	6.93 ± 2.04
Survival rate (%)	0.97 ± 0.10	0.96 ± 0.08
	Nestling condition	
Weight (g)	9.30 ± 0.65	9.25 ± 0.70
Tarsus length (cm)	1.83 ± 0.03	1.82 ± 0.04

Reproductive parameters

No effect of laying date or year was observed in either the number of fledglings or survival rate (Table 3). Treatment also did not significantly affect the number of fledglings or survival rate (Table 3), although higher mean values were observed in aromatic nests for both parameters (Table 2). Nestling survival rate was not significantly affected by brood size (Table 3).

Table 3 - Effects of treatment, brood size and year (fixed factors) on reproductive parameters and nestling condition (dependent variables) using a factorial ANOVA. Laying date was included as a covariable. Significant results are in bold. Df = degrees of freedom, F = F-test, P = p-value.

	Df	**Number of fledglings**		**Survival rate**		**Weight**		**Tarsus length**	
		F	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Laying date	1	1.85	0.18	1.73	0.20	3.62	0.07	0.50	0.48
Year	3	0.87	0.47	0.89	0.45	3.22	**0.03**	0.51	0.68
Treatment	1	0.24	0.63	0.31	0.58	0.002	0.97	1.20	0.17
Brood size	1	16.90	0.002	1.46	0.23	4.50	**0.04**	1.03	0.32
Year x Treatment	3	0.53	0.66	0.83	0.48	0.40	0.76	1.79	0.17
Year x Brood size	2	0.02	0.98	0.51	0.60	3.00	0.06	3.31	0.06
Treatment x Brood size	1	0.91	0.35	0.00	0.97	1.38	0.25	5.03	**0.03**
Year x Treatment x Brood size	1	0.03	0.88	0.14	0.71	3.69	0.06	2.70	0.11

Nestling condition

Laying date did not affect any of the nestling condition parameters (Table 3). The year did not reveal any effect on nestling tarsus length (Table 3), but nestling weight was significantly affected (Table 3), with significant differences between 2015 and 2016 ($P = 0.003$) and 2015 and 2017 ($P = 0.002$).

Treatment did not affect nestling condition (Table 3), although higher values were observed in aromatic nests for both parameters (Table 2). Only nestling weight was significantly affected by brood size (Table 3), with significantly heavier nestlings in small broods as compared to large broods ($P = 0.003$). The ‘treatment x brood size’ interaction revealed significant differences in nestling tarsus length (Table 3), with a clear difference between aromatic and control nests observed in large broods (Figure 5).

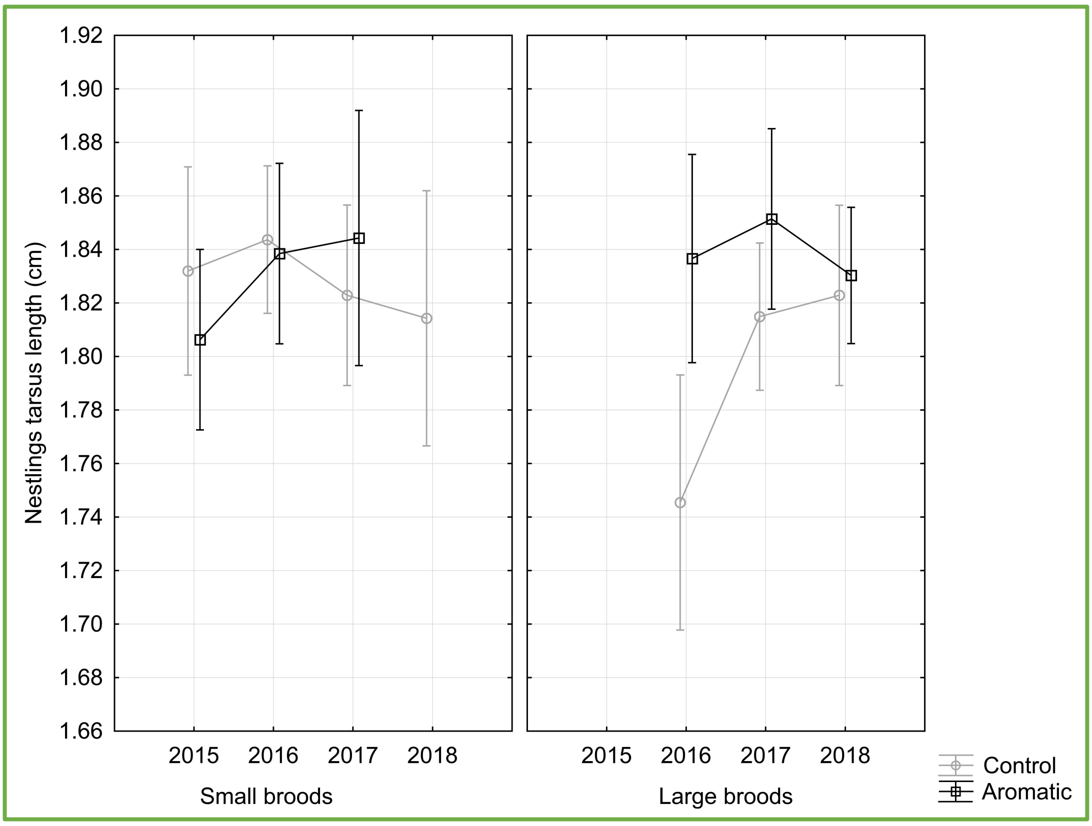

Figure 5 - Variation in nestling tarsus length, through all study years regarding treatment and brood size. Vertical bars denote 95% confidence intervals.

Discussion

Our results showed that the number of fledglings was not significantly affected by either laying date, study year or treatment. In Spotless Starlings and Tree Swallows similar results were found regarding supplementation of nests with aromatic plants (Dawson, 2004; Polo *et al.*, 2015; Shutler & Campbell, 2007). Dawson (2004) did not specifically study the number of fledglings produced but observed that nests supplemented with aromatic plants experienced lower nestling mortality than brome grass or control nests. Similarly, nestling survival rate was also not significantly affected by either laying date, year, brood size or treatment. Mennerat *et al.* (2008) found similar results in a Corsican population of Blue Tits in which nestling survival rate was not affected by the addition of aromatic plants in nests.

Laying date had no significant effect on either weight or tarsus length of nestlings. But although that lack of significance was also observed with tarsus length and study year, weight was affected by year with significant differences between 2015 and 2016 and 2017. Similar differences were also observed in a study with Great Tits and the author suggested that they may be due to weather conditions affecting both parasite populations and food abundance (Allander, 1998). Another study also reported that ectoparasites present in nests and overall weather conditions may impair nestling development (Merino & Potti, 1996).

In our study, nestling weight did not significantly differ between treatments, similarly to what was previously documented (Mennerat *et al.*, 2008, 2009a; Tomás *et al.*, 2012). The same was found for European Starlings (Brouwer & Komdeur, 2004; Clark & Mason, 1988; Fauth *et al.*, 1991), Spotless Starlings (Polo *et al.*, 2015), and Tree Swallows (Dawson, 2004; Shutler & Campbell, 2007), when nestling weight between nests with aromatic plants and controls was compared. However, heavier nestlings were observed in small broods (Mennerat *et al.*, 2008). Similarly, Mennerat *et al.* (2009a) observed that nestlings from large broods had reduced weight gain, as compared to nestlings from small broods.

Nestling's tarsus length was also not significantly affected by treatment, which is in accordance with results for European Starlings and for Spotless Starlings (Fauth *et al.*, 1991; Polo *et al.*, 2015). In our study, however, a clear effect of treatment on nestling tarsus length was observed in large broods. Tarsus length is a body parameter partially inherited and the period in which it grows more rapidly is at the beginning of nestlings' life (Alatalo & Lundberg, 1986; Mazgajski & Kedra, 1997). Moreover, it was also observed that nestling tarsus length may be affected by female mating status, habitat and breeding density, through reduced parental care and low availability of preferred prey (Alatalo & Lundberg, 1986). For example, Blondel *et al.* (1998) observed that nestling tarsus length was significantly higher in smaller broods. However, this is the first time that nestling tarsus length is related to aromatic plants although the effect appears only in large broods. One explanation for this result is that the effect of aromatic plants reducing parasite effects on nestlings allows them to grow longer tarsus in spite of sharing food with more siblings as compared to small broods.

Overall, we cannot statistically validate our initial predictions, even though aromatic nests produced more fledglings, presented higher survival rates and their nestlings were in better condition. Furthermore, although the effect of treatment was not directly observed, an underlying effect of aromatic plants should be considered when its effect interacts with brood size, regarding nestling tarsus length.

Discussion of our results with other Blue Tit studies was difficult, because to our knowledge, only in three papers were studied the same parameters considered in our study (Mennerat *et al.*, 2008, 2009a; Tomás *et al.*, 2012). Other studies considered Blue Tit populations (Lafuma *et al.*, 2001; Mennerat *et al.*, 2008, 2009b), but in all cases reporting the effect of aromatic plants in ectoparasite populations, a parameter not studied here. Discussion of our results with those obtained in other bird species proved to be very important, although species considered showed a different behaviour towards aromatic plants in nests compared to Blue Tits (European Starlings and Spotless Starlings) or no aromatic plant behaviour at all (Tree Swallows). In the specific case of Blue Tits, given the different results obtained, more studies are needed, especially considering alternative parameters such as food availability and environmental conditions, since breeding success and nestlings' condition are most definitely dependent on both parameters. Additionally, we cannot discard the possible effect of the development of parental behaviour, sibling interactions (Michaud & Leonard, 2000), the importance of nest features and adult quality on nestling condition (Álvarez & Barba, 2008).

Chapter 4

Keeping the nest scented: selectivity of aromatic plants in Blue Tit nests and its use through breeding stages

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Abstract

Incorporation of aromatic plants in nests is a behaviour already observed in many bird species. In Blue Tits, this behaviour begins with laying of the first egg, continues throughout all breeding stages and ends when nestlings are ready to fledge. Aromatic plants selected by females are rich in volatile compounds with biological properties important to maintain an aromatic environment that protects the nest, the female and the nestlings. In this study we aim to better understand the mechanisms of selection used by females to incorporate aromatic plants in their nests and also if incorporation of these plants vary through the different breeding stages and how. A set of 25 nest-boxes installed in a forested area dominated by Stone pine was monitored weekly during the breeding seasons of 2017 and 2018. We observed that females are clearly selective regarding the plant species they bring to their nests and the quantities in which they are used. This selectivity is not entirely dependent of such plants availability, since several plants incorporated in nests are not even present in the nests' surroundings. Throughout the breeding season, plants incorporated in nests vary in species and in the amount used. During the incubation and nestling stages, more species were used and in higher quantities, probably because both female and nestlings are bound to the nests for long periods of time.

Keywords

Aromatic plants, selectivity, use, Blue Tits, breeding stages.

Introduction

Nest building is an essential part of the breeding cycle for most bird species (Hansell, 2000). Nests protect the eggs, nestlings and female from predators and/or unfavourable environmental conditions, throughout the different breeding stages (Hansell, 2000). Materials used to build the nests reflect the variety of materials present in the environment (Briggs & Deeming, 2016) and the size of the bird that builds them (Deeming, 2018). In Blue Tits *Cyanistes caeruleus*, apart from standard materials, such as mosses, small twigs and grasses, some females incorporate fresh aromatic plants into their nests (Dubiec *et al.*, 2013; Lambrechts & Dos Santos, 2000). These plants are rich in volatile chemical compounds (Petit *et al.*, 2002; Wimberger, 1984) and often represent a small fraction of all plants available in the nests' surroundings (Lambrechts & Dos Santos, 2000; Mennerat *et al.*, 2009c), suggesting a non-random selection of these plants (Petit *et al.*, 2002).

Blue Tit females clearly show a preference for some aromatic plant species (Lambrechts & Dos Santos, 2000; Pires *et al.*, 2012) and when faced with experimental removal of aromatic plants chosen by them, they quickly react and either replace aromatic plants observed in nests before removal or add new plants (Lambrechts & Dos Santos, 2000). Petit *et al.* (2002) and Mennerat (2008) demonstrated that females easily perceive the change in aromatic plants present in nests using olfaction, a characteristic not widely recognized in passerine birds.

Moreover, Mennerat *et al.* (2009b) found a significant variation in aromatic plant species used among females and that this variation was female-specific, that is, dependent on female identity. Females begin to incorporate aromatic plants into their nests before the start of egg laying and until nestlings are fully developed (Dubiec *et al.*, 2013; Lambrechts & Dos Santos, 2000; Mennerat *et al.*, 2009b).

Number of aromatic plants used increases throughout the breeding season and reaches the highest number in the later stages (Dubiec *et al.*, 2013; Scott-Baumann & Morgan, 2015).

Volatile chemical compounds present in these plants are mainly essential oils, commonly used by humans for pharmaceutical, sanitary, cosmetic, and agricultural purposes, due to their bactericidal, virucidal, fungicidal, anti-parasitical, insecticidal, antimicrobial, anti-inflammatory and other medicinal characteristics (Guimarães *et al.*, 2010). Essential oils are obtained from different plant parts (Proença da Cunha *et al.*, 2012) and constitute complex mixtures of organic compounds that proved to be inhibitors of bacterial growth (Upadhyay, 2010), active in controlling several aspects of insect development (Tripathi *et al.*, 2009) and effective against protozoan pathogens (Upadhyay, 2010). Field experiments revealed that nestlings' bacterial richness and density was significantly reduced (Gwinner & Berger, 2005; Mennerat *et al.*, 2009c) and fledgling growth and condition were significantly increased by the presence of these plants (Mennerat *et al.*, 2009a), but they did not reduce infestation levels in nests (Gwinner *et al.*, 2000; Mennerat *et al.*, 2008).

In this study, we aim to understand if experimental addition of aromatic plants in Blue Tit nests influences the addition of plants of their choice. Specifically, (1) if females choose to incorporate selected aromatic plants in their nests, (2) how do plants used relate with plants present in the nests' surrounding areas and (3) how that incorporation varies across the different breeding stages.

Methods

Study area and field protocols

The study was conducted at Portuguese Air Force Base No. 6 - Montijo located in the left margin of the Tagus Estuary (38º41’40.45’’ N, 9º02’43.55’’ W) in the breeding seasons of 2017 and 2018. The area is covered with stands of *Pinus pinea* L., a few stands of *Quercus suber* L. and *Eucalyptus globulus* Labill.

A total of 25 nest-boxes (14 in 2017 and 11 in 2018) was monitored weekly from March to early June in both years. Nest-boxes were placed in pine trees 25 m apart from each other, facing south-east with the Tagus Estuary in the northwest side. Four breeding stages were considered: laying, in which females lay their eggs (the length of this stage depends on the number of eggs each female lays); incubation, in which females incubate their eggs (this stage lasts between 14 and 15 days); nestlings (from hatch until *pulli* are 15 days-old) and fledglings (from nestlings’ 16th day of age to fledge). During the sampling seasons, nests were checked regularly and the number of eggs, hatchlings and fledglings for each nest-box were recorded.

Aromatic plant treatment

When breeding attempts were confirmed for each nest, and until nestlings left the nests, 3 fragments of aromatic plants (1 fragment with ca. 0.3 cm x 0.5 cm of each plant: *Lavandula dentata* L., *Calamintha nepeta* L. (Savi) and *Dittrichia viscosa* (L.) Greuter) were placed in 12 nests (aromatic group) and grass about the same size was placed in 13 nests (control group). We used these 3 plant species in the aromatic group because in a previous study (Pires *et al.*, 2012) we have found that they were naturally added by female Blue Tits to their nests and used more than expected considering their availability in the nests’ surroundings. The nests were visited every 3-4 days, and the aromatic plants observed in the nest cup (either placed by us or by the female) were removed. The aromatic plants removed were kept in small paper bags and stored for later identification and quantification. Each paper bag was labelled with nest identification and date of removal. This way, we were able to ascertain the specific breeding stage in which each bag was collected from the nest.

Plants availability

The availability of aromatic plants did not change between 2017 and 2018 since they all are perennial plants and land use in the area did not change. Therefore, the sampling undertaken in the second year ensured a reliable evaluation of plants availability. To estimate vegetation cover by aromatic plants, line intercept transects were conducted in the surrounding area of the nests in the breeding season of 2018.

For each nest-box, four line transects of 25 m were established following the four cardinal points with centre in the tree where the nest was placed. With start in the tree and oriented towards each of the cardinal points, species of aromatic plants touching the line were identified, and the length (m) of the projection of the vegetative part of the plant on the line was measured as an estimate of that plant abundance. When a species of aromatic plant was present in the nest, but not recorded in the line intercept transect, a systematic search for that plant was carried out with the purpose of understanding if: i) the species was present in the 25 m circle around the tree, but not in the transect line, ii) the species was present outside the 25 m circle and iii) the species was not observed at all, either inside or outside the 25 m circle and up to 100 m.

Data analysis

The use of aromatic plants by females was evaluated considering the occurrence (presence/absence of added plants) and abundance (number of times each plant was added) of aromatic plants added by females to the nests. The use of each aromatic species was then evaluated through the frequency of occurrence in the nests (number of nests to which the species was added/total number of nests) and relative abundance (total number of times each plant species was added/total number of nests to which the species was added).

The availability of aromatic plants in the surrounding area of the nests was also evaluated considering the frequency of occurrence (number of transects in which the species was observed/total number of transects) and the relative abundance (total length of the species projected area/total length of transects) of each species. The abundance of aromatic plants was calculated for each nest surroundings by summing the total transect length occupied by each species in the four line intercept transects (summing up 100 m), and then standardized into a cover percentage.

The relationship between the frequency of occurrence and the relative abundance of aromatic species was explored graphically and through a Linear Regression Analysis, in order to understand if the more frequent species were also the more abundant, or not. This approach allowed a detailed understanding of the use and availability of aromatic plants. The independence of the aromatic species availability and use (frequency of occurrence and relative abundance) from each treatment (aromatic/control) and sampling year (2017/2018) was evaluated using the *G*-test (Sokal & Rohlf, 1995) for the total data set, and for the different breeding stages.

Jacobs' selectivity index (Jacobs, 1974) was used to relate the aromatic plants used by females and their availability in the surrounding area of the nests, based on the frequency of occurrence of each aromatic species. It was calculated according to the formula

$$D = (r - p)/(r + p - 2rp)$$

where r is the use of the species and p is the availability of the species. It varies from -1 (strong avoidance) to 1 (strong preference) and values close to zero mean that the species is used in accordance to its availability. Significant differences in the selectivity of the females between aromatic and control nests were tested with the Wilcoxon test.

Mann-Whitney test was used to compare number of visits to place the respective treatment, number of aromatic plants used and number of visits in which aromatic plants were collected; it was also used to compare breeding parameters between treatments (aromatic/control) and sampling years (2017/2018). The breeding parameters analysed included clutch size, hatching rate (number of hatchlings/number of eggs), number of fledglings, survival rate (number of fledglings/number of hatchlings) and breeding success (number of fledglings/number of eggs).

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, N.Y., USA). The significance level was set at 0.05 for all the analyses.

Results

Use of extra aromatic plants in aromatic and control nests

Extra aromatic plants were added by females in 100% of the aromatic nests and in 71.4% of the control nests. Fifteen aromatic plant species were used in nests of both groups, six were only used in aromatic nests and one was only used in control nests (Table 4). *Lavandula dentata* (LD)*, Calamintha nepeta* (CN) and *Dittrichia viscosa* (DV) were added to aromatic nests as part of the experiment, but females also added DV as an extra plant in nests of both groups (hereafter mentioned as DV* to differentiate from DV of the experiment). DV added by us and incorporated by females (DV*) were easily differentiated from each other, since the fragment added by us was bigger than those added by females, had a standardized shape and was cut evenly from the leaf; the fragments incorporated by females were much smaller, more numerous and very irregular, either in shape, size or cut. Treatment plants were kept by females in 61.5% of aromatic nests, with CN kept in 100% of nests, DV in 62.5% and LD in 37.5%. Grass was kept in the total number of control nests.

Table 4 - Number of aromatic and control nests where Blue Tit females added aromatic plant species. Abbreviations of species are in parenthesis. DV*: *Dittrichia viscosa* added by Blue Tits in nests of both groups.

Aromatic plant species added by Blue Tit females	**Aromatic nests**	**Control nests**
Cistus salviifolius **(CS)**	5	2
Lavandula stoechas **(LS)**	9	8
Margotia gummifera **(MG)**	7	2
Halimium halimifolium **(HH)**	2	3
Thapsia villosa **(TV)**	4	3
Dittrichia viscosa* **(DV*)**	3	6
Mentha suaveolens **(MS)**	1	1
Erodium cicutarium **(EC)**	1	1
Lithodora prostrata **(LP)**	1	-
Halimium calycinum **(HC)**	2	-
Foeniculum vulgare **(FV)**	1	-
Thymus capitellatus **(TC)**	1	-
Geranium robertianum **(GR)**	1	-
Calluna vulgaris **(CV)**	2	-
Pimpinella villosa **(PV)**	-	1

Overall, the mean number of visits to nests throughout the breeding cycle (aromatic: N = 12, 14.38 ± 0.65; control: N = 13, 14.71 ± 0.91; Mann-Whitney U test: $U = 64.00$; $P = 0.16$), the mean number of extra aromatic plants used (aromatic: N = 12, 3.08 ± 2.29; control: N = 13, 1.93 ± 1.59; Mann-Whitney U test: $U = 67.00$; $P = 0.23$) and the mean number of visits to nests in which aromatic plants were collected (aromatic: N = 12, 4.92 ± 3.28; control: N = 13, 3.79 ± 3.36; Mann-Whitney U test: $U = 71.00$; $P = 0.33$) did not differ significantly between groups. Breeding bird parameters did not differ significantly between nests, when either treatment or year was considered (Table 5).

Table 5 - Breeding parameters of Blue Tits observed in both treatment groups and years.

Breeding parameters	**Treatment**		**Mann Whitney U**	**Year**		**Mann Whitney U**
	Aromatic (N = 12)	**Control (N = 13)**		**2017 (N = 14)**	**2018 (N = 11)**	
Clutch size	8.50 ± 1.09	8.85 ± 1.41	$U = 71.50$ $P = 0.71$	8.64 ± 1.60	8.73 ± 0.65	$U = 68.50$ $P = 0.62$
Hatching rate	0.95 ± 0.07	0.92 ± 0.09	$U = 65.00$ $P = 0.44$	0.93 ± 0.09	0.94 ± 0.06	$U = 76.00$ $P = 0.95$
Number of fledglings	8.00 ± 1.21	7.62 ± 1.39	$U = 61.50$ $P= 0.35$	7.64 ± 1.65	8.00 ± 0.63	$U = 67.00$ $P = 0.57$
Survival rate	0.99 ± 0.03	0.94 ± 0.09	$U = 58.50$ $P = 0.13$	0.95 ± 0.09	0.98 ± 0.04	$U = 71.50$ $P = 0.67$
Breeding success	0.94 ± 0.06	0.87 ± 0.14	$U = 53.50$ $P = 0.17$	0.89 ± 0.15	0.92 ± 0.05	$U = 76.50$ $P = 0.98$

A detailed analysis of the floristic composition used by females revealed different patterns in the use of aromatic species (Figure 6): i) the frequency of use was significantly different between years (G-test$_{2017}$ = 180.2; G-test$_{2018}$ = 139.5; $P < 0.001$) and treatments (G-test$_{aromatic}$ = 443; G-test$_{control}$ = 150.9; $P < 0.001$); ii) the relative abundance significantly differed between years for aromatic (G-test = 33.61; $P = 0.001$) and control nests (G-test = 15.82; $P = 0.025$), as well as between treatments in 2017 nests (G-test = 16.56; $P = 0.05$), but not in 2018 nests (G-test = 8.77; $P > 0.05$). Moreover, the most frequently used aromatic species were not necessarily the most abundantly used by females, as demonstrated by the weak linear relationship between the frequency of occurrence and the relative abundance for all the nest groups (year/treatment), except for control nests in 2017 (R^2 = 0.84) (Figure 6).

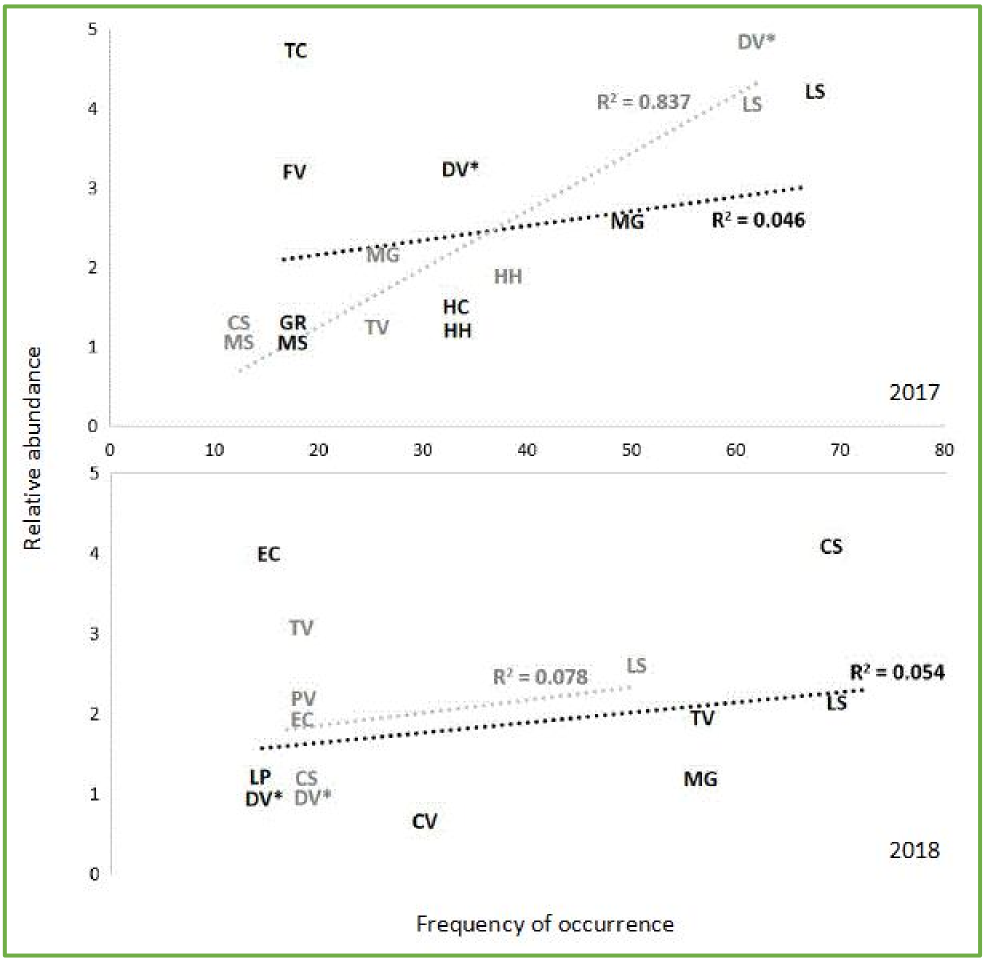

Figure 6 - Frequency of occurrence and relative abundance of extra aromatic plants added by Blue Tit females in aromatic (black line) and control nests (grey line), in 2017 (top) and 2018 (bottom). Coefficient of determination (R^2) is given for relationships between frequency of occurrence and relative abundance of aromatic species. Species abbreviations are in accordance with Table 4.

Availability and selectivity of extra aromatic plants

Ten of the aromatic plant species added by the females were present in the surrounding area of the nests. Other 5 species (DV*, MS, EC, FV, GR) were used in nests by females, but were not quantified by the line intercept transects. The frequency of occurrence and the relative abundance of the available aromatic plants showed a strong linear relationship both in aromatic ($R^2 = 0.63$) and control nests ($R^2 = 0.66$) (Figure 7). The most abundant and frequent aromatic species were HC, LS, and CS, whereas the lowest values were registered for LP. However, the aromatic plants available did not show an identical distribution pattern in the surrounding area of aromatic and control nests (Figure 7). Differences were mostly observed in the relative abundance of the species (*G*-test = 26.53; $P < 0.001$), and not in the frequency of occurrence (*G*-test = 21.28; $P < 0.01$). Furthermore, the presence of MG was only registered near aromatic nests (Figure 7).

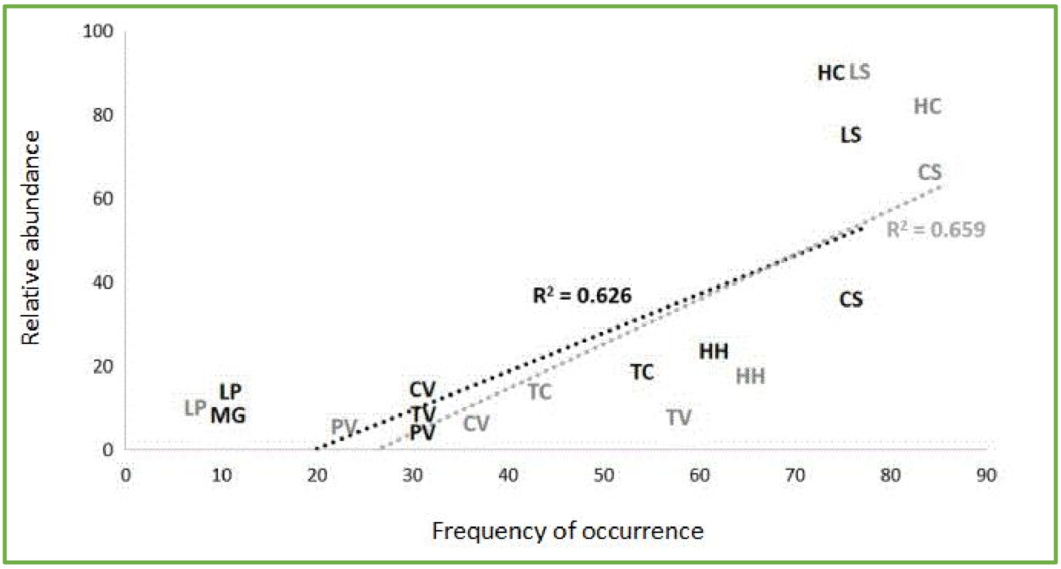

Figure 7 - Availability of extra aromatic plants in the surrounding area of aromatic (black line) and control nests (grey line). Coefficient of determination (R^2) is given for relationships between frequency of occurrence and relative abundance of aromatic species. Species abbreviations are in accordance with Table 4.

Jacobs' selectivity index accounted for the possible influence of the availability of aromatic plants on their selection and use by the females. Results (Figure 8) show the existence of significant differences between aromatic and control nests (Wilcoxon test; $Z = 2.19$; $P = 0.028$) in selection of aromatic plants added by females.

In aromatic nests, a clear preference for MG, DV*, FV, GR, MS and EC is observed, although these plants are not available in the nests' surroundings (DV*, FV, GR, MS and EC) or are available, but in low quantities (MG). Additionally, an avoidance of HH, HC, TC and PV is observed, despite the availability of these plants in the nests' surroundings (Figure 8). In control nests, a selection of MG, DV*, MS and EC is still observed. Clear avoidance of CV, HC, TC and LP is also observed, given that these plants are available in high quantities in the nests' surroundings (Figure 8).

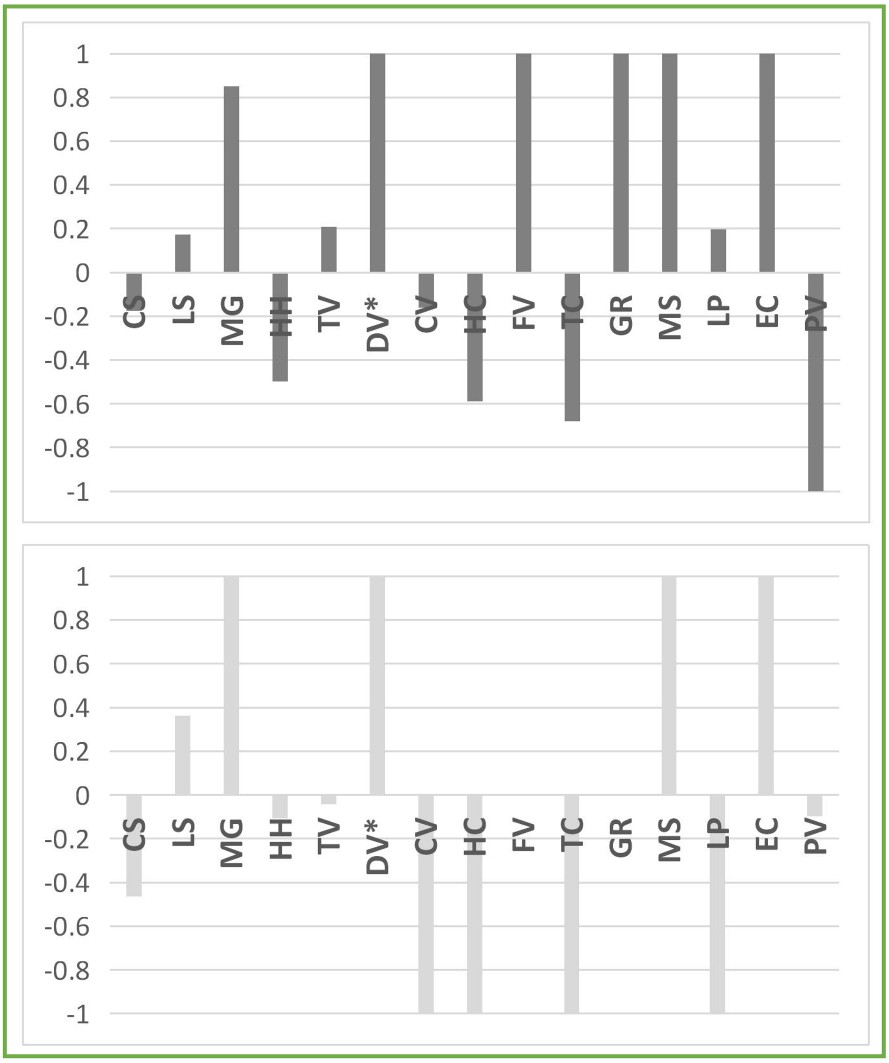

Figure 8 - Aromatic plants preferred (positive) and avoided (negative) by Blue Tit females in aromatic (dark grey, top) and control nests (soft grey, bottom), according to Jacobs' Index. Species abbreviations are in accordance with Table 4.

Extra aromatic plants used in the different breeding stages

Through the different breeding stages, extra aromatic plants incorporated in nests by females in both aromatic and control nests, varied in species, in number of nests where plant fragments were incorporated (occurrence) and in number of times each plant species was incorporated in the nests (abundance) (Figure 9). In the incubation and nestlings stages more aromatic plants were used and in higher number of nests, in both treatments (Figure 9). Aromatic species added to nests by females varied significantly, in all breeding stages, between aromatic and control nests ($G\text{-test}_{\text{laying}} = 73.84$, $P < 0.001$; $G\text{-test}_{\text{incubation}} = 99.11$, $P < 0.001$; $G\text{-test}_{\text{nestlings}} = 97.17$, $P < 0.001$; $G\text{-test}_{\text{fledglings}} = 73.01$, $P < 0.001$); regarding number of times each species was added, differences between aromatic and control nests were also significant ($G\text{-test}_{\text{laying}} = 10.98$, $P < 0.05$; $G\text{-test}_{\text{incubation}} = 11.89$, $P < 0.05$; $G\text{-test}_{\text{nestlings}} = 20.36$, $P < 0.05$; $G\text{-test}_{\text{fledglings}} = 4.01$, $P < 0.05$).

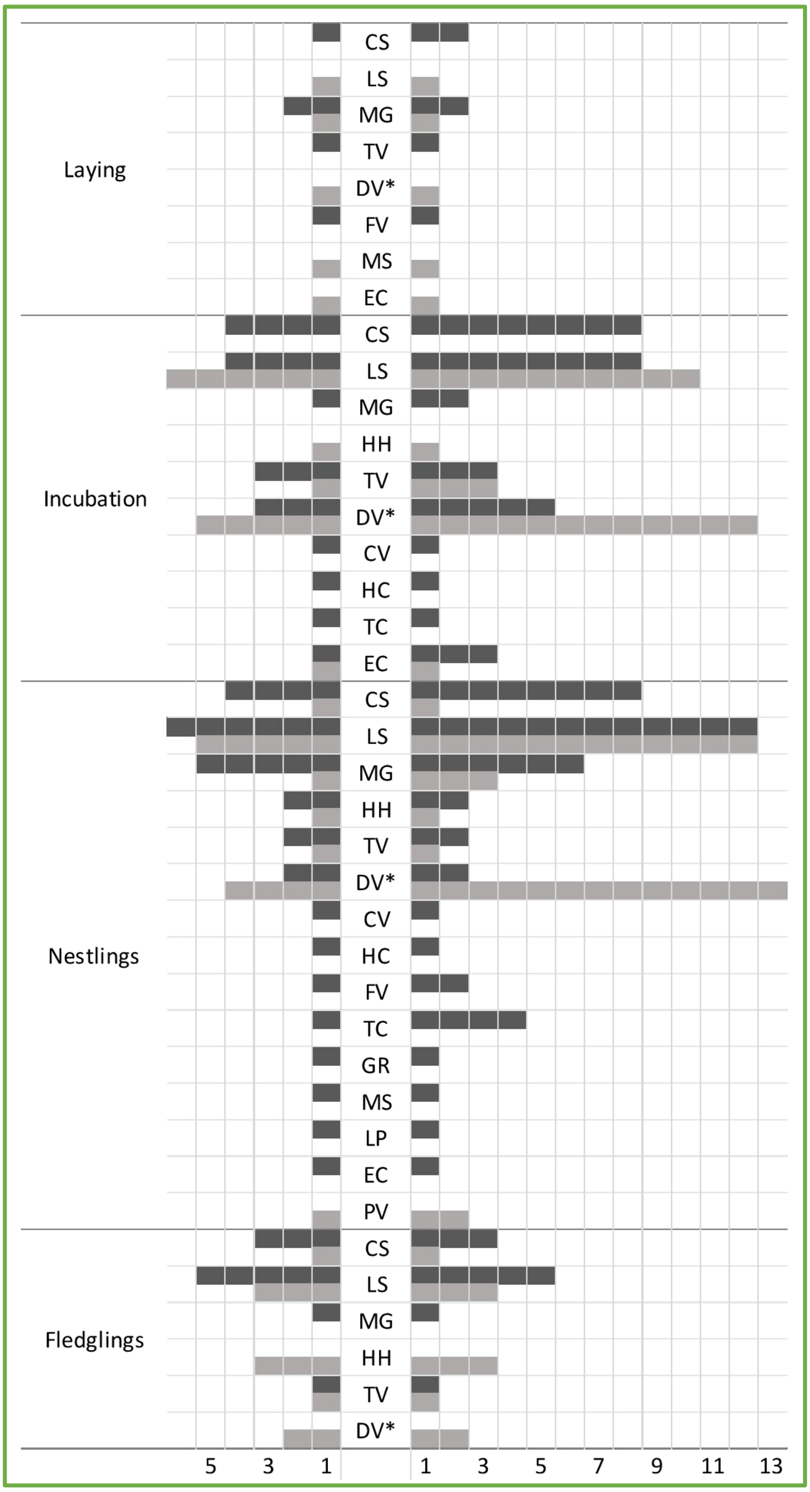

Figure 9 - Occurrence (left axis; number of nests where plant fragments were incorporated) and abundance (right axis; number of times each plant was incorporated in nests) of aromatic plants incorporated by females in the different breeding stages, in aromatic (dark grey) and control nests (soft grey). Species abbreviations are in accordance with Table 4.

Discussion

In this study, we intended to understand and explore the use of aromatic plants by Blue Tits. In all aromatic nests, but only in 71.4% of the control nests, females added extra aromatic plants to their nests. This means that females from the aromatic group found the need to incorporate plants of their choice, although their nests were supplemented with fragments of 3 aromatic plants. This finding is not in accordance with the study by Petit *et al.* (2002), in which females of nests experimentally-supplemented with aromatic plants added a lower proportion of aromatic plants of their choice, compared to females of non-supplemented nests.

Average number of visits, with or without collection of aromatic plants, did not differ significantly between both groups, controlling for potential bias. The average number of aromatic plants incorporated by females in nests of both groups varied, but not significantly. However, the average number of aromatic plants used was similar to that found by Lambrechts and Dos Santos (2000), in which 2.5 to 3 aromatic plants were found per nest on average.

Breeding parameters did not differ significantly between nests of either both groups or study years, although higher results were obtained in nests of the aromatic treatment, in four of the five studied parameters, and in all parameters for nests from 2018.

Our results showed different patterns of use of aromatic plants by females, with significant differences of use observed between years, treatments and in most of the nest groups (year/treatment) considered. Furthermore, the aromatic plants most frequently observed in nests were not necessarily the most abundantly used. This means that those plants were used in a great number of nests but very few times in each. The exception for this trend is control nests in 2017 in which many females chose the plants and used them many times. When we looked to the availability of these plants in the nests' surroundings, the more frequent species in the nests' surroundings were also the more abundant, that is, that with greater coverage. Choosing aromatic plants and incorporating them in nests is accomplished by females alone - they decide which plants are brought to nests and how much of each (Mennerat *et al.*, 2009b; Petit *et al.*, 2002; Tomás *et al.*, 2012). Even though the availability of plants is a necessity for females to use them, differences in use may be explained by an underlying individual preference as previously observed by Mennerat *et al.* (2009b).

In aromatic nests treatment plants were kept in 61.5% of these nests. Contrary to Mennerat (2008) that incorporated aromatic plants in Blue Tit nests hidden from the birds' view, in our study aromatic plants were placed in the nest cup facilitating the removal by the bird, if intended. It should be noted that these three plants were used more than expected considering their availability in other study (Pires *et al.*, 2012), and therefore some kind of importance should be ascribed to them. Essential oils present in these three plants show diversified biological activity: antimicrobial and fungicidal in *Calamintha nepeta* (Proença da Cunha *et al.*, 2012; Flamini *et al.*, 1999; Marongiu *et al.*, 2010); antibacterial, fungicidal and effective in burn cases and insect bites in *Lavandula dentata* (Cavanagh & Wilkinson, 2002, 2005) and antibacterial, antiseptic, healing, anti-inflammatory and antiparasitic in *Dittrichia viscosa* (Aissa *et al.*, 2019; Blanc *et al.*, 2006; Grauso *et al.*, 2019; Parolin *et al.*, 2014). In Corsica, *C. nepeta* is naturally added to nests by females (Lambrechts & Dos Santos, 2000; Mennerat *et al.*, 2009a; Petit *et al.*, 2002), as well as in Portugal (Pires *et al.*, 2012). *D. viscosa* was incorporated in aromatic nests as part of the treatment (DV) but was also naturally incorporated in nests by females (DV*), being one of the species incorporated more often in nests of both treatments. Interestingly, and based on our transects, this plant was not available in the nests' surroundings. Moreover, when searching outside our transects, DV was rare and the few plants found were located far from the nests in which it was used (between 35 and 100 m; B.P. pers. obs.). This is in accordance with the results of the Jacobs' index, in which this plant appears as a highly selected plant in both aromatic and control nests, that is, used in much higher quantities than its availability.

Together with DV* other 5 aromatic plants were the most abundant in nests: *Cistus salviifolius*, *Lavandula stoechas*, *Margotia gummifera*, *Halimium halimifolium* and *Thapsia villosa*. *C. salviifolius*'s essential oils show very low antimicrobial activity rates when tested against several micro-organisms (Guvenç *et al.*, 2005). In our study, *C. salviifolius* was used in both years and in nests from both treatments. *C. salviifolius* was available in the study area and in high quantities. Jacobs' index revealed that *C. salviifolius* is an avoided species in nests of both treatments, since it is much more available than used. We suggest that incorporation of this plant in nests might be more related to its high availability than to its properties as an aromatic plant. In Corsica, another plant belonging to the genus *Cistus* (*Cistus creticus*) is highly used in nests (Lambrechts & Dos Santos, 2000), what is explained by the fact that, among other *Cistus* species, *C. creticus* presents the highest antimicrobial activity (Guvenç *et al.*, 2005). Essential oils studied in *L. stoechas* showed antifungal activity and anti-inflammatory properties (Zuzarte *et al.*, 2013). In the present study, *L. stoechas* was used in both treatment groups and years and was highly available throughout the study area. Nevertheless, the Jacobs' index revealed that *L. stoechas* was a selected plant in nests of both treatments used in slightly higher quantities than its availability. The use of this species by Blue Tit females in our study area is in accordance with previous observations in Corsica, where it is a highly used species (Lambrechts & Dos Santos, 2000; Mennerat *et al.*, 2009a; Petit *et al.*, 2002). *M. gummifera*'s essential oil constitution revealed strong anti-inflammatory properties (Valente *et al.*, 2013). In our study, *M. gummifera* is used in both years and in nests of both treatments. Its availability was sparse in the study area, but according to Jacobs' index it was one of the most selected plant species by females. *H. halimifolium* has antioxidant, anti-inflammatory, anti-allergic and anti-cancer activities (Rebaya *et al.*, 2014) and in our study it was included by females in aromatic and control nests only in 2017. Although it was fairly available in the study area the Jacobs' index revealed that it was an avoided species in nests from both treatments. Essential oils studied in *T. villosa* revealed wide-spectrum fungicidal activity (Pinto *et al.*, 2017) and in our study it was incorporated in nests from both treatments and years. Its availability was sparse in the area and when its use was compared to its availability, the Jacobs' index revealed that it was used in accordance to its availability, being a selected plant in aromatic nests and a slightly avoided plant in control nests.

Other nine aromatic plants were incorporated in nests by Blue Tit females: *Mentha suaveolens*, *Lithodora prostrata*, *Erodium cicutarium*, *Halimium calycinum*, *Foeniculum vulgare*, *Thymus capitellatus*, *Geranium robertianum*, *Calluna vulgaris* and *Pimpinella villosa*. They were incorporated in very few nests and in lower quantities and their use/availability relation shows great variation, from clearly selected to clearly avoided. *H. calycinum* and *T. capitellatus* were used in very few nests and in low quantities, although both species were highly available in the study area. The Jacobs' index revealed that both species were avoided by females. *C. vulgaris*, *L. prostrata* and *P. villosa* were used in very few nests and in low quantities and their availability was also very low. *C. vulgaris* and *P. villosa* were avoided in nests from both treatments, but *L. prostrata* was used according to its availability in aromatic nests and clearly avoided in control nests. *F. vulgare*, *G. robertianum*, *M. suaveolens* and *E. cicutarium* were not available in the study area, but still they were used in aromatic nests (*F. vulgare*, *G. robertianum*) or in nests of both treatments (*M. suaveolens*, *E. cicutarium*). Since none of these species were present in the study area, the Jacobs' index revealed that all species were highly preferable in aromatic nests and *M. suaveolens* and *E. cicutarium* were preferable species in control nests.

The clear collection and identification of extra aromatic plants added by females provided important information on how females incorporate aromatic plants in their nests throughout the different breeding stages. Our observation that females start to incorporate aromatic plants into their nests from the beginning of egg-laying is in accordance with several other studies (Lambrechts & Dos Santos, 2000; Mennerat *et al.*, 2009b; Petit *et al.*, 2002; Tomás *et al.*, 2012). In our study, females stopped adding aromatic plants when nestlings were prepared to fledge, which is in tune with some previous studies (Lambrechts & Dos Santos, 2000; Mennerat *et al.*, 2009a; Petit *et al.*, 2002), while Tomás *et al.* (2012) observed that it stops a few days after hatching.

Extra aromatic plants added by females varied across the different breeding stages in number of species used, in how many nests each species was used (occurrence) and how many times each species was incorporated in nests (abundance). Considering the multitude of biological activities aromatic plants may present, it is understandable that species used vary across breeding stages. Aromatic plants more interesting to females may be chosen several times and incorporated in nests across breeding stages (present study) or even across breeding seasons (Mennerat *et al.*, 2009b). Some aromatic plants may be incorporated in nests several times per breeding stage, which may happen for two different reasons: first, compounds present in those plants, that are what make plants important to females, are volatile and therefore get less intense as time goes by; second, in our aromatic plant procedure, we removed all aromatic plants present in nests every 3 days, hence females might have been compelled to quickly replace the aromatic environment of the nest.

Number of species used and number of times each species was used, was higher in the incubation and nestlings stages, being in accordance with previous results from Lambrechts and Dos Santos (2000). In both stages, females spend long periods of time in nests, either incubating the eggs or providing warm and comfort to its nestlings; nestlings are bound to the nest (most of the time almost completely immobile) for their developing period until they are ready to fledge (Dubiec *et al.*, 2013; Scott-Baumann & Morgan, 2015). Therefore, and considering that aromatic plants are added to nests to reduce bacterial growth (Gwinner & Berger, 2005; Mennerat *et al.*, 2009c) and improve nestling condition (Mennerat *et al.*, 2009a), it is interesting that it is in the most important stages that more aromatic plants are added to nests and in higher quantities.

With this study we were able to show that Blue Tit females in our study area show a selective behaviour regarding aromatic plants they bring to their nests. This selectivity is related to the availability of such plants, but also with a certain preference for specific plants, since some of the plants brought to nests by females are not available in the near vicinity of nests. A clear difference in aromatic plants used and quantities in which they are used is shown for the different breeding stages, with more plants being used in higher quantities during the incubation and nestlings stages. We believe that our findings support the idea of female-selectivity of aromatic plants and the importance of its use regarding both parents and nestlings' protection.

Chapter 5

The heavier the better: nest weight is positively related to reproductive parameters in Blue Tits and aromatic plants in their nests

In preparation for submission as:

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Abstract

Nest construction is the first stage of the breeding cycle and represents a large component of parental investment. It reflects the builder's characteristics and has been shown to be related to several reproductive parameters and to life-history traits. The incorporation of aromatic plants in nests is a characteristic behaviour of Blue Tits. This behaviour has not been considered in studies trying to understand the relation between female investment in nest construction, reproductive parameters and life-history traits in this species. Our study aims to explore the relationships between nest weight, several aspects of breeding performance, laying date, brood mass and aromatic plants present in nests. Females laying earlier, used more aromatic plants in their nests and females that built heavier nests incorporated more aromatic plants in their nests, laid more eggs, hatched more nestlings and produced more fledglings. To our knowledge, this is the first study that considers the presence of aromatic plants in Blue Tit nests and the relation between nest weight and reproductive parameters.

Keywords

Nest weight, aromatic plants, Blue Tits, reproductive parameters, laying date.

Introduction

The primary function of a bird nest is to provide warm and safe conditions for the development of eggs and nestlings (Collias & Collias, 1984; Hansell, 2000). Nest construction is a plastic behaviour that reflects morphology and physiology of the bird that builds it, exhibiting considerable intra-specific variation between individuals (Deeming *et al.*, 2019; Hansell, 2000; Järvinen *et al.*, 2017). It is the first stage of the breeding cycle (Hansell, 2000) and represents a major component of parental investment (Álvarez & Barba, 2008) revealing the builders' quality (Mainwaring *et al.*, 2008), health (Tomás *et al.*, 2006) and body condition (Soler *et al.*, 1998). Nest size is used as a proxy of nest quality (Álvarez & Barba, 2008) and has been shown to be related to several reproductive parameters, such as brood size (Møller *et al.*, 2014), hatching success (Tomás *et al.*, 2006), breeding success (Moreno *et al.*, 2010) and life-history traits like laying date (Lambrechts *et al.*, 2016b).

Nest building is a costly activity due to energetic demands of construction but also because the process of building a nest is subjected to the pressure of optimizing the timing of reproduction, which in turn is subjected to the period when food availability is the greatest (Mainwaring & Hartley, 2008; Williams, 2012). Selection of different nest materials is also a costly activity, highly dependent on availability of materials (Mainwaring & Hartley, 2008), but also on experience to know where to find certain materials and where to use it in the nest, according to construction stage. Builders search for materials that will guarantee nest support (thicker materials, Biddle *et al.*, 2018b), thermal insulation (moss, Biddle *et al.*, 2019) and thermal regulation (lining material, Glądalski *et al.*, 2016), but also specific materials with other functional significance.

Aromatic plants are part of such materials and can be included in nests to reduce ectoparasite loads (Nest Protection Hypothesis; Scott-Baumann & Morgan, 2015), to improve nestling condition (Drug Hypothesis; Gwinner *et al.*, 2000) or to attract a mate (Courtship Hypothesis; Brouwer & Komdeur, 2004).

Here, we explore the relationships among (1) nest weight, (2) several aspects of breeding performance (laying date, brood size, nestling survival rate), (3) brood mass and (4) aromatic plant species used by Blue Tit *Cyanistes caeruleus* females in the nests. Several studies have already considered interactions between the first three variables. However, to our knowledge, the use of aromatic plants in nests has never been considered to be part of an explanatory model of interaction between nest features, reproductive parameters and brood mass. Two papers (Álvarez & Barba, 2008; Lambrechts *et al.*, 2017) have defended the need to include other variables in these studies and see what their part is in explaining results. By including the use of aromatic plants in the model, we predict that if nest weight (i.e. nest size) is an indication of female building effort, (1) a positive relation should be observed between this nest feature and incorporation of aromatic plants in nests, (2) heavier nests should be positively related with higher breeding parameters and brood mass and (3) aromatic plants in nests should be positively related with breeding parameters.

Methods

Between 2015 and 2018, 52 pine wood nest-boxes (width = 12 cm, depth = 15 cm, floor to entrance hole = 11 cm, entrance hole diameter = 2.6 cm) were monitored in the Portuguese Air Force Base N.6, in Montijo. The area is covered with stands of *Pinus pinea* L., and a few stands of *Quercus suber* L. and *Eucalyptus globulus* Labill. Nest-boxes were installed in pine stands accordingly to a grid pattern of roughly 25 m x 25 m, with the entrance hole facing south-east to avoid strong winds from north and west.

Nests were monitored weekly from March to mid-July to follow nest building, laying date (March 1st = day 1), brood size (number of eggs), brood size at hatch (number of eggs that hatched), brood size at fledge (number of nestlings that reached 15 days-old) and nestling survival rates (number of fledglings/number of hatchlings). Nests were removed from nest-boxes 22 days after hatching, once nestlings fledged, and each one was placed in a plastic bag. After removing the materials that were not part of the nest structure such as faeces, prey remains, unhatched eggs and/or dead nestlings, nests were weighted with a Pesola spring scale to the nearest 0.05 g. We used nest weight as a proxy of nest size and building effort (*e.g.* Mainwaring *et al.*, 2008 and Tomás *et al.*, 2006).

Fragments of 3 aromatic plants ca. 0.3 cm x 0.5 cm (one fragment of each species) were introduced in 26 nests (aromatic group) and a non-aromatic grass about the same size in 26 nests (control group). We used *Lavandula dentata* (Ld), *Calamintha nepeta* (Cn) and *Dittrichia viscosa* (Dv) in the aromatic group because in a previous study (Pires *et al.*, 2012) these plants were naturally placed in nests by female Blue Tits and used more than expected considering their availability in each nest surroundings. This procedure was carried out every 3 days from the time the first eggs were observed until nestlings left the nests. In each visit we removed aromatic plants detected in the nest cup, either placed by us or by the female, and stored them in small paper bags for later identification. In the end all aromatic plants were weighted in a Kern ABT 100-5M scale (KERN & SOHN GmbH, Germany) to the nearest 0.00001 g.

When nestlings were 15 days-old, they were weighted with a Pesola spring scale to the nearest 0.1 g and the sum of nestlings weight in each nest was used as brood mass. Brood mass was used instead of other nestling weight approaches, because we expect the total weight of nest occupants to be related with nest weight, considering i) seasonal nest material adjustments made by the female due to material deterioration by nestlings and ii) increasing water contents in nests affected by presence of nestlings (Dubiec & Mazgajski, 2013; Mainwaring & Hartley, 2008).

Statistical analysis

Mann-Whitney Test was used to compare nest weight, laying date, reproductive parameters (brood size, number of nestlings and number of fledglings), survival rate and brood mass between females from aromatic nests that either kept or removed aromatic plants from the treatment from their nests. The analysis was performed using SPSS Statistics for Windows v24.0.

A Redundancy Analysis (RDA) (Jongman *et al.*, 1995) was used to explore relationships between the weight of aromatic plant species collected from nests (either incorporated by us or by the female), reproductive parameters, brood mass and nest weight. This linear ordination method was selected after a preliminary Detrended Correspondence Analysis had shown a gradient length smaller than 3 SD (Ter Braak & Smilauer, 1998). A stepwise forward selection of plant species was used, and the final model was tested with the Monte Carlo test under 999 permutations. Correlations of plant species with the ordination axes higher than |0.4| were used in gradients interpretation. Nest treatment was also included in the analysis by creating a triplot ordination diagram where samples were coded as aromatic or control nests. To account for multicollinearity, plant species were maintained in the models only if their addition did not cause any Variation Inflation Factor (VIF) to exceed the value of 3.

Based on the RDA results, Path analysis with structural equation modelling (SEM) methodology was used to identify the most plausible interaction pathways between nest weight, several reproductive parameters, brood mass and aromatic plant species used by females in the nests. Species richness (S), specific diversity calculated through Shannon diversity index (H') and total weight of aromatic plants collected from each nest were accounted to characterize aromatic plant use by females. Path analysis requires the development of theoretical *a priori* models represented by diagrams specifying different relationships between variables. Multiple related equations are then solved simultaneously to test model fit and estimate parameters.

A large number of configurations were tested, trying to maximize the explanatory power (i.e., retaining as many paths as possible) as long as all paths were significant at $P \leq 0.05$ and the global model was acceptable, presenting good fit values. Each configuration was tested and the weakest paths (or non-significant) were sequentially removed until the best model was achieved (*e.g.* Blanc & Walters, 2008; Spasojevic *et al.*, 2014). The discrepancy function for the Maximum likelihood method was used in parameter estimation. The goodness-of-fit of the models was examined through the chi-square test, the root mean square error of approximation (RMSEA) and the comparative fit index (CFI). The chi-square test indicates the amount of difference between expected and observed covariance matrices. In this case, adequate model fit is indicated by P values greater than 0.05 (Kline, 2010). CFI is equal to the discrepancy function adjusted for sample size and ranges from 0 to 1 with larger values indicating better model fit. Acceptable model fit is indicated by a CFI value of 0.90 or greater (Hu & Bentler, 1999). RMSEA is related to residual in the model. Its values range from 0 to 1 with smaller values indicating better model fit. Acceptable model fit is indicated by an RMSEA value of 0.06 or less (Hu & Bentler, 1999).

Prior to analyses, variables were either log (x+1) (linear measurements) or arcsin [sqrt (x)] (percentages) transformed to improve normality. Redundancy Analysis and Path analysis were performed using the software CANOCO 4.5 and SPSS AMOS v24.0, respectively. The significance level was set at 0.05.

Results

Nest weight varied between 19.38 g and 60.16 g, with a mean of 38.84 g ± 9.24 (SD) (N = 52). Even though aromatic plants were incorporated in aromatic nests, females also incorporated species of their choice (Table 6). *D. viscosa* was added to nests experimentally by us but also by females as an extra plant (mentioned as Dv* to differentiate from Dv of the experiment).

Table 6 - Number of aromatic and control nests where Blue Tit females added aromatic plant species. The last 3 entries refer to number of aromatic nests in which females kept each aromatic plat added by the authors. Abbreviations are indicated in parenthesis. Dv* was naturally added by females.

Aromatic plant species found in Blue Tit nests	**Aromatic nests (N = 26)**	**Control nests (N = 26)**
***Cistus salviifolius* (Cs)**	6	4
***Lavandula stoechas* (Ls)**	17	15
***Margotia gummifera* (Mg)**	8	3
***Halimium halimifolium* (Hh)**	2	6
***Thapsia villosa* (Tv)**	4	3
***Dittrichia viscosa** (Dv*)**	4	11
***Mentha suaveolens* (Ms)**	1	1
***Erodium cicutarium* (Ec)**	1	1
***Lithodora prostrata* (Lp)**	1	1
***Calluna vulgaris* (Cv)**	3	1
***Halimium calycinum* (Hc)**	2	1
***Thymus capitellatus* (Tc)**	1	1
***Geranium robertianum* (Gr)**	1	-
***Foeniculum vulgare* (Fv)**	1	-
***Pimpinella villosa* (Pv)**	-	1
***Calamintha nepeta* (Cn)**	12	-
***Dittrichia viscosa* (Dv)**	10	-
***Lavandula dentata* (Ld)**	8	-

Aromatic plants from the treatment were kept in 58% of aromatic nests (in 15 out of 26 nests). Significant differences in laying date were observed between females that kept aromatic plants from the treatment in their nests (mean laying date: April 5th ± 5 days) and females that removed them (mean laying date: April 14th ± 15 days); no significant differences were observed in any of the other parameters considered in the analysis, although better results were observed in nests of females that kept aromatic plants from the treatment in their nests (Table 7).

Table 7 - Nest weight, laying date, reproductive parameters (brood size, number of nestlings and number of fledglings), survival rate and brood mass of females that kept or removed experimentally added aromatic plants from their nests. Significant results are in bold.

Parameters	**Females that kept aromatic plants from the treatment (N = 15)**	**Females that removed aromatic plants from the treatment (N = 11)**	**Mann-Whitney Test**	
			U	***P***
Nest weight	39.95 ± 6.63	37.78 ± 7.35	64.00	0.36
Laying date	36.27 ± 4.74	44.09 ± 14.28	44.50	**0.047**
Brood size	8.00 ± 1.51	7.82 ± 1.47	79.50	0.88
Number of nestlings	7.53 ± 1.77	6.64 ± 2.66	72.00	0.61
Number of fledglings	7.33 ± 1.72	6.45 ± 2.84	75.50	0.72
Survival rate	97.67 ± 6.78	96.36 ± 12.06	80.00	0.92
Brood mass	63.98 ± 24.35	62.09 ± 23.94	79.00	0.88

The first two axes of the RDA accounted for 40% of the total data variability (Figure 10). These axes also revealed a good association between the weight of aromatic plants collected from nests and the relevant environmental variables (0.65 and 0.55, respectively), explaining most of the species-environmental relation (95.1%), thus supporting the interpretation of the results. Eight significant variables ($P < 0.05$) were included in the ordination model. According to the canonical coefficients and inter-set correlations, axis 1 was mainly defined by Cv (r = 0.45), Ms (r = −0.41), Hc (r = −0.42), and Lp (r = −0.40). Axis 2 was related to Ld (r = 0.5), Mg (r = 0.49), Tv (r = 0.4), and Ec (r = −0.41).

The ordination diagram (triplot) showed a good spatial segregation of the parameters in analysis, especially along the first axis (Figure 10). Conversely, the discrimination of samples was not observed, as indicated by the overlap between aromatic and control nests.

The output of the RDA analysis clearly distinguished three groups of parameters associated with the weight of different aromatic plants used in the nests: (i) Laying date was mostly associated with a high weight of Cv; (ii) Nest weight and survival rate revealed an evident positive relationship with the weight of Mg, Ld and Tv, while showing an inverse association with Ec; (iii) Brood mass and reproductive parameters were positively associated with Ec, Lp, Hc and Ms, but negatively related to Cv (Figure 10). Furthermore, results suggest an association of the second and third groups with a richer and more diverse floristic assemblage, whereas laying date was related to fewer aromatic plants, representing also an overall lower weight.

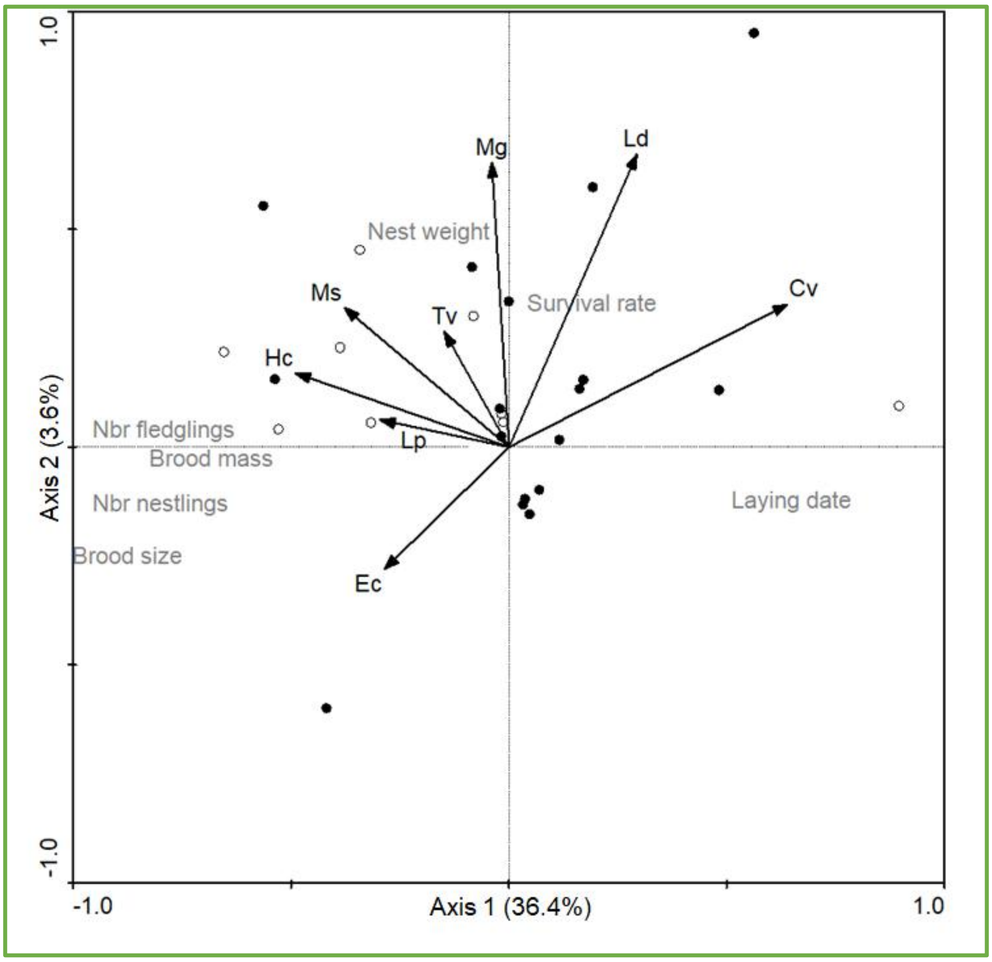


Figure 10 - Ordination diagram (triplot) of the Redundancy Analysis between weight of aromatic plants' species used by females, nest weight, brood mass and reproductive parameters in aromatic (black dots) and control nests (blank dots). Abbreviations are in accordance with Table 6.

Redundancy analysis also revealed that laying date was potentially inversely associated with brood size, number of nestlings and fledglings and brood mass (Figure 10), that is, females laying earlier used more aromatic plants in their nests. Moreover, nest weight showed a relation with brood size, number of nestlings and fledglings and brood mass, that is, heavier nests are associated with higher breeding parameters and nestling weight; survival rate was weakly related to any other variable (Figure 10).

Since we were not able to observe a clear separation between control and aromatic nests in the RDA diagram (blank and black dots, respectively), that is, control and aromatic nests did not produce different associations with the considered parameters, they were included as a single group in the Path analysis (not differentiated by treatment). Furthermore, individual aromatic plants were not included in the Path analysis as individual variables, since the relationships between parameters and aromatic species explored in the RDA are both positive and negative, hampering the construction and interpretation of the tested models. Therefore, total aromatic plant weight, richness and diversity were considered in the models.

Path analysis showed that reproductive parameters and aromatic plants had a positive and highly significant association with nest weight and laying date is negatively (and significantly) linked with aromatic plants present in nests (Figure 11). Laying date had a negative effect on reproductive parameters and aromatic plants had a positive effect on survival rate, but neither of the effects was significant (Figure 11). Path analysis also revealed a negative indirect effect (not in the figure) of laying date on nest weight (-0.27) and brood mass (-0.44).

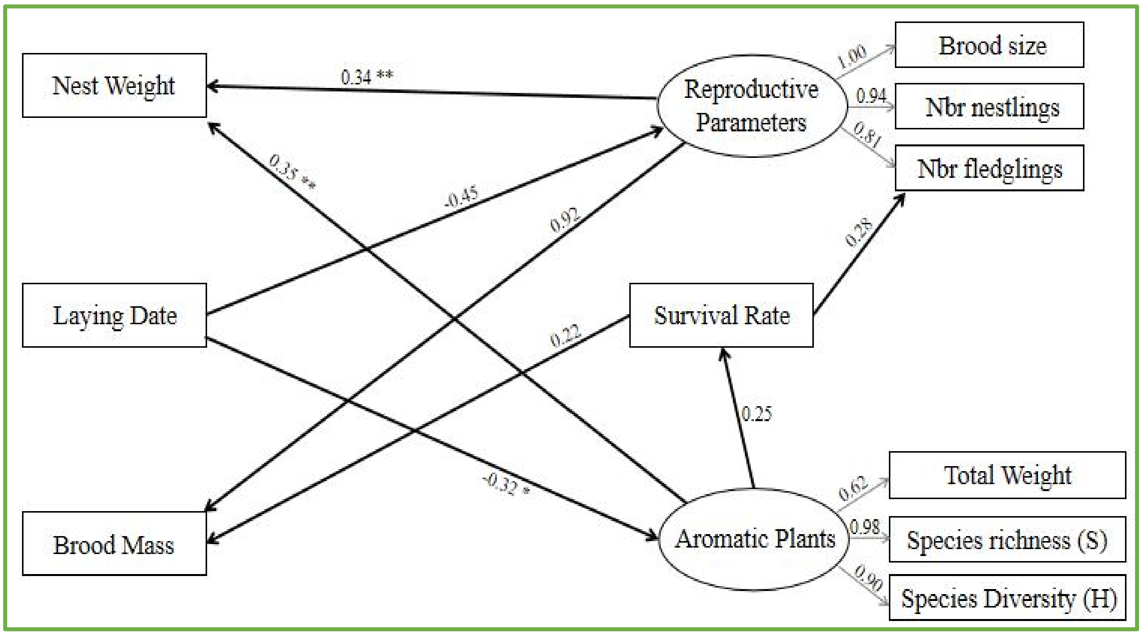


Figure 11 - Path diagram of the selected model explaining the interactions between nest weight, reproductive parameters, laying date, survival rate, brood mass and aromatic plants used in the nests χ^2 (32.93, Df = 31, P = 0.37); RMSEA = 0.03; CFI = 0.99. Direct standardized regression coefficients between variables are shown on each arrow; significant values are indicated: $P < 0.05$ (*) and $P < 0.01$ (**).

Discussion

We aimed to understand how Blue Tits' nest features in nest-boxes are related with several aspects of breeding performance, brood mass and aromatic plant species used by females in the nests. Among the different nest measures, we chose to use nest weight, reported by Mainwaring *et al.* (2008) and Tomás *et al.* (2006) as a reliable indicator of nest size. Other studies used different nest measures, such as nest height (Järvinen *et al.*, 2017; Lambrechts *et al.*, 2016b), nest volume (Lambrechts *et al.*, 2017) and nest base area (Møller *et al.*, 2014). In the context of this discussion, we will compare our results directly with results that used different nest measures, since ultimately all nest measures were considered to be reliable in the framework of the respective studies.

Laying date was negatively and significantly related with the presence of aromatic plants in nests, that is, females laying earlier used more aromatic plants in their nests. This can be explained by the fact that, similar to what Tomás *et al.* (2012) observed, females that breed earlier in our study area, are adult females and therefore more experienced to i) select breeding sites with higher availability of aromatic plants (Álvarez *et al.*, 2013); ii) be more skilled in collecting aromatic plants and incorporating them in their nests; iii) incorporate more aromatic plants in their nests instead of adopting different behaviours of ectoparasite avoidance and nest sanitation (see Hurtrez-Boussès *et al.*, 2000; Simon *et al.*, 2005b). Our findings clearly support the results obtained by Tomás *et al.* (2012), according to which 1st year breeders reproduce later in the season and their nests are more parasitised by blackflies and biting midges; since earlier breeders use more aromatic plants in their nests than later breeders, this can cause the former to be less attacked by parasites than the latter. Nonetheless, Tomás *et al.* (2012) also observed that the incorporation of aromatic plants in nests is independent of female age.

In addition to this, our results also show that females from aromatic nests that kept aromatic plants from the treatment in their nests, started breeding significantly earlier compared to females that removed aromatic plants provided by us from their nests. This finding suggests that females laying earlier used more aromatic plants in their nests. A possible explanation might be that early-breeding females are more stressed to manage resources (find a suitable nest site, food provisioning, etc.) in the first part of the breeding season and therefore chose to keep aromatic plants from the treatment in their nests to save time searching for other aromatic plants.

Ultimately, one final consideration must be made: the behaviour of incorporating aromatic plants in nests is an individual choice of each female and some birds may choose not to use aromatic plants or use lower/higher quantities of aromatic plants in their nests.

In our study, heavier nests were associated with larger broods and higher number of nestlings and fledglings. Assuming that heavier nests take more time to be built it seems that when females invested more time in building a heavier nest they laid more eggs, hatched more nestlings and produced more fledglings. This positive relation between nest weight, productivity and breeding success was also observed by Álvarez and Barba (2008) and Lambrechts *et al.* (2017). Álvarez and Barba (2008) likewise observed that breeding success was positively related to nest quality (measured by its weight and height), pondering whether the nest quality itself will not have a direct effect on breeding success. Nonetheless, other studies reached different results, with number of nestlings and number of fledglings being negatively related to nest size (Lambrechts *et al.*, 2016a; Moreno *et al.*, 2010). Moreno *et al.* (2010) even suggested that females and nestlings benefit from nest size in opposite ways. Females clearly benefit from larger nests, since they allow them to reduce incubation costs; nestlings, on the other hand, benefit from reduced construction costs, since it improves female parental care in the first stages of nestling life.

Females that built heavier nests also used more aromatic plants in their nests, which is in accordance with our initial prediction. As seen before, heavier nests also produced more fledglings and aromatic plants present in nests had a positive effect, although not significant, in nestling survival rate. Nest building and the incorporation of aromatic plants in nests are carried out by females alone and both activities are considered female-traits to alter features of parental investment (Tomás *et al.*, 2013). Ultimately, nest construction and incorporation of aromatic plants in nests are costly behaviours that can be positively or negatively influenced by many female-related traits (see Mainwaring *et al.*, 2008; Mainwaring & Hartley, 2009; Tomás *et al.*, 2006).

After nest construction, offspring provisioning may be impaired by females and, therefore, hamper nestling development and reproductive success as previously shown by Merino *et al.* (1998) and Moreno *et al.* (2010).

Path analysis also reported direct and indirect effects that were not significant. From those, the negative indirect effect of laying date on nest weight and brood mass requires some explanation. First, both relations were included in the theoretical Path model created initially, but since the model's goodness-of-fit was impaired, they were removed; nonetheless, the final model included both relations as indirect effects. Second, results on nest weight being negatively related to laying date are in accordance with Møller *et al.* (2014) but differ from those obtained by Britt and Deeming (2011) and Lambrechts *et al.* (2016b, 2017). Regarding relation between brood mass and nest weight, our results are in agreement with Järvinen *et al.* (2017). Ultimately, these results are explained by the fact that females that breed later in the season experience a decline in reproductive success (Verhulst & Nilsson, 2008) and nestling condition (de Lope *et al.*, 1998).

In last, this study provided new and important insights on the relations between nest weight, reproductive parameters and aromatic plants in Blue Tit nests. Nest weight proved to be a reliable predictor of female building effort, establishing positive and significant relations with reproductive parameters and with presence of aromatic plants in nests. Considering these results (and others similar), future studies should focus on particular nest features (*e.g.* materials used in nest construction, building structure) and investigate which relations are established in an explanatory model similar to the one used in this study.

Chapter 6

General Discussion

Since the seminal work of Wimberger (1984), the use of aromatic plants by some bird species has been intensively studied (*e.g.* Dubiec *et al.*, 2013). Throughout the years several hypotheses have been formulated to explain this behaviour, but it is now widely accepted that birds incorporate aromatic plants in their nests as a defence mechanism to avoid presence of ectoparasites and/or pathogens in nests. Aromatic plants are believed to decrease parasite numbers (Nest Protection Hypothesis; Scott-Baumann & Morgan, 2015) and enhance nestling immune function (Drug Hypothesis; Gwinner *et al.*, 2000), improving nestling growth and condition.

The need to supplement nests with aromatic plants has its origin in the fact that parasites in nests produce detrimental effects on their hosts, mainly tissue damage and blood consumption (chapter 2; Allander, 1998; Hurtrez-Boussès *et al.*, 1997; Johnson & Clayton, 2003; Tomás *et al.*, 2007b). Nestling condition parameters (especially nestling weight and tarsus length) have already been shown to be negatively affected by presence of ectoparasites in nests (chapter 2).

Nest site choice, and especially the avoidance of nest sites with old nest material are defence mechanisms adopted by parents to reduce the chances of arrival and/or persistence of parasites in their nests (chapter 2; Mazgajski, 2007a, 2007b).

Regarding the presence of aromatic plants in Blue Tit nests, many studies have focused on how they affect nestling condition and development (*e.g.* Mennerat *et al.*, 2008, 2009a, 2009c). Presence of aromatic plants in nests positively affected nestlings' mass gain, feather development and hematocrit levels (Mennerat *et al.*, 2009a) and significantly reduced nestling bacterial loads, especially under high infestation of Protocalliphora blow fly larvae (Mennerat *et al.*, 2009c). When brood size is taken into consideration, nestlings are heavier in smaller broods (Mennerat *et al.*, 2008; chapter 3), have increased mass gain, feather development and body size (Mennerat *et al.*, 2009a). When presence of aromatic plants interacts with brood size, nestling tarsus length is positively and significantly affected by presence of aromatic plants in large broods (chapter 3). That is, aromatic plants might work by reducing parasite loads, which allowed nestlings to grow longer tarsi, even though food and parental care available for each nestling was much less in large broods as compared to small broods.

In all these studies - Mennerat *et al.* (2008, 2009a, 2009c) and chapter 3 - aromatic plants considered in aromatic nests were experimentally incorporated in nests by the authors through an aromatic plant procedure and were chosen because they are often found in Blue Tit nests, naturally placed by the female. In chapter 3, *Lavandula dentata*, *Calamintha nepeta* and *Dittrichia viscosa* were chosen because they were naturally incorporated by females in a previous study (Pires *et al.*, 2012) and were found in nests in higher quantities than expected, considering their availability in the surrounding areas of nests.

In chapter 3, no effect of presence of these aromatic plants in nests was observed in either reproductive parameters (number of fledglings and nestling survival rate) or nestling condition (nestling weight and tarsus length). However, in chapter 4 we found that an importance should be attributed to these plants, since they were kept in 61.5% of the studied aromatic nests; that is, given the choice of keeping or removing these three aromatic plants from their nests, females chose to keep them. In fact, *Calamintha nepeta* was kept by the females in 100% of nests, *Dittrichia viscosa* in 62.5% and *Lavandula dentata* in 37.5%. Essential oils present in these plants show various biological activities, such as antimicrobial, fungicidal, antibacterial, antiseptic, healing, anti-inflammatory and antiparasitic (Cavanagh & Wilkinson, 2005; Grauso *et al.*, 2019; Proença da Cunha *et al.*, 2012), which can explain why they were kept in nests.

In chapter 4, it was also observed that, even though aromatic nests were supplemented with specific aromatic plants, females still added aromatic plants of their choice, which is exactly the opposite of what was observed in a previous study (Petit *et al.*, 2002). The pattern of use of aromatic plants incorporated in nests by females was very diverse, either between years (2017 and 2018) or treatments (aromatic and control nests). Comparison between the use of some species and their availability revealed that (1) some of the plants incorporated in nests by females were not present in the nests' surroundings, (2) some plants were highly available, but females chose not to use them and (3) significant differences were observed for the use/availability relation between aromatic and control nests. A total of 15 aromatic plants were used in aromatic and control nests, but six were incorporated in a higher number of nests (*Cistus salviifolius*, *Lavandula stoechas*, *Margotia gummifera*, *Halimium halimifolium*, *Thapsia villosa* and *Dittrichia viscosa*). The relation between use and availability of each aromatic plant in aromatic and control nests, studied through Jacobs' Index, revealed preferable and avoided plants in aromatic and control nests.

The study presented in chapter 4, also allowed the understanding of how aromatic plants are used across the different breeding stages. Results obtained were very similar to others achieved by several other studies (Lambrechts & Dos Santos, 2000; Mennerat *et al.*, 2009a, 2009b; Petit *et al.*, 2002; Tomás *et al.*, 2012), reinforcing the idea that more aromatic plants (and in higher quantities) are added to nests when females and nestlings are present in nests for longer periods of time, that is, in the incubation and nestlings stages.

Results achieved with this study (chapter 4) reinforce two major notions regarding use and selectivity of aromatic plants by female Blue Tits. First, biological activities identified from the essential oils present in the aromatic plants used by females, clearly explain why females actively searched for them. Second, the process of choosing and incorporating aromatic plants in nests is made by females alone and, since in our study availability of these plants did not entirely explain why they were used, an underlying effect of female individual preference must be considered (Mennerat *et al.*, 2009b).

In a way, similar to nest construction, the incorporation of aromatic plants in nests represents an aspect of female effort. In Blue Tits, if a study aims to understand how female building effort relates to several aspects of breeding performance, aromatic plants should be included in that relation.

In chapter 5, the use of aromatic plants by females was associated to parameters related to female building effort (i.e. nest weight), laying date, reproductive parameters (brood size, number of nestlings, number of fledglings and nestlings' survival rate) and brood mass. Relations of nest weight with some of the parameters mentioned above, or similar, were already investigated in previous studies (Álvarez & Barba, 2008; Lambrechts *et al.*, 2016b, 2017; Møller *et al.*, 2014; Moreno *et al.*, 2010; Tomás *et al.*, 2006). However, to our knowledge, specific studies that focused on Blue Tits did not acknowledge the behaviour displayed by this species of incorporating aromatic plants in nests, which are an important component of the nest.

This study used two different but complementary methodologies to understand how the different parameters considered are related: Redundancy Analysis (RDA) and Path analysis with Structural Equation Modelling. The RDA worked as a preliminary analysis of the relation between all parameters considered and the different aromatic plants incorporated in nests by the authors and by the female. This methodology allowed us to understand that in the Path analysis (1) aromatic and control nests should be considered as a single group, since we did not observe differences in the associations that each group established with the considered parameters and (2) aromatic plants should be considered as a single group and not individually, since the relations between the different aromatic plants and the parameters considered were, most of the times, antagonistic (either positive or negative). Nonetheless, four results of the RDA alerted us to important relations to take into account, while constructing the Path model: i) laying date was related to fewer aromatic plants (lower weights); ii) laying date was related to reproductive parameters and brood mass; iii) nest weight was related to reproductive parameters and brood mass and iv) survival rate was weakly related to any other variable. All these relations were included in the Path analysis. Even though the model suffered several changes to guarantee the best model fit possible, results produced were in tight accordance with the results observed in the RDA: i) nest weight was positively and significantly related to reproductive parameters and aromatic plants; ii) laying date was negatively and significantly related to aromatic plants and negatively related to reproductive parameters; iii) aromatic plants were positively related to survival rates and iv) laying date was indirectly and negatively related to nest weight and brood mass.

From these, two significant relations were extracted from the selected model. First, females laying earlier in the season, use more aromatic plants in their nests. This can be explained by the fact that perhaps females breeding earlier in our study area, may be older females (following Tomás *et al.*, 2012) and, due to experience, may select breeding sites with more aromatic plants available (Álvarez *et al.*, 2013), may be more skilled at collecting aromatic plants and/or may choose to incorporate more aromatic plants in their nests instead of adopting other behaviours of ectoparasite avoidance and nest sanitation (see Hurtrez-Boussès *et al.*, 2000; Simon *et al.*, 2005a). Additionally, our results also show that females from aromatic nests that kept aromatic plants from the treatment in their nests, started laying significantly earlier as compared to females that removed aromatic plants from the treatment from their nests, which can contribute to explain this first main result.

Second, nest weight was significantly and positively related to weight of aromatic plants and reproductive parameters. And this is an expected result, assuming that incorporation of aromatic plants in nests is a trait of female building effort, that is, females that increase building effort in nest construction will invest more time in aromatic plant collection (Tomás *et al.*, 2013). Similarly, females that invested more time building a nest laid more eggs, hatched more nestlings and produced more fledglings as also observed by Álvarez and Barba (2008) and Lambrechts *et al.* (2017). A non-significant result, relevant in explaining these relations, was that aromatic plants present in nests had a positive effect on nestlings' survival rate. Apart from this non-significant result, relation between these three parameters was highly expected.

Conclusions and future research

The set of studies presented in this thesis allowed a better understanding of two major issues that have led to a large number of studies in recent years: how nestlings of cavity-nesting birds are affected by parasites (chapter 2) and how aromatic plants are used by Blue Tits in their nests (chapter 3, 4 and 5).

Regarding the first issue, literature reviewed helped us to understand that nestlings are affected by parasites in a multitude of ways: i) ectoparasites present in nests that establish a direct contact with nestlings, ii) blood parasites, transmitted by ectoparasites present in nests, and iii) through parents' infection status and condition. To mitigate the negative impacts of parasites, both parents and nestlings adopt different defence mechanisms that will either reduce the probability of arrival of parasites or alleviate contact with parasites. Nonetheless, our review also showed that more studies are needed because the information is still sparse, fragmented and contradictory. Topics such as how nestlings are affected by blood parasites require more studies, because the existing ones are few, making it difficult to reach firm conclusions. Adoption of standardized methods will also allow more comparable results.

Regarding the second issue, we believe the three studies carried out enriched the current knowledge on the topic. The results obtained gave scientific support to previous studies, but have also considerably improved our knowledge of the subject to date by including new study variables, new methodologies and new ideas. Thanks to the joint data from previous studies and those presented here, it is now clear that aromatic plants in nests benefit nestlings' development and condition. Selectivity of aromatic plants by females and the periodicity with which aromatic plants are included in nests were already known, but not with the level of detail that the studies presented here provided. The relation between nest features (*e.g.* nest size) and several reproductive parameters was also previously known but, in Blue Tits, the addition of parameters related to aromatic plants, proved to be useful and interesting to better understand how these variables, that are fundamental to nestlings' condition and development, interact.

For a better understanding of how female Blue Tits use aromatic plants and how their inclusion in the nests influences reproductive parameters and nestlings' body condition and development, the variation in weather conditions must be considered in future studies. Not only because biodiversity is facing a crisis, amplified by the effects of climate change, but also because the variation in weather conditions will surely affect the availability of resources (food, materials used in nest construction, etc.) and it will be important to witness how species with such specific behaviours will adapt.

References

Aissa, I., Nimbarte, V. D., Zardi-Bergaoui, A., Znati, M., Flamini, G., Ascrizzi, R. & Jannet, H. B. (2019). Isocostic acid, a promising bioactive agent from the essential oil of *Inula viscosa* (L.): Insights from drug likeness properties, molecular docking and SAR analysis. *Chemistry & Biodiversity*, *16*(4), e1800648. https://doi.org/10.1002/cbdv.201800648

Alatalo, R. V. & Lundberg, A. (1986). Heritability and selection on tarsus length in the pied flycatcher (*Ficedula hypoleuca*). *Evolution*, *40*(3), 574-583. https://doi.org/10.1111/j.1558-5646.1986.tb00508.x

Allander, K. (1998). The effects of an ectoparasite on reproductive success in the great tit: a 3-year experimental study. *Canadian Journal of Zoology*, *76*(1), 19-25. https://doi.org/10.1139/z97-162

Álvarez, E. & Barba, E. (2008). Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits *Parus major*. *Acta Ornithologica*, *43*(1), 3-9. https://doi.org/10.3161/000164508X345275

Álvarez, E., Belda, E. J., Verdejo, J. & Barba, E. (2013). Variation in Great Tit nest mass and composition and its breeding consequences: a comparative study in four Mediterranean habitats. *Avian Biology Research*, *6*(1), 39-46. https://doi.org/10.3184/175815513X13609517587237

Arriero, E. (2009). Rearing environment effects on immune defence in Blue Tit *Cyanistes caeruleus* nestlings. *Oecologia*, *159*(4), 697. https://doi.org/10.1007/s00442-008-1253-1

Atkinson, C. T., Thomas, N. J. & Hunter, D. B. (Eds.) (2008). *Parasitic diseases of wild birds*. Wiley-Blackwell.

Bańbura, J., Perret, P., Blondel, J., Sauvages, A., Galan, M-J. & Lambrechts, M. M. (2001). Sex differences in parental care in a Corsican Blue Tit *Parus caeruleus* population. *Ardea*, *89*(3), 517-526.

Bańbura, J., Perret, P., Blondel, J., Thomas, D. W., Cartan-Son, M., & Lambrechts, M. M. (2004). Effects of *Protocalliphora* parasites on nestling food composition in Corsican Blue Tits *Parus caeruleus*: consequences for nestling performance. *Acta Ornithologica*, *39*(2), 93-103.

https://doi.org/10.3161/068.039.0206

Bauchau, V. (1997). Do parasitic mites decrease growth of nestling Pied Flycatchers Ficedula hypoleuca? Ardea, 85(2), 243-247.

Bennett, G. F., Peirce, M. A. & Ashford, R. W. (1993). Avian haematozoa: mortality and pathogenicity. *Journal of Natural History*, *27*(5), 993-1001. https://doi.org/10.1080/00222939300770621

Bennett, G. F., Squires-Parsons, D., Siikamäki, P., Huhta, E., Allander, K. & Hillström, L. (1995). A comparison of the blood parasites of three Fenno-Scandian populations of the Pied Flycatcher *Ficedula hypoleuca*. Journal of Avian Biology, 26(1), 33-38. https://doi.org/10.2307/3677210

Biddle, L. E., Broughton, R. E., Goodman, A. M. & Deeming, D. C. (2018a). Composition of bird nests is a species-specific characteristic. *Avian Biology Research*, *11*(2), 132-153. https://doi.org/10.3184/175815618X15222318755467

Biddle, L. E., Deeming, D. C. & Goodman, A. M. (2018b). Birds use structural properties when selecting materials for different parts of their nests. *Journal of Ornithology*, *159*(4), 999-1008. https://doi.org/10.1007/s10336-018-1571-y

Biddle, L. E., Dickinson, A. M., Broughton, R. E., Gray, L. A., Bennett, S. L., Goodman, A. M. & Deeming, D. C. (2019). Construction materials affect the hydrological properties of bird nests. *Journal of Zoology*, *309*(3), 161-171. https://doi.org/10.1111/jzo.12713

Blanc, L. A. & Walters, J. W. (2008). Cavity excavation and enlargement as mechanisms for indirect interactions in an avian community. *Ecology*, *89*(2), 506-514. https://doi.org/10.1890/07-0219.1

Blanc, M. C., Bradesi, P., Gonçalves, M. J., Salgueiro, L. & Casanova, J. (2006). Essential oil of *Dittrichia viscosa* ssp. *viscosa*: analysis by ^{13}C-NMR and antimicrobial activity. *Flavour and Fragrance Journal*, *21*(2), 324-332. https://doi.org/10.1002/ffj.1605

Blondel, J., Maistre, M., Perret, P., Hurtrez-Boussès, S. & Lambrechts, M. M. (1998). Is the small clutch size of a Corsican blue tit population optimal? *Oecologia*, *117*(1-2), 80-89. https://doi.org/10.1007/s004420050634

Bouslama, Z., Chabi, Y. & Lambrechts, M. M. (2001). Chicks resist parasite intensities in an Algerian population of blue tits. *Écoscience*, *8*(3), 320-324. https://doi.org/10.1080/11956860.2001.11682659

Bouslama, Z., Lambrechts, M. M., Ziane, N., Djenidi, R. & Chabi, Y. (2002). The effect of nest ectoparasites on parental provisioning in a north-African population of the Blue Tit *Parus caeruleus*. *Ibis*, *144*(2), 73-78. https://doi.org/10.1046/j.1474-919X.2002.00070_5.x

Briggs, K. B. & Deeming, D. C. (2016). Use of materials in nest construction by Pied Flycatchers *Ficedula hypoleuca* reflects localized habitat and geographical location. *Bird study*, *63*(4), 516-524. https://doi.org/10.1080/00063657.2016.1238867

Britt, J. & Deeming, D. C. (2011). First-egg date and air temperature affect nest construction in Blue Tits *Cyanistes caeruleus*, but not in Great Tits *Parus major*. *Bird Study*, *58*(1), 78-89. https://doi.org/10.1080/00063657.2010.524916

Brommer, J. E. (2004). Immunocompetence and its costs during development: an experimental study in blue tit nestlings. *Proceedings of the Royal Society B. Biological Sciences*, *271*(Suppl_3), 110-113. https://doi.org/10.1098/rsbl.2003.0103

Brouwer, L. & Komdeur, J. (2004). Green nesting material has a function in mate attraction in the European starling. *Animal Behaviour*, *67*(3), 539-548. https://doi.org/10.1016/j.anbehav.2003.07.005

Buechler, K., Fitze, P. S., Gottstein, B., Jacot, A. & Richner, H. (2002). Parasite-induced maternal response in a natural bird population. *Journal of Animal Ecology*, *71*(2), 247-252. https://doi.org/10.1046/j.1365-2656.2002.00591.x

Burkett-Cadena, N. D., Ligon, R. A., Liu, M., Hassan, H. K., Hill, G. E., Eubanks, M. D. & Unnasch, T. R. (2010). Vector-host interactions in avian nests: do mosquitoes prefer nestlings over adults? *The American Journal of Tropical Medicine and Hygiene*, *83*(2), 395-399. https://doi.org/10.4269/ajtmh.2010.10-0048

Cantarero, A., López-Arrabé, J., Rodríguez-García, V., González-Braojos, S., Ruiz-de-Castañeda, R., Redondo, A. J. & Moreno, J. (2013). Factors affecting the presence and abundance of generalist ectoparasites in nests of three sympatric hole-nesting bird species. *Acta Ornithologica*, *48*(1), 39-54. https://doi.org/10.3161/000164513X669982

Cavanagh, H. M. & Wilkinson, J. M. (2002). Biological activities of lavander essential oil. *Phytotherapy Research*, 16(4), 301-308. http://doi.org/10.1002/ptr.1103

Cavanagh, H. M. & Wilkinson, J. M. (2005). Lavander essential oil: a review. *Australian Infection Control*, *10*(1), 35-37. https://doi.org/10.1071/HI05035

Chapman, B. R. & George, J. E. (1991). The effects of ectoparasites on cliff swallow growth and survival. In J. E. Loye & M. Zuk (Eds.), *Bird-ectoparasite interactions: Ecology, evolution and behaviour* (pp. 69-92). Oxford University Press.

Christe, P., Richner, H. & Oppliger, A. (1996). Begging, food provisioning and nestling competition in great tit broods infested with ectoparasites. *Behavioral Ecology*, *7*(2), 127-131. https://doi.org/10.1093/beheco/7.2.127

Christe, P., Møller, A. P. & de Lope, F. (1998). Immunocompetence and nestling survival in the house martin: The Tasty Chick Hypothesis. *Oikos*, *83*(1), 175-179. https://doi.org/10.2307/3546559

Clark, L. & Mason, J. R. (1985). Use of nest material as insecticidal and anti-pathogenic agents by the European Starling. *Oecologia*, *67*(2), 169-176. https://doi.org/10.1007/BF00384280

Clark, L. & Mason, J. R. (1988). Effect of biological active plants used as nest material and the derived benefit to starling nestlings. *Oecologia*, *77*(2), 174-180. https://doi.org/10.1007/BF00379183

Collias, N. E. & Collias, E. C. (1984). *Nest building and bird behavior*. Princeton University Press.

Cosgrove, C. L., Knowles, S. C. L., Day, K. P. & Sheldon, B. C. (2006). No evidence for avian malaria infection during the nestling phase in a passerine bird. *The Journal of Parasitology*, *92*(6), 1302-1304. https://doi.org/10.1645/GE-878R.1

Dawson, R. D. (2004). Does fresh vegetation protect avian nests from ectoparasites? An experiment with tree swallows. *Canadian Journal of Zoology*, *82*(7), 1005-1010. https://doi.org/10.1139/z04-076

Deeming, D. C. & Reynolds, S. J. (Eds.) (2015). *Nests, eggs, and incubation: New ideas about avian reproduction*. Oxford University Press.

Deeming, D. C. (2018). Use of woody materials in nests reflects body mass in passerines. *Bird Study*, *65*(3), 417-421. https://doi.org/10.1080/00063657.2018.1504876

Deeming, D. C., Morton, F. E. M. & Laverack, K. L. (2019). Nestbox size affects mass and proportions of materials used in Blue Tit *Cyanistes caeruleus* nests. *Bird Study*, *66*(1), 130-135. https://doi.org/10.1080/00063657.2019.1618243

De Lope, F., Møller, A. P., & De la Cruz, C. (1998). Parasitism, immune response and reproductive success in the house martin *Delichon urbica*. *Oecologia*, *114*(2), 188-193. https://doi.org/10.1007/s004420050435

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S. & Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, *47*(1), 15-25. https://doi.org/10.1111/j.1365-2664.2009.01744.x

Dubiec, A. & Cichoñ, M. (2001). Seasonal decline in health status of Great Tit (*Parus major*) nestlings. *Canadian Journal of Zoology*, *79*(10), 1829-1833. https://doi.org/10.1139/z01-151

Dubiec, A. & Mazgajski, T. D. (2013). Nest mass variation over the nesting cycle in the Pied Flycatcher (*Ficedula hypoleuca*). *Avian Biology Research*, *6*(2), 127-132. https://doi.org/10.3184/175815513X13612847142708

Dubiec, A., Góźdź, I. & Mazgajski, T. D. (2013). Green plant material in avian nests. *Avian Biology Research*, *6*(2), 133-146. https://doi.org/10.3184/175815513X13615363233558

Du Feu, C. (2002). *The BTO Nestbox Guide - BTO Guides v. 23*. British Trust for Ornithology.

Dufva, R. (1996). Blood parasites, health, reproductive success and egg volume in female Great Tits *Parus major*. *Journal of Avian Biology*, *27*(1), 83-87. https://doi.org/10.2307/3676964

Dunn, J. C., Stockdale, J. E., Bradford, E. L., McCubbin, A., Morris, A. J., Grice, P. V., Goodman, S. J. & Hamer, K. C. (2017). High rates of infection by blood parasites during the nestling phase in UK Columbids with notes on ecological associations. *Parasitology*, *144*(5), 622-628. https://doi.org/10.1017/S0031182016002274

Dykstra, C. R., Hays, J. L. & Simon, M. M. (2009). Selection of fresh vegetation for nest lining by red-shouldered hawks. *The Wilson Journal of Ornithology*, *121*(1), 207-210. https://doi.org/10.1676/08-035.1

Eastman, M. D., Johnson, L. S. & Kermott, L. H. (1989). Ectoparasitism of nestling House Wrens, *Troglodytes aedon*, by larvae of the blow fly *Protocalliphora braueri* (Diptera: Calliphoridae). *Canadian Journal of Zoology*, *67*(10), 2358-2362. https://doi.org/10.1139/z89-333

Fair, J., Whitaker, S. & Pearson, B. (2007). Sources of variation in haematocrit in birds. *Ibis*, *149*(3), 535-552. https://doi.org/10.1111/j.1474-919X.2007.00680.x

Fargallo, J. A. & Merino, S. (1999). Brood size manipulation modifies the intensity of infection by Haematozoa in female Blue Tits *Parus caeruleus*. *Ardea*, *87*(2), 261-268.

Fargallo, J. A. & Merino, S. (2004). Clutch size and haemoparasite species richness in adult and nestling blue tits. *Écoscience*, *11*(2), 168-174. https://doi.org/10.1080/11956860.2004.11682821

Fauth, P. T., Krementz, D. G. & Hines, J. E. (1991). Ectoparasitism and the role of green nesting material in the European starling. *Oecologia*, *88*(1), 22-29.

https://doi.org/10.1007/BF00328399

Flamini, G., Cioni, P. L., Puleio, R., Morelli, I. & Panizzi, L. (1999). Antimicrobial activity of the essential oil of *Calamintha nepeta* and its constituent pulegone against bacteria and fungi. *Phytotherapy Research*, *13*(4), 349-351. https://doi.org/10.1002/(SICI)1099-1573(199906)13:4<349::AID-PTR446>3.0.CO;2-Z

Flegg, J. (1987). *The Blue Tit (Shire Natural History)*. Shire Publications Ltd.

Garnett, M. C. (1981). Body size, its heritability and influence on juvenile survival among Great Tits *Parus major*. *Ibis*, *123*(1), 31-41. https://doi.org/10.1111/j.1474-919X.1981.tb00170.x

Glądalski, M., Bańbura, M., Kaliński, A., Markowski, M., Skwarska, J., Wawrzyniak, J., Zieliński, P., Cyżewska, I. & Bańbura, J. (2016). Effects of nest characteristics on reproductive performance in Blue Tits *Cyanistes caeruleus* and Great Tits *Parus major*. *Avian Biology Research*, 9(1), 37-43. https://doi.org/10.3184/175815516X14447556559088

Granthon, C., & Williams, D. A. (2017). Avian malaria, body condition, and blood parameters in four species of songbirds. *The Wilson Journal of Ornithology*, *129*(3), 492-508. https://doi.org/10.1676/16-060.1

Grauso, L., Cesarano, G., Zotti, M., Ranesi, M., Sun, W., Bonanomi, G., & Lanzotti, V. (2019). Exploring *Dittrichia viscosa* (L.) Greuter phytochemical diversity to explain its antimicrobial, nematicidal and insecticidal activity. *Phytochemistry Reviews*, 1-31. https://doi.org/10.1007/s11101-019-09607-1

Grindstaff, J. L., Hasselquist, D., Nilsson, J-Å., Sandell, M., Smith, H. G., & Stjernman, M. (2006). Transgenerational priming of immunity: maternal exposure to a bacterial antigen enhances offspring humoral immunity. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1600), 2551-2557. https://doi.org/10.1098/rspb.2006.3608

Guimarães, R., Sousa, M. J. & Ferreira, I. C. F. R. (2010). Contribution of essential oils and phenolics to the antioxidant properties of aromatic plants. *Industrial Crops and Products*, *32*(2), 152-156.

https://doi.org/10.1016/j.indcrop.2010.04.011

Güvenç, A., Yıldız, S., Özkan, A. M., Erdurak, C. S., Coşkun, M., Yılmaz, G., Okuyama, T. & Okada, Y. (2005). Antimicrobiological studies on Turkish *Cistus* species. *Pharmaceutical Biology*, *43*(2), 178-183. https://doi.org/10.1080/13880200590919537

Gwinner, H., Oltrogge, M., Trost, L. & Nienaber, U. (2000). Green plants in starling nests: effects on nestlings. *Animal Behaviour*, *59*(2), 301-309. https://doi.org/10.1006/anbe.1999.1306

Gwinner, H. & Berger, S. (2005). European starlings: nestling condition, parasites and green nest material during the breeding season. *Journal of Ornithology*, *146*(4), 365-371. https://doi.org/10.1007/s10336-005-0012-x

Gwinner, H. & Berger, S. (2006). Parasite defence in birds: the role of volatiles. *Acta Zoologica Sinica*, *52*(Suppl.), 280-283.

Gwinner, H., Capilla-Lasheras, P., Cooper, C. & Helm, B. (2018). 'Green incubation': avian offspring benefit from aromatic nest herbs through improved parental incubation behaviour. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1880), 20180376. https://doi.org/10.1098/rspb.2018.0376

Hansell, M. (2000). *Bird nests and construction behaviour*. Cambridge University Press.

Harrison, C. and Castell, P. (2002). *Collins Field Guide: Bird nests, eggs and nestlings*. HarperCollins Publishers Limited.

Heeb, P., Werner, I., Kölliker, M., & Richner, H. (1998). Benefits of induced host responses against an ectoparasite. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1390), 51-56. https://doi.org/10.1098/rspb.1998.0263

Heeb, P., Kölliker, M. & Richner, H. (2000). Bird-ectoparasite interactions, nest humidity, and ectoparasite community structure. *Ecology*, *81*(4), 958-968. https://doi.org/10.1890/0012-9658(2000)081[0958:BEINHA]2.0.CO;2

Hu, L. & Bentler, P. M. (1999). Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural Equation Modeling: a Multidisciplinary Journal*, *6*(1), 1-55. https://doi.org/10.1080/10705519909540118

Hurtrez-Boussès, S., Perret, P., Renaud, F. & Blondel, J. (1997). High blowfly parasitic loads affect breeding success in a Mediterranean population of blue tits. *Oecologia*, *112*(4), 514-517. https://doi.org/10.1007/s004420050339

Hurtrez-Boussès, S., Blondel, J., Perret, P., Fabreguettes, J. & Renaud, F. (1998). Chick parasitism by blowflies affects feeding rates in a Mediterranean population of blue tits. *Ecology Letters*, *1*(1), 17-20. https://doi.org/10.1046/j.1461-0248.1998.00017.x

Hurtrez-Boussès, S., Renaud, F., Blondel, J., Perret, P. & Galan, M-J. (2000). Effects of ectoparasites of young on parents' behaviour in a Mediterranean population of blue tits. *Journal of Avian Biology*, *31*(2), 266-269. https://doi.org/10.1034/j.1600-048X.2000.310219.x

Jacobs, J. (1974). Quantitative measurements of food selection. *Oecologia*, *14*(4), 413-417. https://doi.org/10.1007/BF00384581

Järvinen, P., Kluen, E., Tiiri, M. & Brommer, J. E. (2017). Experimental manipulation of Blue Tit nest height does not support the thermoregulation hypothesis. *Ornis Fennica*, *94*(2), 82-91.

Johnson, L. S. & Albrecht, D. J. (1993). Effects of haematophagous ectoparasites on nestling house wrens *Troglodytes aedon*: Who pays the cost of parasitism? *Oikos*, *66*(2), 255-262. https://doi.org/10.2307/3544812

Johnson, K. P. & Clayton, D. H. (2003). The biology, ecology and evolution of chewing lice. In Price, R. D., Hellenthal, R. A., Palma, R. L., Johnson, K. P. & Clayton, D. H. (Eds.), *The chewing lice: world checklist and biological overview* (pp. 1-25). Illinois Natural History Survey Special Publication 24.

Jongman, R. H. G., Ter Braak, C. J. F. & van Tongeren, O. F. R. (1995). *Data analysis in community and landscape ecology*. Cambridge University Press.

Kline, R. B. (2010). Promise and pitfalls of structural equation modeling in gifted research. In Thompson, B. & Subotnik, R. F. (Eds.), *Methodologies for conducting research on giftedness*. American Psychological Association.

Knowles, S. C., Palinauskas, V. & Sheldon, B. C. (2010). Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology*, *23*(3), 557-569. https://doi.org/10.1111/j.1420-9101.2009.01920.x

Koul, O., Walia, S. K. & Dhaliwal, G. S. (2008). Essential oils as green pesticides: potential and constraints. *Biopesticides International*, *4*(1), 63-84.

Krams, I., Cīrule, D., Krama, T., Hukkanen, M., Rytkönen, S., Orell, M., Iezhova, T., Rantala, M. J. & Tummeleht, L. (2010). Effects of forest management on haematological parameters, blood parasites, and reproductive success of the Siberian tit (*Poecile cinctus*) in northern Finland. *Annales Zoologici Fennici*, *47*(5), 335-347. https://doi.org/10.5735/086.047.0504

Labocha, M. K. & Hayes, J. P. (2012). Morphometric indices of body condition in birds: a review. *Journal of Ornithology*, *153*(1), 1-22. https://doi.org/10.1007/s10336-011-0706-1

Lafuma, L., Lambrechts, M. M. & Raymond, M. (2001). Aromatic plants in bird nests as a protection against blood-sucking flying insects? *Behavioural Processes*, *56*(2), 113-120. https://doi.org/10.1016/s0376-6357(01)00191-7

Lambrechts, M. M. & Dos Santos, A. (2000). Aromatic herbs in Corsican Blue Tit nests: the 'Potpourri' hypothesis. *Acta Oecologica*, *21*(3), 175-178. https://doi.org/10.1016/S1146-609X(00)00122-3

Lambrechts, M. M., Adriaensen, F., Ardia, D. R., Artemyev, A. V., Atiénzar, F., Bańbura, J., Barba, E., Bouvier, J-C., Camprodon, J., Cooper, C. B., Dawson, R. D., Eens, M., Eeva, T., Faivre, B., Garamszegi, L. Z., Goodenough, A. E., Gosler, A. G., Grégoire, A., Griffith, S. C., ... & Ziane, N. (2010). The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. *Acta Ornithologica*, *45*(1), 1-26.

https://doi.org/10.3161/000164510X516047

Lambrechts, M. M., Marrot, P., Fargevieille, A., Giovannini, P., Lucas, A., Demeyrier, V., Midamegbe, A., Perret, P., Grégoire, A., Charmantier, A. & Doutrelant, C. (2016a). Nest size is not closely related to breeding success in Blue Tits: A long-term nest-box study in a Mediterranean oak habitat. *The Auk*, *133*(2), 198-204. https://doi.org/10.1642/AUK-15-214.1

Lambrechts, M. M., Blondel, J., Bernard, C., Caro, S. P., Charmantier, A., Demeyrier, V., Doutreland, C., Dubuc-Messier, G., Fargevieille, A., de Franceschi, C., Giovannini, P., Grégoire, A., Hurtrez-Boussès, S., Lucas, A., Mainwaring, M., Marrot, P., Mennerat, A., Perret, S. & Perret, P. (2016b). Exploring biotic and abiotic determinants of nest size in Mediterranean Great Tits (*Parus major*) and Blue Tits (*Cyanistes caeruleus*). *Ethology*, *122*(6), 492-501. https://doi.org/10.1111/eth.12494

Lambrechts, M. M., Blondel, J., de Franceschi, C. & Doutrelant, C. (2017). Nest size is positively correlated with fledging success in Corsican Blue Tits (*Cyanistes caeruleus*) in an insular oak-dominated habitat mosaic. *Journal of Ornithology*, *158*(1), 125-132. https://doi.org/10.1007/s10336-016-1377-8

Lill, A., Yachou-Wos, L. & Johnstone, C. P. (2013). Are haematocrit and haemoglobin concentration reliable indicators of body condition in adult Welcome Swallows? *Emu - Austral Ornothology*, *113*(2), 93-98. https://doi.org/10.1071/MU12106

Mainwaring, M. C., Benskin, C. M. H. & Hartley, I. R. (2008). The weight of female-built nests correlates with female but not male quality in the Blue Tit *Cyanistes caeruleus*. *Acta Ornithologica*, *43*(1), 43-48. https://doi.org/10.3161/000164508X345310

Mainwaring, M. C. & Hartley, I. R. (2008). Seasonal adjustments in nest cup lining in Blue Tits *Cyanistes caeruleus*. *Ardea*, *96*(2), 278-283. https://doi.org/10.5253/078.096.0213

Mainwaring, M. C. & Hartley, I. R. (2009). Experimental evidence for state-dependent nest weight in the blue tit, *Cyanistes caeruleus*. *Behavioural Processes*, *81*(1), 144-146. https://doi.org/10.1016/j.beproc.2009.02.001

Mainwaring, M. C. (2017). Causes and consequences of intraspecific variation in nesting behaviours: insights from Blue Tits and Great Tits. *Frontiers in Ecology and Evolution*, *5*, 39. https://doi.org/10.3389/fevo.2017.00039

Mappes, T., Mappes, J. & Kotiaho, J. (1994). Ectoparasites, nest site choice and breeding success in the pied flycatcher. *Oecologia*, *98*(2), 147-149. https://doi.org/10.1007/BF00341466

Markowski, M., Bańbura, M., Glądalski, M., Kaliński, A., Skwarska, J., Wawrzyniak, J., Zieliński, P. & Bańbura, J. (2015). Variation in haematocrit of nestling Blue Tits (*Cyanistes caeruleus*) in central Poland. *Avian Biology Research*, *8*(3), 179-184. https://doi.org/10.3184/175815515X14375499328034

Marongiu, B., Piras, A., Porcedda, S., Falconieri, D., Maxia, A., Gonçalves, M. J., Cavaleiro, C. & Salgueiro, L. (2010). Chemical composition and biological assays of essential oils of *Calamintha nepeta* (L.) Savi subsp. *nepeta* (Lamiaceae). *Natural Product Research*, *24*(18), 1734-1742. https://doi.org/10.1080/14786410903108944

Martínez-de la Puente, J., Merino, S., Lobato, E., Rivero-de Aguilar, J., Del Cerro, S., Ruiz-de-Catañeda, R. & Moreno, J. (2009). Does weather affect biting fly abundance in avian nests? *Journal of Avian Biology*, *40*(6), 653-657. https://doi.org/10.1111/j.1600-048X.2009.04726.x

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., García-Fraile, S. & Belda, E. J. (2010). The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. *Biology Letters*, *6*(5), 663-665. https://doi.org/10.1098/rsbl.2010.0046

Martínez-de la Puente, J., Martínez, J., Rivero-de Aguilar, J., Del Cerro, S. & Merino, S. (2013). Vector abundance determines *Trypanosoma* prevalence in nestling blue tits. *Parasitology*, *140*(8), 1009–1015. https://doi.org/10.1017/S0031182013000371

Marzal, A., de Lope, F., Navarro, C. & Møller, A. P. (2005). Malarial parasites decrease reproductive success: an experimental study in a passerine bird. *Oecologia*, *142*(4), 541-545. https://doi.org/10.1007/s00442-004-1757-2

Mazgajski, T. D. & Kedra, A. H. (1997). Are nestlings of hole-nesting birds affected by ectoparasites? A review. *Wiadomosci Parazytologiczne*, *43*(4), 347-355.

Mazgajski, T. D., Kedra, A. H., Modlińska, E. & Samborski, J. (1997). Fleas *Siphonaptera* influence the condition of Starling *Sturnus vulgaris* nestlings. *Acta Ornithologica*, 32(2), 185-189.

Mazgajski, T. D. (2007a). Effect of old nest material on nest-site selection and breeding parameters in secondary hole nesters - a review. *Acta Ornithologica*, *42*(1), 1-14. https://doi.org/10.3161/068.042.0107

Mazgajski, T. D. (2007b). Effect of old nest material in nestboxes on ectoparasite abundance and reproductive output in the European Starling (*Sturnus vulgaris* L.). *Polish Journal of Ecology*, *55*(2), 377-385.

Mennerat, A. (2008). Blue tits (*Cyanistes caeruleus*) respond to an experimental change in the aromatic plant odour composition of their nest. *Behavioural Processes*, *79*(3), 189-191. https://doi.org/10.1016/j.beproc.2008.07.003

Mennerat, A., Perret, P., Caro, S. P., Heeb, P. & Lambrechts, M. M. (2008). Aromatic plants in blue tit *Cyanistes caeruleus* nests: no negative effect on blood-sucking *Protocalliphora* blow fly larvae. *Journal of Avian Biology*, *39*(2), 127-132. https://doi.org/10.1111/j.0908-8857.2008.04400.x

Mennerat, A., Perret, P., Bourgault, P., Blondel, J., Gimenez, O., Thomas, D. W., Heeb, P. & Lambrechts, M. M. (2009a). Aromatic plants in nests of Blue Tits: positive effects on nestlings. *Animal Behaviour*, *77*(3), 569-574. https://doi.org/10.1016/j.anbehav.2008.11.008

Mennerat, A., Perret, P. & Lambrechts, M. M. (2009b). Local individual preferences for nest materials in a passerine bird. *PLoS One*, *4*(4), e5104. https://doi.org/10.1371/journal.pone.0005104

Mennerat, A., Mirleau, P., Blondel, J., Perret, P., Lambrechts, M. M. & Heeb, P. (2009c). Aromatic plants in nests of the blue tit *Cyanistes caeruleus* protect chicks from bacteria. *Oecologia*, *161*(4), 849-855. https://doi.org/10.1007/s00442-009-1418-6

Merilä, J. & Andersson, M. (1999). Reproductive effort and success are related to haematozoan infections in blue tits. *Écoscience*, *6*(3), 421-428. https://doi.org/10.1080/11956860.1999.11682542

Merino, S. & Potti, J. (1995a). Mites and blowflies decrease growth and survival in nestling pied flycatchers. *Oikos*, *73*(1), 95-103. https://doi.org/10.2307/3545730

Merino, S. & Potti, J. (1995b). Pied Flycatchers prefer to nest in clean nest boxes in an area with detrimental nest ectoparasites. *The Condor*, *97*(3), 828-831. https://doi.org/10.2307/1369195

Merino, S. & Potti, J. (1995c). High prevalence of hematozoa in nestlings of a passerine species, the Pied Flycatcher (*Ficedula hypoleuca*). *The Auk*, *112*(4), 1041-1043. https://doi.org/10.2307/4089037

Merino, S. & Potti, J. (1996). Weather dependent effects of nest ectoparasites on their bird hosts. *Ecography*, *19*(2), 107-113. https://doi.org/10.1111/j.1600-0587.1996.tb00161.x

Merino, S., Potti, J. & Moreno, J. (1996). Maternal effort mediates the prevalence of trypanosomes in the offspring of a passerine bird. *Proceeding of the National Academy of Sciences of the United States of America*, *93*(12): 5726-5730. https://doi.org/10.1073/pnas.93.12.5726

Merino, S., Potti, J. & Fargallo, J. (1997). Blood parasites of passerine birds from Central Spain. *Journal of Wildlife Diseases*, *33*(3), 638-641. https://doi.org/10.7589/0090-3558-33.3.638

Merino, S. & Potti, J. (1998). Growth, nutrition and blow fly parasitism in nestling Pied Flycatchers. *Canadian Journal of Zoology*, *76*(5), 936-941. https://doi.org/10.1139/z98-013

Merino, S., Moreno, J., Potti, J., de Léon, A. & Rodríguez, R. (1998). Nest ectoparasites and maternal effort in Pied Flycatchers. *Biologia Conservazione della Fauna*, 102, 200-205.

Merino, S., Moreno J., Sanz, J. J. & Arriero, E. (2000). Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proceedings of the Royal Society B: Biological Sciences*, *267*(1461), 2507-2510. https://doi.org/10.1098/rspb.2000.1312

Merino, S. (2010). Immunocompetence and parasitism in nestlings from wild populations. *The Open Ornithology Journal*, *3*, 27-32. https://doi.org/10.2174/1874453201003010027

Michaud, T. & Leonard, M. (2000). The role of development, parental behaviour, and nestmate competition in fledging of nestling Tree Swallows. *The Auk*, *117*(4), 996-1002. https://doi.org/10.1642/0004-8038(2000)117[0996:TRODPB]2.0.CO;2

Milton, S. & Dean, R. (1999). Nesting thyme. The use of aromatic plants in Cape Sparrow nests. *Africa - Birds and Birding*, *February/March*, 37-39.

Møller, A. P. (1989). Parasites, predators and nest boxes: Facts and artefacts in nest box studies of birds? *Oikos*, *56*(3), 421-423. https://doi.org/10.2307/3565628

Møller, A. P. (1990). Effects of parasitism by a haematophagous mite on reproduction in the Barn Swallow. *Ecology*, *71*(6), 2345-2357. https://doi.org/10.2307/1938645

Møller, A. P. (1997). Parasitism and the evolution of host life history. In Clayton, D. H. & Moore, J. (Eds.). *Host-parasite evolution. General principles and avian models* (pp. 105-127). Oxford University Press.

Møller, A. P., Arriero, E., Lobato, E. & Merino, S. (2009). A meta-analysis of parasite virulence in nestling birds. *Biological Reviews*, *84*(4), 567-588. https://doi.org/10.1111/j.1469-185X.2009.00087.x

Møller, A. P., Adriaensen, F., Artemyev, A., Bańbura, J., Barba, E., Biard, C., Blondel, J., Bouslama, Z., Bouvier, J-C., Camprodon, J., Cecere, F., Charmantier, A., Charter, M., Cichoń, M., Cusimano, C., Czeszczewik, D., Demeyrier, V., Doligez, B., Doutrelant, C., ... & Lambrechts, M. M. (2014). Variation in clutch size in relation to nest size in birds. *Ecology and Evolution*, 4(18), 3583-3595. https://doi.org/10.1002/ece3.1189

Moreno, J., Lobato, E., Morales, J., Merino, S., Martínez-de la Puente, J. & Tomás, G. (2008). Pre-laying nutrition mediates maternal effects on offspring immune capacity and growth in the pied flycatcher. *Oecologia*, *156*(4), 727-735. https://doi.org/10.1007/s00442-008-1029-7

Moreno, J., Lobato, E., González-Braojos, S. & Ruiz-de-Castañeda, R. (2010). Nest construction costs affect nestling growth: a field experiment in a cavity-nesting passerine. *Acta Ornithologica*, *45*(2), 139-145. https://doi.org/10.3161/000164510X551291

Morrison, B. L. & Johnson, L. S. (2002). Feeding of house wren nestlings afflicted by hematophagous ectoparasites: a test of the Parental Compensation Hypothesis. *The Condor*, *104*(1), 183–187. https://doi.org/10.1093/condor/104.1.183

Moyer, B. R. & Clayton, D. H. (2004). Avian defences against ectoparasites. In van Emden, H. F. & Rothschild, M. (Eds.), *Insect and bird interactions* (pp. 243-259). Intercept.

Nadolski, J., Skwarska, J., Kaliński, A., Bańbura, M., Śniegula, R. & Bańbura, J. (2006). Blood parameters as consistent predictors of nestling performance in great tits (*Parus major*) in the wild. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *143*(1), 50-54. https://doi.org/10.1016/j.cbpa.2005.10.021

Nilsson, J-Å. (2003). Ectoparasitism in marsh tits: costs and functional explanations. *Behavioural Ecology*, *14*(2), 175-181. https://doi.org/10.1093/beheco/14.2.175

Norris, K., Anwar, M. & Read, A. F. (1994). Reproductive effort influences the prevalence of haematozoan parasites in great tits. *Journal of Animal Ecology*, *63*(3), 601-610. https://doi.org/10.2307/5226

Ontiveros, D., Caro, J. & Pleguezuelos, J. M. (2008). Green plant material versus ectoparasites in nests of Bonelli's Eagle. *Journal of Zoology*, *274*(1), 99-104.

https://doi.org/10.1111/j.1469-7998.2007.00364.x

Oppliger, A., Richner, H. & Christe, P. (1994). Effect of an ectoparasite on lay date, nest-site choice, desertion, and hatching success in the great tit (*Parus major*). *Behavioural Ecology*, *5*(2), 130-134. https://doi.org/10.1093/beheco/5.2.130

Orell, M., Rytkönen, S. & Ilomäki, K. (1993). Do pied flycatchers prefer nest boxes with old nest material? *Annales Zoologici Fennici*, *30*(4), 313-316.

Parolin, P., Scotta, M. I. & Bresch, C. (2014). Biology of *Dittrichia viscosa*, a Mediterranean ruderal plant: a review. *Phyton-International Journal of Experimental Botany*, *83*, 251-262.

Petit, C., Hossaert-McKey, M., Perret, P., Blondel, J. & Lambrechts, M. M. (2002). Blue Tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecology Letters*, *5*(4), 585-589. https://doi.org/10.1046/j.1461-0248.2002.00361.x

Pinto, E., Gonçalves, M. J., Cavaleiro, C. & Salgueiro, L. (2017). Antifungal activity of *Thapsia villosa* essential oil against *Candida*, *Cryptococcus*, *Malassezia*, *Aspergillus* and Dermatophyte species. *Molecules*, *22*(10), E1595. https://doi.org/10.3390/molecules22101595

Pires, B. A., Belo, A. F. & Rabaça, J. E. (2012). Aromatic plants in Eurasian blue tit nests: The ‘Nest Protection Hypothesis’ revisited. *Wilson Journal of Ornithology*, *124*(1), 162-165. https://doi.org/10.1676/11-102.1

Podmokła, E., Dubiec, A., Drobniak, S. M., Arct, A., Gustafsson, L. & Cichón, M. (2014). Avian malaria is associated with increased reproductive investment in the blue tit. *Journal of Avian Biology*, *45*(3), 219-224. https://doi.org/10.1111/j.1600-048X.2013.00284.x

Polo, V., Rubalcaba, J. G. & Veiga, J. P. (2015). Green plants in nests reduce offspring recruitment rates in the spotless starling. *Behavioural Ecology*, *26*(4), 1131-1137. https://doi.org/10.1093/beheco/arv056

Proença da Cunha, A. P., Roque, O. R. & Nogueira, M. T. (2012). *Plantas aromáticas e óleos essenciais: Composição e aplicações*. Fundação Calouste Gulbenkian.

Rebaya, A., Belghith, S. I., Baghdikian B., Leddet, V. M., Mabrouki, F., Olivier, E., Cherif, J. K. & Ayadi, M. T. (2014). Total phenolic, total flavonoid, tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae). *Journal of Applied Pharmaceutical Science*, *5*(1), 52-57. https://doi.org/10.7324/JAPS.2015.50110

Reid, J. M., Arcese, P., Keller, L. F. & Hasselquist, D. (2006). Long-term maternal effect on offspring immune response in song sparrows *Melospiza melodia*. *Biology Letters*, *2*(4), 573-576. https://doi.org/10.1098/rsbl.2006.0544

Rendell, W. B. & Verbeek, N. A. M. (1996a). Old nest material in nest boxes of tree swallows: Effects of nest-site choice and nest building. *The Auk*, *113*(2), 319-328. https://doi.org/10.2307/4088898

Rendell, W. B. & Verbeek, N. A. M. (1996b). Are avian ectoparasites more numerous in nest boxes with old nest material? *Canadian Journal of Zoology*, *74*(10), 1819-1825. https://doi.org/10.1139/z96-203

Richner, H., Oppliger, A. & Christe, P. (1993). Effect of an ectoparasite on reproduction in great tits. *Journal of Animal Ecology*, *62*(4), 703-710. https://doi.org/10.2307/5390

Richner, H. & Heeb, P. (1995). Are clutch and brood size patterns in birds shaped by ectoparasites? *Oikos*, *73*(3), 435-441. https://doi.org/10.2307/3545973

Roby, D. D., Brink, K. L. & Wittmann, K. (1992). Effects of bird blowfly parasitism on eastern Bluebird and Tree Swallow nestlings. *The Wilson Bulletin*, *104*(4), 630-643.

Roulin, A., Brinkhof, M. W. G., Bize, P., Richner, H., Jungi, T. W., Bavoux, C., Boileau, N. & Burneleau, G. (2003). Which chick is tasty to parasites? The importance of host immunology vs. parasite life history. *Journal of Animal Ecology*, *72*(1), 75-81. https://doi.org/10.1046/j.1365-2656.2003.00677.x

Ruiz-de-Catañeda, R., Morales, J., Moreno, J., Lobato, E., Merino, S., Martínez-de la Puente, J. & Tomás, G. (2009). Costs and benefits of early reproduction:

Haemoproteus prevalence and reproductive success of infected male Pied Flycatchers in a montane habitat in Central Spain. *Ardeola*, *56*(2), 271-280.

Sanz, J. J., Arriero, E., Moreno, J. & Merino, S. (2001). Female hematozoan infection reduces hatching success but not fledging success in Pied Flycatchers *Ficedula hypoleuca*. *The Auk*, *118*(3), 750-755. https://doi.org/10.1093/auk/118.3.750

Scheuerlein, A. & Ricklefs, R. E. (2004). Prevalence of blood parasites in European passeriform birds. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1546), 1363-1370. https://doi.org/10.1098/rspb.2004.2726

Scott-Baumann, J. F. & Morgan, E. R. (2015). A review of the nest protection hypothesis: does inclusion of fresh green plant material in birds' nests reduce parasite infestation? *Parasitology*, *142*(8), 1016-1023. https://doi.org/10.1017/S0031182015000189

Sheldon, B. C. & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, *11*(8), 317-321. https://doi.org/10.1016/0169-5347(96)10039-2

Shutler, D. & Campbell, A. A. (2007). Experimental addition of greenery reduces flea loads in nests of a non-greenery using species, the tree swallow *Tachycineta bicolor*. *Journal of Avian Biology*, *38*(1), 7-12. https://doi.org/10.1111/j.2007.0908-8857.04015.x

Simon, A., Thomas, D., Blondel, J., Perret, P. & Lambrechts, M. M. (2004). Physiological ecology of Mediterranean blue tits (*Parus caeruleus* L.): effects of ectoparasites (*Protocalliphora* spp.) and food abundance on metabolic capacity of nestlings. *Physiological and Biochemical Zoology*, *77*(3), 492-501. https://doi.org/10.1086/383512

Simon, A., Thomas, D., Bourgault, P., Blondel, J., Perret, P. & Lambrechts, M. M. (2005a). Between-population differences in nestling size and hematocrit level in blue tits (*Parus caeruleus*): a cross-fostering test for genetic and environmental effects. *Canadian Journal of Zoology*, *83*(5), 694-701. https://doi.org/10.1139/z05-059

Simon, A., Thomas, D., Speakman, J. R., Blondel, J., Perret, P. & Lambrechts, M. M. (2005b). Impact of ectoparasitic blowfly larvae (*Protocalliphora* spp.) on the behavior and energetics of nestling Blue Tits. *Journal of Field Ornithology*, *76*(4), 402-410. https://doi.org/10.1648/0273-8570-76.4.402

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry: the principles and practice of statistics in biological research*. WH Freeman and Co.

Soler, J. J., Cuervo, J. J., Møller, A. P. & de Lope, F. (1998). Nest building is a sexually selected behaviour in the barn swallow. *Animal Behaviour*, *56*(6), 1435-1442. https://doi.org/10.1006/anbe.1998.0938

Spasojevic, M. J., Grace, J. B., Harrison, S. & Damschen, E. I. (2014). Functional diversity supports the physiological tolerance hypothesis for plant species richness along climatic gradients. *Journal of Ecology*, 102(2), 447-455. https://doi.org/10.1111/1365-2745.12204

Ter Braak, C. J. F. & Smilauer, P. (1998). *CANOCO Reference Manual and User's Guide to CANOCO for Windows: Software for Canonical Community Ordination*. Microcomputer Power.

Tomás, G., Merino, S., Martínez, J., Moreno, J. & Sanz, J. J. (2005). Stress protein levels and blood parasite infection in blue tits (*Parus caeruleus*): a medication field experiment. *Annales Zoologici Fennici*, *42*(1), 45-56.

Tomás, G., Merino, S., Moreno, J., Sanz, J. J., Morales, J. & García-Fraile, S. (2006). Nest weight and female health in the Blue Tit (*Cyanistes caeruleus*). *The Auk*, *123*(4), 1013-1021. https://doi.org/10.1642/0004-8038(2006)123[1013:NWAFHI]2.0.CO;2

Tomás, G., Merino, S., Moreno, J. & Morales, J. (2007a). Consequences of nest reuse for parasite burden and female health and condition in blue tits, *Cyanistes caeruleus*. *Animal Behaviour*, *73*(5), 805-814. https://doi.org/10.1016/j.anbehav.2006.06.016

Tomás, G., Merino, S., Moreno, J., Morales, J. & Martínez-de la Puente, J. (2007b). Impact of blood parasites on immunoglobulin level and parental effort: a medication field experiment on a wild passerine. *Functional Ecology*, *21*(1), 125-133.

https://doi.org/10.1111/j.1365-2435.2006.01214.x

Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J. & Lobato, E. (2007c). Simple trapping method to estimate abundances of blood-sucking flying insects in avian nests. *Animal Behaviour*, *75*, 723-729. https://doi.org/10.1016/j.anbehav.2007.08.018

Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J. & Lobato, E. (2008). Determinants of abundance and effects of blood-sucking flying insects in the nest of a hole-nesting bird. *Oecologia*, *156*(2), 305-312. https://doi.org/10.1007/s00442-008-1001-6

Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J., Lobato, E., Rivero-de Aguilar, J. & del Cerro, S. (2012). Interacting effects of aromatic plants and female age on nest-dwelling ectoparasites and blood-sucking flies in avian nests. *Behavioural Processes*, *90*(2), 246-253. https://doi.org/10.1016/j.beproc.2012.02.003

Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J. & Rivero-de Aguilar, J. (2013). Nest size and aromatic plants in the nest as sexually selected female traits in blue tits. *Behavioural ecology*, *24*(4), 926-934. https://doi.org/10.1093/beheco/art015

Tomás, G., & Soler, J. J. (2016). Begging and ectoparasite attraction. *Animal Behaviour*, *113*, 93-98. https://doi.org/10.1016/j.anbehav.2015.12.026

Tompkins, D. M., Jones, T. & Clayton, D. H. (1996). Effect of vertically transmitted ectoparasites on the reproductive success of Swifts (*Apus apus*). *Functional Ecology*, *10*(6), 733-740. https://doi.org/10.2307/2390508

Tripathi, A. K., Upadhyay, S., Bhuiyan, M. N. & Bhattacharya, P. R. (2009). A review on prospects of essential oils as biopesticide in insect-pest management. *Journal of Pharmacognosy and Phytotherapy*, *1*(15), 00-00.

Tripet, F. & Richner, H. (1997). Host responses to ectoparasites: food compensation by parent blue tits. *Oikos*, *78*(3), 557-561. https://doi.org/10.2307/3545617

Tschirren, B., Siitari, H., Saladin, V. & Richner, H. (2009). Transgenerational immunity in a bird-ectoparasite system: do maternally transferred antibodies affect parasite fecundity or the offspring's susceptibility to fleas? *Ibis*, *151*(1), 160-170. https://doi.org/10.1111/j.1474-919X.2008.00880.x

Upadhyay, R. K. (2010). Essential oils: anti-microbial, antihelminthic, antiviral, anticancer and anti-insect properties. *Journal of Applied Bioscience*, *36*(1), 1-22.

Valente, J., Zuzarte, M., Liberal, J., Gonçalves, M. J., Lopes, M. C., Cavaleiro, C., Cruz, M. T. & Salgueiro, L. (2013). *Margotia gummifera* essential oil as a source of anti-inflammatory drugs. *Industrial Crops and Products*, *47*, 86-91. https://doi.org/10.1016/j.indcrop.2013.02.036

Valera, F., Hoi, H. & KrištÍn, A. (2006). Parasite pressure and its effects on blood parameters in a stable and dense population of the endangered Lesser grey shrike. *Biodiversity & Conservation*, *15*(7), 2187-2195. https://doi.org/10.1007/s10531-004-6902-z

Valkiünas, G. (2005). *Avian malaria parasites and other haemosporidia*. CRC Press.

Verhulst, S. & Nilsson, J-Å. (2008). The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1490), 399-410. https://doi.org/10.1098/rstb.2007.2146

Votýpka, J. & Svobodová, M. (2004). *Trypanosoma avium*: experimental transmission from black flies to canaries. *Parasitology Research*, *92*(2), 147-151. https://doi.org/10.1007/s00436-003-1034-z

Williams, T. D. (2012). *Physiological adaptations for breeding in birds*. Princeton University Press.

Wimberger, P. H. (1984). The use of green plant material in bird nests to avoid ectoparasites. *The Auk*, *101*(3), 615-618. https://doi.org/10.1093/auk/101.3.615

Zangari, L., Ferraguti, M., Luiselli, L., Battisti, C. & Bologna, M. A. (2013). Comparing patterns in abundance and diversity of hole-nesting birds in Mediterranean habitats. *Revue d'écologie*, *68*(3), 267-274.

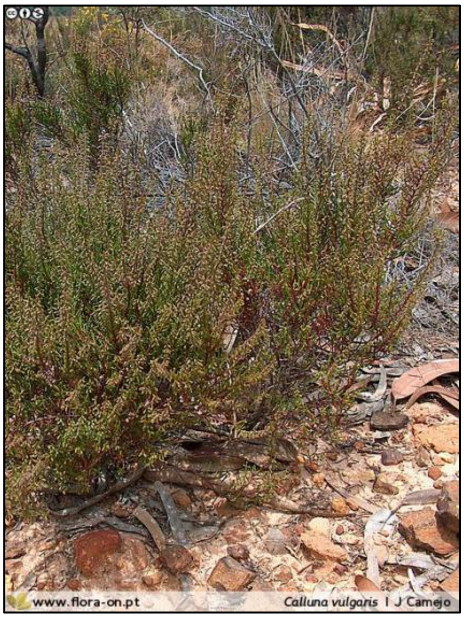
Zuzarte, M., Gonçalves, M. J., Cavaleiro, C., Cruz, M. T., Benzarti, A., Marongiu, B., Maxia, A., Piras, A. & Salgueiro, L. (2013). Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. *Industrial Crops and Products*, *44*, 97-103. https://doi.org/10.1016/j.indcrop.2012.11.002

Appendix

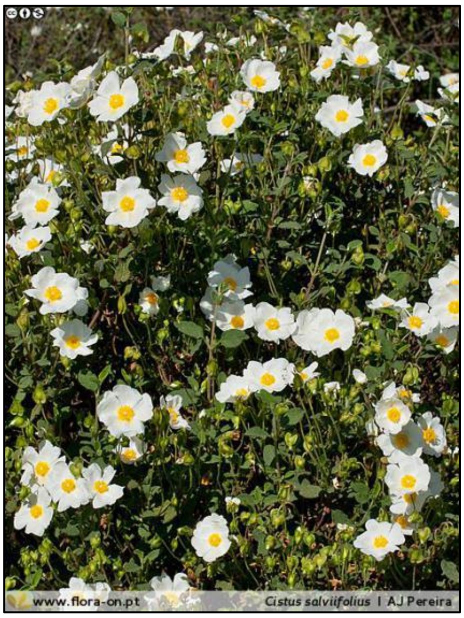
List of aromatic plants incorporated in nests by the authors and by the females. Species are in alphabetical order.

Calamintha nepeta

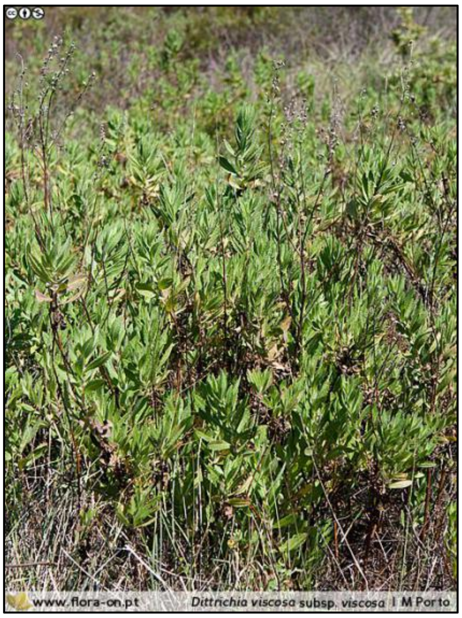
Calluna vulgaris



Cistus salviifolius

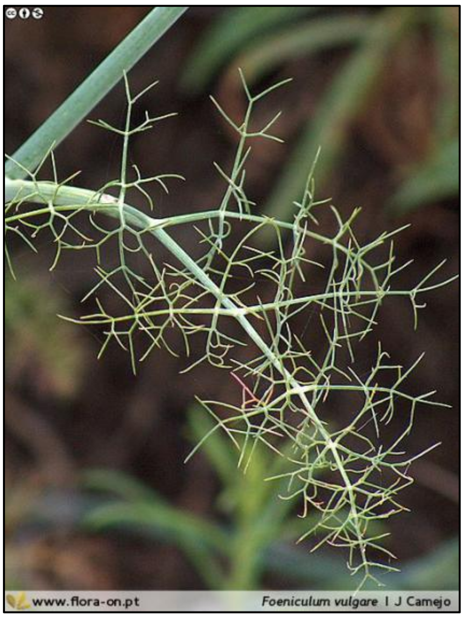


Dittrichia viscosa



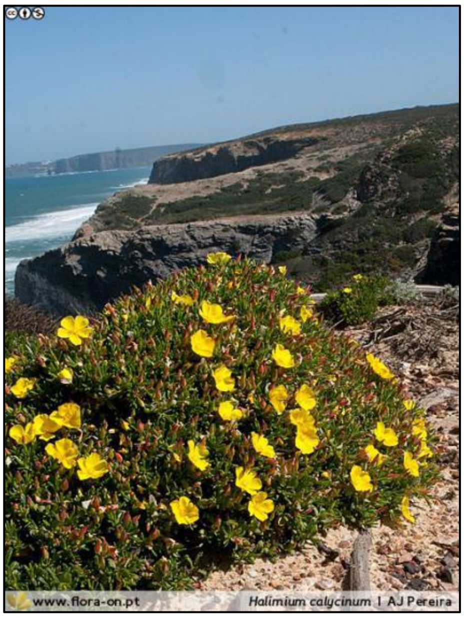
Erodium cicutarium

Foeniculum vulgare



Geranium robertianum

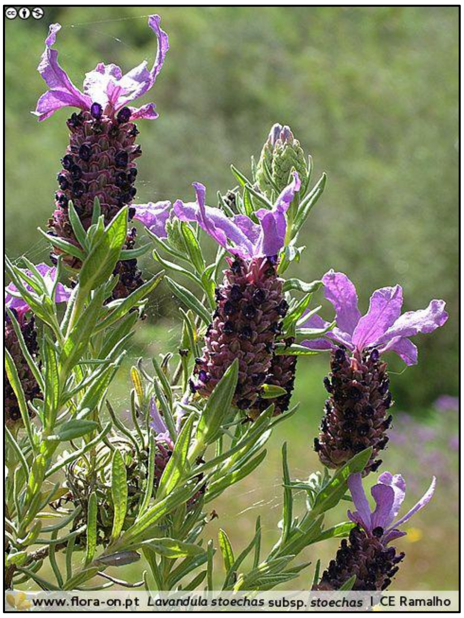
Halimium calycinum



Halimium halimifolium

Lavandula dentata

Lavandula stoechas

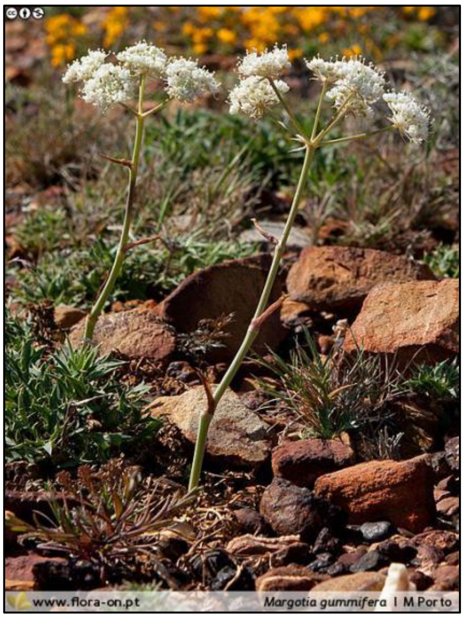


Lithodora prostrata


www.flora-on.pt *Lithodora prostrata* subsp. *lusitanica* | C Aguiar (CIMO)

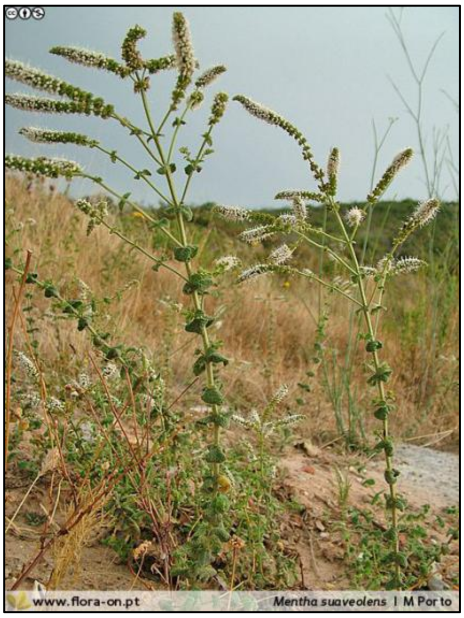

www.flora-on.pt *Lithodora prostrata* subsp. *lusitanica* | M Porto

Margotia gummifera


www.flora-on.pt *Margotia gummifera* | M Porto

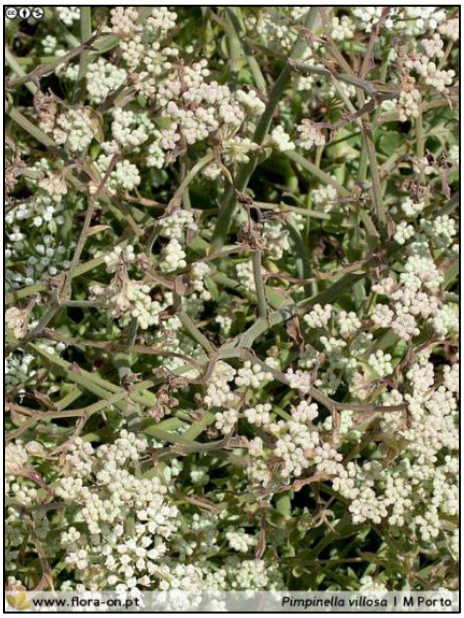

www.flora-on.pt *Margotia gummifera* | AJ Pereira

Mentha suaveolens


www.flora-on.pt *Mentha suaveolens* | M Porto

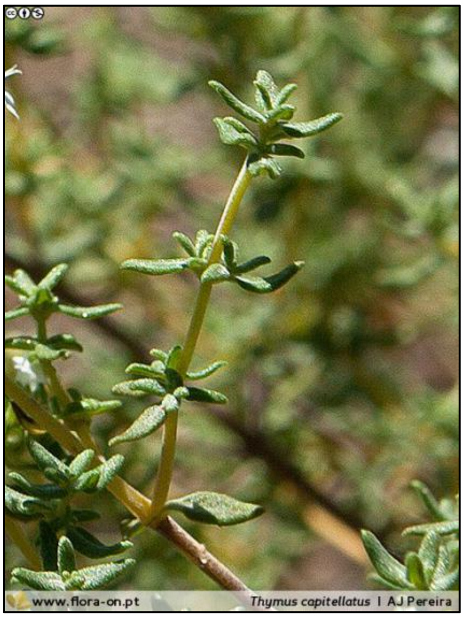

www.flora-on.pt *Mentha suaveolens* | J Camejo

Pimpinella villosa



Thapsia villosa

Thymus capitellatus



Photos of *Calamintha nepeta*, *Calluna vulgaris*, *Cistus salviifolius*, *Dittrichia viscosa*, *Erodium cicutarium*, *Foeniculum vulgare*, Geranium robertianum, *Halimium calycinum*, *Halimium halimifolium*, *Lavandula stoechas*, *Lithodora prostrata*, *Margotia gummifera*, *Mentha suaveolens*, *Pimpinella villosa*, *Thapsia villosa* and *Thymus capitellatus* are from www.flora-on.pt, accessed on February 19, 2020.

Photos of *Lavandula dentata* are from https://jb.utad.pt/especie/Lavandula_dentata, accessed on February 19, 2020.