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ENTOMOLOGICAL MONITORING IN THE CULTURAL HERITAGE FACILITIES AS PREREQUISITE FOR A SUCCESSFUL IPM PROGRAMME

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Summary

Background

The European Committee for Standardization, or to be more precise, the Technical Committee CEN / TC 346, Working Group 4 "Environment", is working on the standardization of an IPM (Integrated Pest Management) programme in museums and cultural heritage facilities.

Entomological monitoring will represent a crucial part of the IPM programme.

At present, the scientific "**basic-research"** on entomological traps, whether light or pheromones, for "cultural heritage pests" is extremely poor, and only recently the behaviour and/or the physiology of the "museums insects" have been investigated.

Also, **applied research** to design specific traps for cultural heritage facilities do not exist.

Thus, it is clear how studies in this area are necessary to improve the practice of monitoring (using entomological traps) in museums in order to increase awareness about prevention against insects and to expand its routine application.

1 INTRODUCTION

"Cultural heritage is used in societies to construct and reconstruct identities and multiple cultural and social values […]. Preservation of cultural heritage has been seen as a moral responsibility in societies because it maintains and strengthens a nation's identity and understanding of its past. In general, preservation and conservation of cultural heritage of a country aims to safeguard the existence of cultural heritage of all mankind. The preserved cultural heritage from different centuries indicates that societies have valued aspects of both their past and contemporary cultures: all the cultural phenomena are first contemporary culture and if they are valued and preserved they may become past culture representatives" (Wirilander, 2012).

In addition, cultural heritage represents, or should represent, an economic resource. In Italy, "museums, monuments, archaeological sites are not only the focus of major tourist flows which support an economic field that, despite the internal difficulties, has 10.3% of GDP. But they are also powerful attractors of foreign investments, which find in the beauty of our country a reason to set up business and factories" (http://www.beniculturali.it/mibac).

Therefore, conservation means to keep and to preserve (Petzet, 2004) not only the artistic and historic objects, but also the identity and the economy of a country.

The aim of preventive conservation is that of minimizing and/or retarding the deterioration and consequently the loss of historical and artistic documents, safeguarding the authenticity and the integrity of cultural heritage in order to guarantee its accessibility in the present and in the future (The Venice Charter, 1964).

To be effective, preventive conservation methods require a multidisciplinary approach and awareness of everyday actions.

Progress in scientific research at the beginning of the 20th century provided new means that could be applied to cultural heritage preservation (Lambert, 2010). It may be considered that modern conservation started in 1930, when the International Museum Office organized the first International Conference for the Study of Scientific Methods for the Examination and Conservation of Works of Art (Wirilander, 2012).

In the 1970's, the theoretical concepts of conservation started to evolve into those of minimum intervention for the care of cultural heritage objects, which subsequently

developed into the preventive conservation theory. This development and the re-evaluation of the reversibility question resulted in the minimalist tendencies becoming dominant in conservation (Redondo, 2008).

Preventive conservation is based on the concept that restoration, even if well executed, does not neutralize the damage suffered by the object, and often changes its appearance (Carlini, 2009). Besides, the restoration does not eliminate the need for future preservation.

In Italy it is exactly the opposite point of view that prevails: restoration is deemed much more important than conservation as it ensures greater visibility and publicity than a regular conservation/prevention activity. More visibility means more sponsors, especially if great artworks are involved. Pompili (2009) regards "economic and bureaucratic problems" as the reasons why preventive conservation is not applied.

Collections care involves a range of activities including housekeeping, insect pest management, environmental monitoring, analysis and management of temperature, relative humidity, light, dust and pollutants, emergency planning and training, outline of protection strategies relating to visitors or contractor such as access during functions, filming, photographing, building work and digitisation of photographic archives, technical design and manufacture of display cases, objects moving and transport, storage environment, packaging methods and storage materials, condition surveys and risk assessments, care of outdoor sculpture, historic garden furniture and ordinance.

The main risk factors for indoor collections are climate, gases, pollution and biological causes (insects, fungi, bacteria). Significant risks are posed by inappropriate relative humidity, temperature and light conditions, which are affected by wall thickness, air leakage, ventilation and heating systems, solar radiation and number of visitors (Dahlin, 2002).

Parameters such as food, habitat, access points, housekeeping and indoor climate affect insects infestations in historic properties and conservation facilities, even if it is not easy to predict their impact in terms of infestation and damage to historic collections. Stuffing levels, visitor numbers, the introduction of food and availability of habitat (such as dark and undisturbed spaces), and loans between collections may be important factors to control (Brimblecombe & Brimblecombe, 2014).

More quick and easy objects transfers together with climate changes which alter the insects species distribution in different geographic areas, are also relevant to the management of historic properties (Alexander, 2007; Stengaard Hansen et al., 2012; Brimblecombe & Lankester, 2013). In recent years the concern about increasing insect populations in historic houses is increasing (Pinniger, 2011; Xavier-Rowe & Lauder, 2011). Although there are no studies that quantify the extent of damage due to pests, it is obvious that they represent a major threat for all organic property. Pest species can feed on animal fur, textiles made with animal fibres, feathers or felt as well as wood or paper. Insects and other animals are also found within buildings where they feed on dust or waste so that their presence does not always mean a threat for museum objects or, on the opposite, they could be ignored while being harmful (Querner & Morelli, 2009; Querner, 2013).

"Insects are mobile and, given the right conditions, can spread and move rapidly into uninfested areas. The most common methods of introducing infestation are:

(i) Introduction into collections of new material infested at a low level, which is undetected. The infestation may be on new acquisitions or on material on loan from another museum. Material which had been sent out on loan may return with infestation acquired while it was away.

(ii) Spread of infestation from another source in the museum.

(iii) Introduction of insects from outside, via windows and doors" (Child, 1999).

The diversity of properties to be preserved is an additional complicating factor. Even when properties are broadly similar, insect numbers and infestations can vary widely. Infestations can be specific to a room or set of rooms within a property.

The destruction wrought by the woodworm common furniture beetle *Anobium punctatum* (DeGeer, 1774) (Bletchly, 1953; Matei & Teodorescu, 2011; Schöller & Prozell, 2011), the drugstore beetle *Stegobium paniceum* (Linnaeus, 1761) (Fohrer et al., 2006; Baslé et al., 2009; Ignatowicz et al., 2011), and termites (Ferrari et al., 2011; Evans et al., 2013) to wood and paper, the irreparable damage to textiles caused by the moths *Tineola bisselliella* (Hummel, 1823) (Wudtke, 2002; Cox & Pinniger, 2007; Evans, 2011; Querner, 2013), and the less frequent case-bearing clothes moth *Tinea pellionella* Linnaeus, 1758 (Key & Common, 1959) are real problems. Carpet beetles (*Attagenus* spp. or *Anthrenus* spp. *Trogoderma* spp.) (Chiappini et al., 2001; Stengaard

Hansen et al., 2012) and silverfish (*Lepisma saccharina* Linnaeus, 1758) (Antonie & Teodorescu, 2009; Querner et al., 2011) also cause damages in the European museums.

In addition, some species which attack the cultural heritage objects can create health problems to the staff and the end users. The Dermestidae larvae *hastisetae* are irritating and can cause allergies, as well as *fungi* (Florian, 1997; Florian & Manning, 2000), while the Acarina of the genus *Pyemotes* and the Hymenoptera of the genus *Scleroderma*, both of which live on woodborers' larvae, can sting humans, causing highly itchy wheals (Chiappini et al., 2001).

The problem is further complicated by the fact that often the managers and their staff do not know the strategies to be adopted against biodeteriogens, are not able to detect their presence and to recognise them. Frequently the infestation is noticed when it has already reached a major level and the damage is high. Later the problem is usually solved with a **"self-made"** approach**,** or relying on **restorers**, who possibly have a high knowledge as how to repair the damage caused by pests but a lower one on defence strategies that should be applied against biodeteriogens, or on **pest control operators** who know how to kill off biodeteriogens, but do not have the necessary knowledge of the complications regarding the application of treatments to cultural heritage objects.

In addition, even if the problem is momentarily eliminated, it is not permanently solved as the same environmental conditions persist, and the level of risk remain the same.

Since the introduction of the Control of Pesticides Regulations 1986, attitudes and practices have significantly changed and very few museums in Europe regularly use chemicals against insect pests. These were substituted by physical methods like heating, freezing or anoxic treatments, mainly with nitrogen or CO_2 (Gilberg & Roach, 1991; Maekawa & Elert, 2003; Child & Pinniger, 2008; Berzolla et al., 2011). Chemicals are only used in emergencies or when no other method can be applied as, for example, when only few days remain before an exhibition opening (Åkerlund et al., 1998; Jessup, 1998; Kingsley et al., 2001; Querner et al., 2013).

The current technologies employed in disinfestation of works of art show limitations and critical points.

- First of all the health and safety of humans, environment and artefacts (Child, 1999; Mosneagu, 2012; Querner et al., 2013). In many cases, the treatments were

unsuccessful and occasionally, positively damaging, both to the material being treated and to people working with it.

- Secondly the overall cost that includes the real cost of treatment (products, equipment, electric-powered) and the "indirect" costs such as insurances, the building closing for varying times (depending on the kind of treatment), the transfer of materials in different locations, and eventually the rent of premises to conserve the artefacts during the disinfestation, etc.. The gas treatments, for example, are fast, effective but costly, being executed by specialised firms, with trained personnel that can use the appropriate technology and must guarantee for the safety of the personnel and the environment.
- Thirdly, logistic aspects which depend on the location where the treatments are necessary (a whole room, a whole building, a structural part of a building). It is impossible to always use all kinds of treatment.
- Last but not least the effectiveness of the treatment. Sometimes treatments are used which do not guarantee the result desired in that specific environmental conditions and for those species of insects. For too many years, and still frequently today, the insect problems have been/is solved with curing actions, not getting at the root cause. Adopting such a simplistic approach often leads to wrong or unnecessary solutions (Berzolla et al., 2011).

The best approach then is that of prevention and rational management of pests problem, as it should be not only for biodeteriogens, but for any problem that may occur.

1.1 IPM IN CULTURAL HERITAGE FACILITIES

Integrated Pest Management (IPM), a concept developed in the 50s for field cultivation, later applied to the food industry, and since the 80s successfully used in museums (Kingsley et al., 2001), is a holistic approach to pest problems solving, taking into account the environment where the objects are displayed or stored, and the risk of them to be damaged by the pests that could attack the materials trey are made of. It considers the possible preventive strategies and the environmental impact and toxicological risk of effective treatments (chemical or physical) necessary to control pests (Doyle et al., 2011; Nilsen, 2011).

The most recent and complete reviews on IPM principles in museums are those by Pinniger (2008) and Brokerhof et al. (2007).

The most important aspect of integrated pest management is preventive conservation, also called collection care, which involves all the actions taken to prevent or delay the deterioration of the artefacts, and requires a multidisciplinary collaboration between different specialists including physicists, chemists, biologists, entomologists, architects, restorers (Pompili, 2009; Chiappini et al., 2014).

This is implemented by sealing the building, regulating the climate, periodical general cleaning (maintaining high hygienic standards), staff training and well-chosen housekeeping, introducing quarantining for new and incoming objects, inspecting the objects and the storage modules, and regularly monitoring pest infestations with traps.

These are manageable factors that can limit infestations (Brimblecombe & Brimblecombe, 2014). The most important factors necessary to form a collection IPM scheme are:

- "a knowledge of the main insect pests and their basic biology;
- sources of infestation in a building or collection;
- detection, inspection and monitoring methods;
- control through prevention of access and modification of the environment, etc;
- treatment options that minimize deleterious effects" (Child, 1999).

The logical and rational sequence of steps in a correct IPM procedure is to avoid, block, detect, monitor, confine, treat and eventually repair (Michalski et al., 1992; Strang, 1996a; Strang, 1996b; Child, 1999; Boylan, 2004; Strang & Kigawa, 2006; Strang & Kigawa, 2009; Chiappini et al., 2014).

It also seems important to examine the effect of previous responses to infestations, for example if an insect species was reduced for long periods as a consequence of a specific action, such as a treatment of the infested object or a deep cleaning. It would be useful to analyse one-off events such as deep cleaning, maintenance, changing room-use in comparison with continuous climate variables. It can be difficult to parameterise these irregular events and issues such as food and niche availability, but these are likely to be significant for control on the prevalence of insects. This is possible only with a correct and rational management which finds its fundamentals in an IPM program.

In the last fifteen years, the IPM practice has become very common in several European cultural heritage facilities (Swedish, German, Austrian, Switz, and U.K. structures) (Querner 2013) as well as in Canadian and American ones (Strang & Kigawa, 2006).
Nevertheless, most museums have not put a long term monitoring protocol in place and IPM is not a standard for all large collections yet (Wudtke, 2002; Querner, 2013).
IPM has to be a group effort that requires the joining in of a diverse group of stakeholders, including the highest levels of institution administration, the facilities managers, housekeeping staff, groundskeepers, security managers and patrol staff, pest management experts either within or contracted from outside the organization, curatorial/collection manager and support staff, caterers and shop managers, exhibit designers, and human resources staff, as well as conservation/preservation staff. Well-written, approved, distributed, promoted, and enforced policies and procedures are vital to a successful IPM programme.

1.2 THE EUROPEAN STANDARD

A specific European standardisation activity in the field of conservation of cultural heritage is essential to obtain a common unified scientific approach to the problems relevant to the preservation and conservation of the cultural property against biodeterioration.

Standards are established on a need-based approach, in the fields of the processes, practices, methodologies and documentation of conservation of tangible cultural heritage to support its preservation, protection, and maintenance and to enhance its significance.

Since 2010, the European Committee for Standardization, in particular the Task Group 6 of the CEN / TC 346 (Conservation of Cultural Heritage) is working to develop a standard on "Integrated Pest Management for Protection of Cultural Heritage", an effort of collaboration and mediation between representatives of 33 European countries.

The scope was to define procedures for avoiding and/or managing pest infestations within the cultural property area (Nilsen, 2011).

The justifications for creating a standard were the following.

- This standard will not repeat work already done but will draw on and make reference to such work and combine it into one unified European document.

- Although IPM exists in other areas, the sensitivity and unique nature of cultural material requires a separate standard.

The standard is likely to be used by public and private organizations, as well as by commercial services.

This standardization is addressed to all parties concerned with the individual subjects covered by the standard including owners, stakeholders and users of cultural heritage (monuments, museums, archives, libraries and collections) as well as peer groups such as architects, custodians, archaeologists, engineers, planners, conservators-restorers, craftsmen, conservation scientists, energy advisers, national authorities, transport and insurance companies, etc.

These normative documents may concern:

- Movable and immovable cultural heritage.
- All materials constituting tangible cultural heritage.
- All aspects of the environment of tangible cultural heritage that could impact its conservation.
- All aspects or stages of the conservation process, such as terminology, examination.
- Documentation accompanying the conservation operations, diagnosis, investigations.
- Conservation work, monitoring, etc.

At present, the text is being proposed to the European Union member states for the last comments and then, if published, will become a standard at Community level and therefore valid for all the countries in CEN and to be used by all business operators who will handle the problem of biodeteriogens using an approach of integrated pest management. It was presented in Italy first at the international "Meeting on Cultural Heritage Pests" (Nielsen, 2011) and again at the round table "IPM for cultural heritage, a standard not a utopia" (CPBC - Centro per la Protezione dei Beni Culturali dagli organismi dannosi - Piacenza – 24th of May 2013) when its applicability in Italy was discussed.

1.3 ENTOMOLOGICAL MONITORING IN CULTURAL HERITAGE FACILITIES

The focal points in the application of an IPM programme include all the aspects that regulate the systems involved, i.e. the monitoring of harmful populations and climate

conditions, the storage of historical data and the integrated use of such information in order to manage infestations in an appropriate and effective way.

In general the entomological monitoring is an unavoidable step when a prevention program is adopted. The base of a successful integrated pest management plan is an effective monitoring system that provides information on species and density of insects, foci of infestation, routes of insect entry, problems associated with the building, changes in pest population over time and treatment efficacy (Burkholder, 1990; Mueller et al., 1990; Child & Pinniger, 1993; Pinniger et al., 2004; Mahroof & Phillips, 2008). Insects (species) identification is crucial in order to eradication and future prevention; it informs on the feeding patterns, reproductive cycles, behaviour, and environmental conditions that can be targeted/controlled to ensure successful trapping. Entomological monitoring allows the original source of an infestation to be identified at a very early date and pest control measures to be implemented with maximum efficiency. Currently, the monitoring activity is planned and supervised by external pests control professionals (more or less qualified) or by curators, keepers, archivists and librarians who are particularly sensitive to the problem, but, in most cases, not learned about insects. In fact, the cultural heritage facilities staff is rarely trained about pests biology and ethology.

At present, the traps managing is completely manual, based exclusively on the reliability of the workers, also in those case in which is implemented to send the captures data of traps, identified with a code bar, by mobile phone.

Monitoring is based on traps which attract insects of different taxonomic groups by using different cues (such as pheromones, light, colours or food substrates), and capture them. These traps are designed to permit the observation and count of the insects caught in order to identify the specie and to establish the density of infestation. The control of the traps is manual and is made at specific time intervals. Development of traps to be used for public health and food industry has benefited museums and archives because these traps can be adapted also for the detection of some cultural heritage pests (Child, 1999). Nevertheless the "rules" and conditions of the monitoring in the food warehouses and storages are not the same as in the museums as most species of insects attacking cultural heritage objects are different and have different behaviours with respect to those that attack food.

The traps normally used in the museums are typically “sticky traps” (Child & Pinniger, 1994) sometimes called “blunder traps”. Pheromone traps are occasionally used (Brimblecombe et al., 2013) to catch either the webbing clothes moth or the case bearing clothes moth, dermestid and anobiid beetles (Gilberg & Roach, 1991; Brimblecombe & Brimblecombe, 2014). Light traps are rarely used.

Usually a good entomological monitoring programme at first considers an inspection of the environment and the microclimate conditions in which the objects are kept, and of the objects conserved. Then, with the local staff, a practicable programme will be setup, followed by traps positioning and periodical control.

Once pests have been identified, it is possible to have a pretty good idea their ideal environment in which to thrive; to decide eventual disinfestation treatments and/or restoration; to modify the environmental conditions which have favoured infestations.

To complement entomological monitoring, regular inspections should also be undertaken by the IPM officer to verify if there are other visual signs of pest infestations. In addition, the assistance of all staff should be requested to report sightings of any insects.

Sometimes, in support of monitoring and/or visual inspections, devices with audio sensors (Querner et al., 2011), CO_2 measuring (Querner & Biebl, 2011) X-raying and CT scanning (Schöller & Prozell, 2011; Mosneagu, 2012) are utilized.

1.4 THE AIM OF RESEARCH

The main goals of this project are two.

- To understand what is **the state of art of IPM application in Italian** cultural heritage facilities (**museums**, archives, libraries), and which is their approach in the management of pests, with particular reference to insects.
- **To carry out scientific research in order to understand the response of insects towards attractive systems**, especially the light, to develop advanced technologies using new light sources such as LEDs.

The research will develop in the following steps:

- survey on IPM in Italian cultural heritage facilities,
- flight tests with *Stegobium paniceum*, a dangerous species for cultural heritage,,

- preliminary capture tests with “Anobiids pheromone” performed using *Stegobium paniceum*,
- tests with different light sources, to evaluate the influence of the wavelength on *Stegobium paniceum*.

2 IPM IN ITALIAN CONSERVATION FACILITIES

2.1 INTRODUCTION

It is only in recent years that prevention principles have started to spread. Italian regions have undertaken projects in this direction: «self-assessment questionnaire» project in Lombardia, the «declaration of conformity» in Piemonte, the «preventive concrete measures» project in Liguria, the «vocational training» project in Toscana, the «MUSA» project for «certified quality museums» (IBC-Emilia Romagna) in Emilia Romagna; the «ISTAT survey» in Marche, and the «Museum High quality brand» in Lazio. However, in none of these projects any of the tasks focused on IPM; in fact, while temperature, relative humidity, dust, and light were considered as degradation factors (Chiappini et al., 2014) pests were never taken into account.

In recent years, microclimate (temperature, relative humidity and light) monitoring inside cultural heritage facilities, as well as pollution surveys, have been given much attention (VV.AA, 2007) and reference parameters, to which environmental conditions should conform, have been specified in a specific standard (Chiappini et al., 2014). On the contrary, at present, there are no normative references with regards to insects, fungi and other biodeterioration agents.

Nevertheless, the CEN standard on IPM for Protection of Cultural Heritage which has just being sent for Public Enquiry will be an important hint.

The main aim of our research was to verify the cultural heritage protection approach applied by conservation institutions in our country, pointing out the "deficiencies" and evaluating the possible usefulness of an IPM standard for museums and cultural heritage facilities all together. To perform this investigation, in order to validate the protocol, a pilot study was first realized in Emilia Romagna, an Italian region characterized by a high number of cultural heritage facilities (over 500 museums, libraries, and cultural institutions) (http://ibc.regione.emilia-romagna.it/servizi-online/biblioteche) . Afterword, the survey was extended to all Italian cultural heritage conservation facilities.

2.2 Materials and Methods

2.2.1 Questionnaire project

After considering French and German questionnaires (Querner et al., 2004; Nicosia, 2011) realized on the same topics, we decided to produce a new one better adapted to the Italian situation.

Completeness, clearness and brevity were the parameters considered in order to encourage respondents to complete the questionnaire.

One of the objectives was that the questionnaire was adapted to any cultural heritage facility with display or storage areas.

The questionnaire consisted of 31 questions about pest problem awareness, perception and management; training on pests, and resources spent on pest prevention; the final section collected data on the structural characteristics of conservation facilities and personal information about respondents. Pest problem awareness, perception and management were assessed on a 5-point scale.

2.2.2 Methodology

A first version of the questionnaire was pre-tested with nine cultural heritage facilities fitting into different categories of conservation structures. This pre-test was developed to discover any possible weaknesses in the questionnaire.

After the pre-test, the questionnaire was revised and, in September 2012, a final version was delivered to all managing directors of cultural heritage facilities in Emilia Romagna of which it was possible to find an email address. 850 questionnaires were sent out and, 123 were completed and returned, corresponding to a percentage of 14.5% (level of significance 98% with a maximum allowable error of 0.05).

After the analysis of this pilot study results and protocol validation, at the end of June 2013, 3055 questionnaire were sent out to all Italian cultural heritage facilities of which was possible to find an email address, addressed to the managing directors.

2.2.3 Statistical analysis

A descriptive analysis was conducted, obtaining the means, standard deviations, and correlations for all the variables. Factor and cluster analyses were also applied to analyse data. First, factor analysis (with the varimax rotation method) was used to group different types of chemical and physical treatments, eradication and restoration actions. Based on the factors identified, non-hierarchical clustering (with K-means method) was performed to obtain segments. Bivariate analyses including cross-tabulation with Chi square-statistics, Independent Samples T-test and One-Way ANOVA comparison of means were then used to profile the clusters. All analyses were performed using the statistical software SPSS 15.0 for Windows.

2.3 RESULTS AND DISCUSSION

The results of the survey of all Italian cultural Heritage Facilities replicated exactly those of the Emilia Romagna pilot study. Therefore, only the national results will be reported and discussed.

The "Italian" questionnaires completed and returned were 477 out of 3055 (15.6%) (level of significance 98% with a maximum allowable error of 0.05), of those 69.9% were museums 16.1% libraries, 5.2% archives, 4.10% galleries, and 4.50% historic houses.

All the Italian regions were represented: Lombardia 19.5%, Emilia Romagna 17.4%, Toscana 11.0%, Piemonte 9.2%, Veneto 8.0%, Campania 6.4%, Lazio 5.0%, Liguria 4.1%, Umbria 3.4%, Puglia 3.0%, Marche 3.0%, Trentino Alto Adige 2.1%, Sicilia 2.1%, Basilicata 1.6%, Sardegna 1.4%, Friuli Venezia Giulia 0.9%, Calabria 0.7%, Abruzzo 0.7%, Molise 0.2%, Valle d'Aosta 0.2%).

The majority of facilities which answered the survey, were public (60.4%).The questionnaire respondents were represented by cultural heritage facility director (28.7%), curators (25.4%), museum operators (22.2%), archivists/librarians (19.4%), restorers (4,3%). Pests are a problem for 76% of the cultural heritage facilities that took part in this survey (441 out 477 of participants answered to this question).

Nevertheless the **environmental control** is not a frequent operation; in fact the results show that only the 40% of the respondents measures temperature and the 26% relative

humidity; of these, only the 37% and the 57% respectively (which correspond to the 14.8% of the total in both cases) has a data register.

Monitoring environmental climate conditions is the basis of an IPM programme, but it is essential that a continuous and complete data collection is recorded. Historical data series allow to correlate abnormal situations with any possible change that had been recorded. Pests develop at different rates in different temperatures and R.H. conditions and therefore they can represent a risk either high or low, depending on the different situation. In the few cases in which these data are reported, the most frequent temperature is 20°C in winter and 25°C in summer and the relative humidity is about 50%. However, dangerous values are sometimes recorded, especially in summer, i.e. 28-32°C and 70-75% R.H., which are very favourable conditions for many pest species.

Cleaning is another important issue for IPM, which is regularly carried out (83%) in the conservation facilities, even though are performed by staff untrained on biodeteriogens, both when outsourced (69% of the respondents) or internal (31%). Nevertheless, the 41% (426 out of 477) and the 35% (424 out of 477) of the respondents stated that it allowed to recognize conservative problems and infestation or pest problems, respectively.

Visual inspections are regularly performed by the 81% of the interviewees (477), but of these only 48% opportunely trained on the pests problems. In fact, this activity is up to "general" staff of the museum (66.9%), to curators (26.2%), and to housekeeping staff 7%. The inspection activity also permitted to detect conservation problems (63%) and insects infestations (52%).

As we stated above, most cultural heritage facilities consider that pests are a problem (75%) but when they are asked **how often insects and frass are encountered** in the museums, the majority of them answer never or rarely (54% for insects and 75% for frass), in both the exhibition and storage areas. The insects can be easily recognizable, also without a specific training (although, probably, their importance in relation to the risk posed by the different species is not perceived), but the frass is often confused with powder.

So, the absence of pests is because they are not there or because they are not seen and recognized? If the 75% of the respondents, according to their experience, affirms that

insects represent a problem but their presence is not equally detected, it means that their presence is deduced from the damage they cause on the objects.

The knowledge of pests is considered little or not important only by 10-12% of the respondents, which is in line to what was expressed above. If the “insects” are considered a big problem and their presence is detected on the damage they cause, obviously the respondents must be aware of the inadequacy of their knowledge to solve it. Also the knowledge of chemicals and physical treatments adopted against pests is considered very important (89%).

Generic prevention measures (blocking all the routes of entry i.e. installing brush sweepers, sealing all of the cracks and holes in the building walls, and installing nets on the windows to prevent access of insects; cleaning the outside areas; screening or sealing off the unused pipes or drains etc.) and pests monitoring are never or rarely adopted respectively by the 53% and 62% of the respondents, and regularly adopted (often and ever) only in the 25% and 23% of the cases respectively .

Therefore, monitoring and general prevention are considered and employed as exceptional or emergency measures whereas they are useful only when performed as ordinary activities like for dusting and routine maintenance operations which are regularly adopted (often and ever) by the 65% of respondents and never or rarely only by the 10%.

Similarly to generic prevention and monitoring, chemical or physical disinfestation treatments are adopted never or rarely in 63% and 62% of cases, respectively; the respondents use often these methods only in the 12% and 13% respectively. In the same way, the restoration due to damages caused by insects, is an exceptional event as the 66% of respondents applies it never or rarely and only the 10% often.

There is not a relationship between the answers to the question “Are pests a problem?” (the respondents could answer choosing in a 5 scale from “not at all” to “a great deal”) and the frequency at which chemical and/or physical treatments were used, nevertheless in the most of cases the respondents which affirm that pests are a problem are those which “restore” as a consequence of damages caused by insects. This could mean that respondents who think that pests are a problem, as a consequence of the fact that they suffered their damages, react trying at most to repair the damages (restoring the objects) but not to solve the problem at its bases.

Cluster analysis shows that it is possible divide the survey sample into two groups: the first one includes those that operate against pests, either through prevention or eradication, while the second one includes those that do not take any action against pests but are almost exclusively involved in routine and emergency maintenance (Fig. 1).

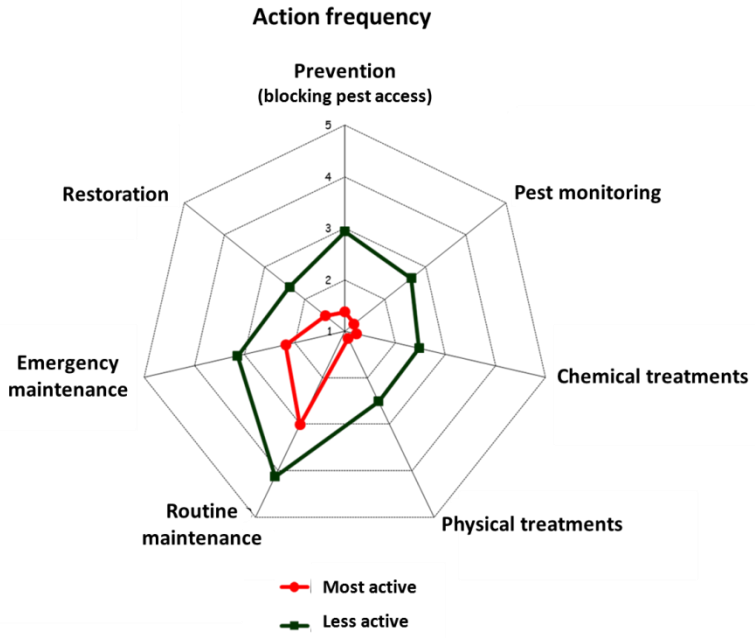


Fig. 1 - The cluster analysis shows that we can divide the survey sample into two groups: the "most active" and the "less active". Most active are those that operate against pests both by prevention and eradication. The answer scale goes from 1 to 5 (1= never, 2= rarely, 3= sometimes, 4= often, 5= always).

Insects, frass, bacteria and fungi are found more frequently by "most active" cluster than "less active" cluster, while the two clusters detect powder with the same frequency. Moreover, there is a positive correlation between the most active cluster and the respondents who regularly carry out visual inspections. Essentially this means that there is a strong correlation between the most active and the "most careful" respondents.

This indicates that a greater attention results in the identification of problems otherwise ignored or underestimated, in agreement with what previously stated that the biodeteriogens danger is evaluated on their damages and so when it is too late.

The average percentage of budget (relative to the last 5 years) which an cultural heritage institution allocates to preventive measures (i.e. entomological monitoring),

disinfestation treatments, or restoration is extremely limited (between 0 and 1% in the 80%, 78% and 72% of the respondents respectively).

In the “less active cluster” the budget allocated is near 0% for both preventive actions and restoration. Instead, in the “most active cluster”, there is a positive correlation between those that expend money for preventive conservation and those which expend money for disinfestation treatments.

It is clear that, even though the insect problem is a real problem, the general tendency is not to prevent and not to disinfest mainly because of lack of funds that is indicated as a major cause by the 87% of the respondents. About the same percentage (86%) indicates the need to invest in other areas that means that, as there is not enough money for everything, they use it to dispose of what they judge prior necessities.

Nevertheless, 75% of the respondents think that a valid pest management programme could also be limited because there is no real knowledge of the problems caused by pests (75%) and because of the absence of a standard which could give indications for a correct and efficient pest control (70 %).

The results show that only 12 facilities out of 477 apply IPM (2.5%) and the 80% does not even know what IPM is.

Very few respondents have taken courses on pests during (18%) or after their university courses or during their job activity (12%). In fact, 74% of them are conscious that they have a low knowledge (47%) or no-knowledge (27%) at all of the methods for pest detection in the environment. However, those who have received at least a minimal training consider very important the knowledge of all the aspects concerning pests control and eradication (Fig. 2).

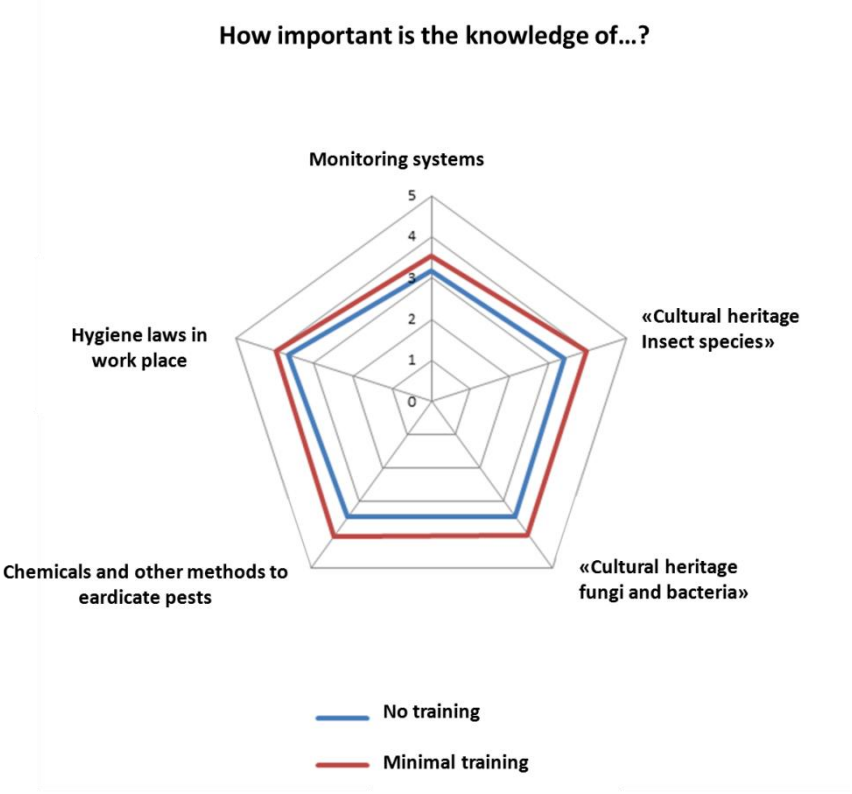


Fig. 2 - The cluster analysis shows that the survey sample can be divided into two groups: those that received (minimal) training about pests problems and those that did not receive training. The respondents which received a minimal training about pests are more conscious of the importance of the pest knowledge. The answer scale goes from 1 to 5 (1= not at all, 2= very little, 3= quite a lot, 4= a lot, 5= a great deal).

When a disinfestation and/or disinfection treatment was needed, the cultural heritage institutions found out to whom they might outsource the work, through local authorities responsible for cultural heritage protection (Superintendents, etc.) (36%), colleagues of other institutions (27%), internet (12%), or trusted companies (9%).

If, having not ever had the need, they will in future, they would refer to local authorities responsible for cultural heritage protection in most cases (70%).

This is a very significant result because it stresses the importance of the education in this field of local authorities responsible for cultural heritage protection that usually have a humanistic education.

3 TRAP TESTS

3.1 INTRODUCTION (TRAPS TYPES, ATTRACTANTS, AND EFFICIENCY)

Trap selection is a key point of any monitoring within an IPM program. Different parameters must be considered when considering a trap. The position and design of the trap, for example, could affect the numbers and identities of insects caught in it (Baker & Sadovy, 1978). Improper choice of trap type can lead to a misrepresentation of the true pest numbers in an area. There is not a single best type of traps to use in a pest monitoring program. It is important to choose the traps considering the environmental conditions and the possible insects species that could be present in that situation. Some considerations include dusty or non-dusty area, hot or cold temperatures, crawling or flying insects, the size or the capacity of the trap may also be a factor to be considered for the choice.

Traps designs, including colours and shapes, are essential to obtain a high efficacy in insects catches (Navarro-Llopis et al., 2008). This is primarily due to the fact that pest may:

- not be able to enter a trap because of an inability to fly or crawl into the device because of its design.
- be able to escape the trap once it has entered.

Traps use cues to attract insects towards the caching apparatus where they will be confined and die.

Entomological traps have various shapes, sizes, attractive - light, pheromone and food/substrates - and capture system – a vessel with liquid where the insect will sink and drown, a closed container such as in a funnel trap from which the insect cannot escape and will die for exhaustion, a close container with a fan that will suck the insect inside, glue board or a sticky surface, as in 'fly-paper' and sticky traps, that will capture the insects when lending on them, a high voltage grid that will kill the insect by electrocution. This last capture method is to be excluded because destroying the insects by electrocution precludes their identification. In fact it is necessary to identify the captured insects in order to decide which will be the most effective and less risky (for

human, environment, and object safety) defence strategy. In general, capture methods with container allow to better control and identify the species. On the opposite, the traps most widely used are those with a sticky surface as capture method, even if it is not always easy to identify the insects caught up in the glue. In most cases, when the intention is to see which pests may be present in storage or exhibit area, without having any idea of what may be there, a simple **“blunder” sticky trap** is used. The term “blunder” comes from the fact that these are passive traps; there is no specific stimulus that attracts insects to the traps, and the trap captures any insect that is just passing by chance on the trap, and is caught on the sticky board. This kind of trap, catching both crawling and flying insects, works well to give an indication of what insects are moving through an area. The value of sticky traps has been clearly demonstrated in food and public health facilities for the detection of beetles, moths and cockroaches. In addition, the use of these traps has allowed evaluating cleaning procedures and pest control applications.

These traps can be paper traps, having triangular shape, that lay flat on the ground or shelf and that have a thin glue layer (1 mm or less) that will trap most insects that wander into it. Small triangular prism shaped traps typically with a base size of 2.5 x 3.0 cm are now manufactured by a number of companies using a synthetic sticky material of inert polybutenes. Most insect traps used in museums are based on the familiar sticky cockroach trap (Detector, Roach Hotel, Hoy-Hoy etc.) where the sticky surface forms the base of an open-ended box. In this structure the sticky surface does not get accidentally attached to objects, visitors or staff and is protected from dust and debris which would reduce its efficacy. The critical factor in effective monitoring plans using sticky traps appears to be the correct location of the trap rather than the trap type or design. They must be placed in "strategic" positions where pests are likely to be found (e.g. along skirting boards, behind cupboards, beside entrances and fire escapes), basing on the characteristics of the environment, objects and insects which are suspected to be there.

Sticky traps are produced by many manufacturers and in several designs. The flat "glue board," the box-shaped "motel," and the triangular-shaped "pup tent" are common forms.

They are very cheap and easy to use and therefore extremely widespread among cultural heritage facilities conservators. Detection of insects to provide early warning of their presence and monitoring of infestation levels using sticky traps has been a crucial point of the pest management program implemented by a major museum in London (Hillyer & Blyth, 1992). However sticky traps for flying insects are not so popular because of their exposed sticky surfaces and their ugliness.

Sticky traps may be baited with a food lure, sometimes mixed with the glue, or pheromones.

Pheromones designed to attract insects can be used with blunder traps. A sexual pheromone lure mimics the scent exuded by one of the sexes of a specific species in order to attract the opposite one. As they are species specific it is necessary to hypothesize which insect could be present before purchasing the **pheromone trap**. The pheromone is placed in the centre of the trap so that the sticky adhesive surface surrounding it will catch the insect landing on it.

Though pheromone traps have been widely employed in agriculture and the stored product industry for detecting and monitoring a variety of insect pests, they have found only limited application for the control of museum pests, because they are available for few species only (Klassen et al., 1982; Gilberg & Roach, 1991). In fact, there has been minor commercial development of the pheromones of pests which are specific to harmful in museums and houses (Child & Pinniger, 1994). The cost and difficulties of synthesizing pheromones, together with the relatively small market of cultural properties monitoring, did not stimulate the research in this field.

Pheromones are non-toxic molecules and the possibility of resistance development in response to prolonged exposure to pheromones is highly improbable (Gilberg & Roach, 1991).

Nevertheless, pheromones of Tineidae (*Tineola bisselliella*, *Tineola pellionella*), Anobiidae (*Lasioderma serricorne*), Dermestidae (*Trogoderma* spp.) which infest cultural heritage facilities are common and easy to find (Hammack et al., 1973; Plarre & Kruger-Carstensen, 2011).

”Over the past four decades pheromones have been identified for about 40 species of stored-product insects. A variety of different dispensers are employed, ranging from simple rubber tubing or septa, polyethylene capsules, and glass or plastic beads in

plastic capsules covered by specific membranes into or onto which the active pheromone can be embedded into the glue of the sticky trap surface. The performance of a pheromone product is defined by the choice of formulation, its age and release rate, and population pressure. Although useful, advances are needed in the fields of pheromone synthesis, blend quality (purity and composition), stabilization and longevity. They are environmentally labile molecules and must be protected from photochemical, thermal, oxidative and hydrolytic degradation, as well as from isomerisation and polymerisation. Adequate determination of the release rates of dispensers should become a part of the standard protocol of dispenser evaluation trials to introduce hard facts rather 'guesstimates' for the interpretation of field trial results" (Trematerra, 2013).

However, as the structure of pheromone traps has been developed on empirical basis, their performances show some limits and some critical aspects in indoor application (Trematerra, 2011).

The effectiveness of pheromones traps depends on saturation level reached in closed environments (and consequent possible "confusion" for the insects), on temperature and relative humidity of the room in which they are positioned (in relation to the chemical nature of the substances), on the pheromone conservation conditions before its using. These aspects are not well known and therefore it is not always easy to manage and to interpret trap captures, which are hampered by factors associated with the performance of traps, insect species, trapping method, or trapping environment (Nansen et al., 2008).

Light traps attract by the light adult insects, they are not species specific, and their efficacy depends mainly on the behaviour of insects towards the light.

Currently, light traps are used to catch Diptera and nocturnally active insects in flour mills. Generally these traps use broad spectrum fluorescent bulbs and consequently are large and must be attached to mainline power (Duehl et al., 2011).

At present, the most common capture mechanism in commercial light traps for museums monitoring is the adhesive panel, but also a close container with a fan that sucks the insect inside is becoming more frequent.

Light traps commonly present in commerce present different shapes:

1. trap hanging from the ceiling, with the lamp suspended on the top and facing down and the adhesive panel , positioned horizontally above it, with the glued surface facing upward;
2. trap hanging from the ceiling, with the lamp positioned on the bottom, in the centre of the adhesive panel, positioned horizontally, with the glued surface facing upward;
3. trap hanging on the wall with the lamp turned towards the wall and the adhesive panel placed vertically, with the glued surface facing the lamp, towards the room;
4. trap hanging on the wall with the lamp oriented towards the room, protected by screens or slotted panels and the adhesive panel positioned vertically behind the lamp, with the glued surface turned towards the room.

The area controlled by each trap and indicated by the manufacturer is expressed as "coverage area" or as "operating range" and varies depending on the trap type (shape and light source). The operating range is the most accurate information as it expresses the distance at which a particular lamp, with a given power, is capable of attracting the insects while the coverage area varies depending on the environment in which it is placed (for example, if the trap is located in the centre of a very large local, the lamp light is diffused at 360° and the cover corresponds to the area of the circle). Anyhow, at present, the companies that produce the lamps provide information relating to measurable parameters, such as electrical or brightness ones, but not on the distance at which the light "can be seen". Therefore, when this information is provided for light traps is clearly indicative and mainly has a commercial purpose. Moreover wavelengths are not always accurately reported even if they generally are in the ultraviolet spectral region.

In recent years, considerable interest has been raised by technologies that utilize the responses of insects to light as a “clean” form of pest control that allows limiting the use of synthetic pesticides. Therefore, light traps have been widely studied for agricultural insect species (Antignus, 2000; Emura & Tazawa, 2004; Duehl et al., 2011; Honda, 2011; Johansen et al., 2011; Ben-Yakir et al., 2013). Nevertheless, researches for light traps applications in cultural heritage facilities for the capture of “museum insect species” are still needed.

Empirical experiences reports that light traps could be useful to capture and monitor insects in cultural heritage environments, but methods and efficacy are not scientifically verified. Electrical grid traps positioned inside the building have been used to capture and to kill anobiids, dermestids (*Anthrenus* spp.), and cloth moths (*Tineola* spp.) (Mosneagu, 2012) after their attacks had already been identify trough previous visual inspections. Belmain et al. (1999) used an ultra-violet light trap with electrocution, designed to catch walking and flying insects (NPW 80 Insectocutor, Pest Westr Electronics Ltd., UK), to catch deathwatch beetles - *Xestobium rufovillosum* de Geer (Coleoptera: Anobiidae) - and other arthropods in infested buildings (Salisbury Cathedral, Winchester Cathedral and Kew Palace).

Zaitseva (1989) reports the results of the experiments that she realized to verify the possible use of light traps in museums. All the five tested traps with different UV light sources and containers showed that could be used to verify the existence of insect infestation (Dermestidae species, clothe moths, *Stegobium paniceum*),

Since very few bibliographic data regarding scientific research and use of light traps in museums are available, an informal survey was carried out among the most affordable and known operators responsible of the cultural heritage conservation against pests in different parts of the world (Colin Macgregor – Manager Material Conservation and Analytical Resources of Australian Museum, Alex Roach – Australian pest management professional, Dee Lauder – works for English Heritage Collection Conservation, Sylviane Vaucheret - Documentation Officer of Natural History collections and National Museum of Ireland, Blyth Valley, Capucine Korenberg - Conservator of British Museum, Sophie Rowe - Conservator Scott Polar Research Institute, responsible for the pest management, Fabien Fohrer - Entomologist at CICRP –Interdisciplinary Center of Conservation and Restoration of Cultural Heritage, Marseille, France. It was asked if they used light traps for entomological monitoring in museums and if not, to explain the reasons. This investigation showed that light traps are not routinely used for the monitoring of museum insect pests, but, as found in bibliography, blunder and pheromone sticky traps are mainly used. The respondents reported that “sticky traps” (sometimes associated with pheromone lures) are efficient enough for their needs and they have no plans to change to light traps in the near future. At the Victoria and Albert Museum, for example, an integrated pest management programme has been adopted for

over 20 years. Here both blunder sticky traps for *Anthrenus* and pheromone sticky traps for webbing clothes moth have been used. The English Heritage (officially known as the Historic Buildings and Monuments Commission for England, is an executive Public Body of the Department for Culture) currently recommends to deploy museum sticky traps, plastic floor traps and bat-protecting traps as well as pheromone confusion technique for moth such as the Exosex CL and Killgerm AF Demi-Diamond. These have been used for the last ten years and proved to be effective.

In most cases, when light traps are utilized within museums, they tend to be used in catering areas for the control of flying insects that pose a threat to food safety. Where light control units are installed, by chance they may capture museum insect pests such as adults of *Anthrenus*, *Anobium*, *Stegobium* and *Dermestes* spp. etc.. Nevertheless, the captures and the results trends are not usually registered or analysed. They are always ineffective in capturing any clothes moths species.

Sometimes, light traps are empirically tested to verify their possible usefulness by the operators responsible for pest management.

Alex Roach wrote that during the 1990s some trials were performed with a conventional light trap (a type used outdoors to control 'nuisance pests') in the storage areas of the Australian Museum to verify if they would attract cigarette beetles (*Lasioderma serricorne*). The light source was a black light (~350nm). The trap was positioned in the middle of the collection area and regulated by a timer so as to operate outside staff presence hours. Any beetles were caught over a two-week period, although they were caught in pheromone traps during the same time. Moreover, between 1997 and early 2000, several other different types of light traps for moths and other pests were tested, but any was found that would attract museum pests. However, in 2012 he (Alex Roach) found a cigarette beetle infestation in display cases, at the museum and he observed that the beetles were concentrated around the low-energy bulbs installed in the display cases.

As an alternative, Fabien Fohrer is working with green neon tubes (500-550 nm). He wrote that they are also very effective for trapping *Stegobium paniceum* (unpubl. data) adding that this insect seeks the light coming from outside and that is why it is found very often on the edge of the window.

From the results of the survey, it emerged that light traps (fly control units) are not incorporated as part of a museum IPM monitoring system due to the following factors.

- Costs. Are they effective enough to justify their cost? (Sticky blunder traps, including pheromone traps, are considerably cheaper).
- Regular maintenance and management. Bulbs need to be changed on an annual basis for maximum efficiency.
- Aesthetic problems. Shape and structure do not suit exhibition environments as usually their aspect is very poor.
- Potential risks. The traps in commerce use UV light (usually short wavelengths 350- 370nm) that can discolour hit surfaces (over a period of time).

In fact, UV are not recommended in environments such as archives and libraries, museums or other cultural heritage environments because this electromagnetic radiation can be a source of damage such as fading, discoloration and embrittlement to most textiles, watercolours, pastels, prints and drawings, manuscripts, miniatures, paintings in distemper media, wallpaper, and most natural history objects, including botanical specimens, fur and feathers (Zaitseva, 1989; Child & Pinniger, 1994), that have a "medium sensitivity" (CIE 157: 2004) or even a "high sensitivity" as silk, colorants known to be highly fugitive, most graphic art and photographic documents (CIE 157: 2004). These damages are cumulative and irreversible: no conservation treatment can restore change of colour or loss in strength of materials damaged by light.

The risk of damage exponentially increases with the decreasing of light wavelength; the energy radiation of UV is much more damaging than blue light, blue light is more damaging than green light, and so on. "Accordingly, it is recommended to minimise the presence of UV in display lighting. The maximum acceptable relative level of UV is 75μW/lm. Indeed, lower relative levels of UV (such as 10μW/lm) can be attained either by using UV absorbers, on windows and electric light sources, or by employing sources with minimal or zero UV output, such as most white LEDs" (CIE 157: 2004).

Light-emitting diodes (LEDs) have become a widely available and popular substitute for incandescent light over the past 15 years. At present, LEDs are more and more widely used for lighting museums and galleries. They are based on semiconductors, which emit light after application of a suitable voltage. Advantages of using LEDs over incandescent light bulbs include greatly reduced power consumption, a cooler operating temperature, an adjustable light intensity, a low weight, a prolonged lifetime, a small size, and a minor susceptibility to shock damage.

Light emitting diodes have only recently been tested as substitutes for incandescent light in insect light traps (Burkett & Butler, 2005; Hoel et al., 2007)..

The solid state physics of LEDs allows light traps to be deployed in harsh and varied environments. Adding colour-specific LEDs to traps designed for crawling insects might enhance surveillance, increase trap life, and lower operating costs all at the same time (Duhel et al., 2011).

The new researches on light traps, mainly carried out in the food safety field, are primarily aimed to verify the behaviour of the insects towards LEDs. Specific LED wavelengths were reported to be potential pest control agents due to their high attractive or repellent effects towards many pests (Bishop et al., 2004; Hoel et al., 2007; McQuate, 2014).

LED devices with various wavelengths can now be manufactured due to recent technological advances, and new agricultural technology using light is starting to attract attention. Advances are also expectable in the use of light for pest control in the cultural heritage facilities.

3.2 SPECIES TEST

In order to verify the possibility to develop and efficiently use light traps for insects monitoring in the cultural heritage facilities, behavioural tests have been performed on the species *Stegobium paniceum* (Linnaeus) (Coleoptera: Anobiidae, at the adult instar.

This species is one of the most dangerous and common insects in museums, archives and libraries and historic building, where, at larval instar, attacks old books, wooden objects and even materials of animal origin including horns, leather, wool and hair (Gămălie & Mustață, 2006; Schöller & Prozell, 2011; Mosneagu, 2012; Querner et al., 2013).

Stegobium paniceum infests also (principally) a wide variety of dry and durable stored food products including flour, dried bread, biscuits, chocolate, grain, and granular feed for animals as well as spices, drugs, pharmacological products. It is very easy to find it in food processing facilities, grain stores, warehouses, museums, houses and in bird and insect nests (Jacob & Ushakumary, 1991; Trematerra & Sciaretta, 2004). It has a broad, cosmopolitan distribution; it has been found throughout the tropical and subtropical parts of the world, as well as in warmed buildings in temperate countries (Kuwahara et

al., 1975; Gilberg & Brokerhof, 1991; Mahroof & Phillips, 2008; Kalaitzakis & Smonou, 2012).

Even though the optimum development conditions for *S. paniceum* are 28-30 °C and 70 to 90% R.H., it can develop at conditions variable from 15 to 35 °C and 30% R.H. (Lefkovitch, 1967). Moreover it is very resistant to the several disinfestation treatments which commonly can be applied to cultural heritage objects (Gilberg & Brokerhof, 1991).

Attacking food, unlike most insects that attack cultural heritage objects alone, *S. paniceum* is easy to breed in controlled laboratory conditions on easy to use and readily available food substrates. This aspect is essential to have always available a lot of insects to replicate our tests.

S. paniceum flight behaviour in relation to the temperature, the attraction to commercial pheromone (preliminary test) and its orientation and response towards the light have been studied to improve its monitoring and capture effectiveness of the traps.

3.2.1 Breeding

Stegobium paniceum was bread on an egg pasta in the shape of little butterfly (Figure 3) at 28±2 °C and 70±5 % R.H. and 12 L : 12 D photoperiod.

Fig. 3 - Egg pasta used for the breeding of *S. paniceum.*

This proved to be the ideal kind of substrate as, growing the larvae in the centre of the "butterfly", when pupae are necessary, it is possible to break the piece of pasta in the middle and pull out them. At the same way, it is possible to see when the adults are about to emerge and isolate them.

3.2.2 Adults used for the test

Selection between male and female adults was based on morphological criteria and, in particular, on the basis of body length and width (measured at the widest part of the thorax), in general varying respectively from 1.6 mm to 3.7 mm and from 0.46 mm and 2.7 mm. Females are longer and wider than males (Kashef, 1955).

Just emerged adults were separated by sieving them. Females were selected using a sieve with 1.5 mm meshes, as they could not pass through the mesh. The males were selected letting them pass through a sieve with 1 mm meshes. Those adults that passed through 1.5 mm meshes but not through 1.0 mm ones were not considered. At the beginning, to verify the method, the genitalia of the adults separated as described above were prepared on glass slides to be checked under a light stereo-microscope.

Genitalia of sixty smaller and sixty bigger insect adults, that were hypothesized to be males and female respectively, were prepared on glass slides and observed. Microscopic observations of genitalia showed that 54 out of 60 (90%) smaller adults were males and that 56 out of 60 (93.3%) bigger were females.

Therefore, it was confirmed the affordability of the separation method.

Males and mated females were obtained isolating the pupae and separating them (males and females) through morphological characters (Halstead, 1963) or selected by sieving them.

Some females were isolated in little boxes and used 24 hours for the trials (virgin females). Some others were putted with males (one male and one female) in a little box until copulation took place (Ward, 1981). After 24 hours from the end of the copulation the insects were used for the tests.

3.3 FLIGHT TESTS

Entomological traps efficiency in capturing flying and/or crawling insects can vary depending on the different attitude to move in relation to species or environmental conditions. Movement of insects can be influenced by the temperature, relative humidity, and light (Hagstrum et al., 1996; Trematerra & Sciarretta, 2004).

Temperature is one factor that may limit insect flight because body temperature must be high enough for muscles and enzymes to work efficiently (Chapman, 1971). Thus, there

is a minimum body temperature below which an insect is incapable of flight. Throne and Cline (1994) using sticky traps observed that most of the captured *S. paniceum* were flying when temperatures were at least 20 °C. They also reported that *S. paniceum* can fly also between 10 and 20 °C, but, above all, between 20 e 30° C.
Therefore, in order to better establish its flight behaviour in relation to temperature, fly tests with males and female were performed.

3.3.1 Materials and Methods

3.3.1.1 Test

In the flying experiments 40 mated females and 40 males were tested. The tests were conducted in a climate chamber at different temperature (18, 21, 25, 28 °C) and 50% R.H. Illumination was provided by a 60-W tungsten lamp suspended 0.5 m above the experimental area. Tendency to fly was assessed by allowing adults to climb up a glass tube 120 mm high and 50 mm large. Once they had reached the rim of the tube they were observed for 5 min distinguishing: no flight behaviour, pre-flight behaviour and spontaneous flight (Ward, 1980).

3.3.1.2 Statistical analysis

Data are presented as N (%) with 95% confidence intervals (95%CI). Chi-square test was used to evaluate the differences between categorical variables.
All the post-hoc tests were adjusted for multiple comparisons using Bonferroni correction.
In order to evaluate the role of different temperatures on *S. paniceum* flight activity, Kaplan-Meier survival estimates were used to analyse the probability (chance) of flying in *Stegobium* at 18, 21, 25, 28 °C. The time period considered in the analyses was 5 minutes.
The alpha level was set at 0.05. Analyses were carried out using SPSS, version 20 for Windows.

3.3.2 Results

During the experiments, it was possible to distinguish the behaviour described by Ward (1980) and known as “no flight behaviour”, “pre-flight behaviour” and “spontaneous flight” (Figure 4).

Fig. 4 - From left to right, flight phases: preparing, pre-flight behaviour, flight.

In Figure 5 the percentages of mated females and males which flew is reported for the four considered temperatures.

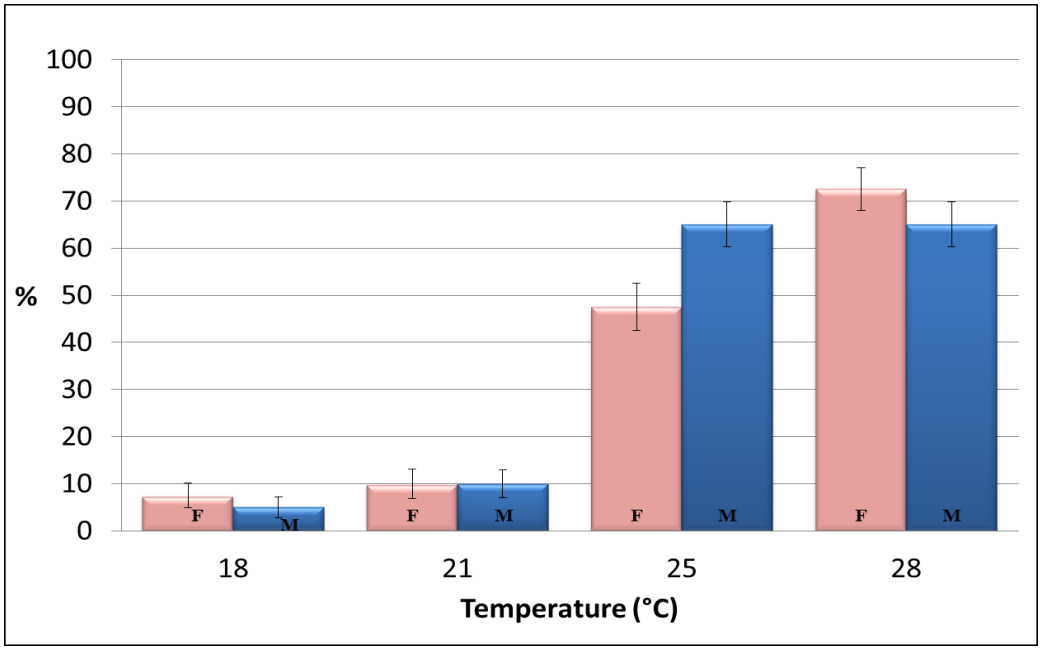


Fig. 5 - Percentage of females (n=40 for each tested temperature) and males (n=40 for each tested temperature) which flew within five minutes from the beginning of the test, at the four considered temperatures. Vertical lines indicate 95%CIs.

The percentages distribution was not homogeneous ($X^2_7 = 109.76$ $P < 0.001$). The found differences were due to the temperature ($X^2_3 = 106.53$ $P < 0.001$) and not sexes.

Post-hoc analysis showed that the flying insects percentage at 28 °C was significantly higher than at 18 °C ($X^2_1 = 66.67$ $P < 0.001$) and at 21 °C ($X^2_1 = 57.84$ $P < 0.001$), while

it was not at 25 °C (X^2_1 = 2.67 P = 0.10). The flying insects percentage at 25 °C was higher than at 18 °C (X^2_1 = 46.54 P < 0.001), and at 21 °C (X^2_1 = 38.62 P < 0.001). There was no difference between flying insects percentage at 18 °C and at 21 °C (X^2_1 = 0.75 P = 0.38) (Figure 5).

Kaplan-Meier analysis on flight starting time at the four considered temperatures, showed that the 28 °C curve was significantly different from the others three (median time = 271 s for 18 °C, 268 s for 21 °C, 208 s for 25 °C and 164 s for 28 °C) (Figure 6).

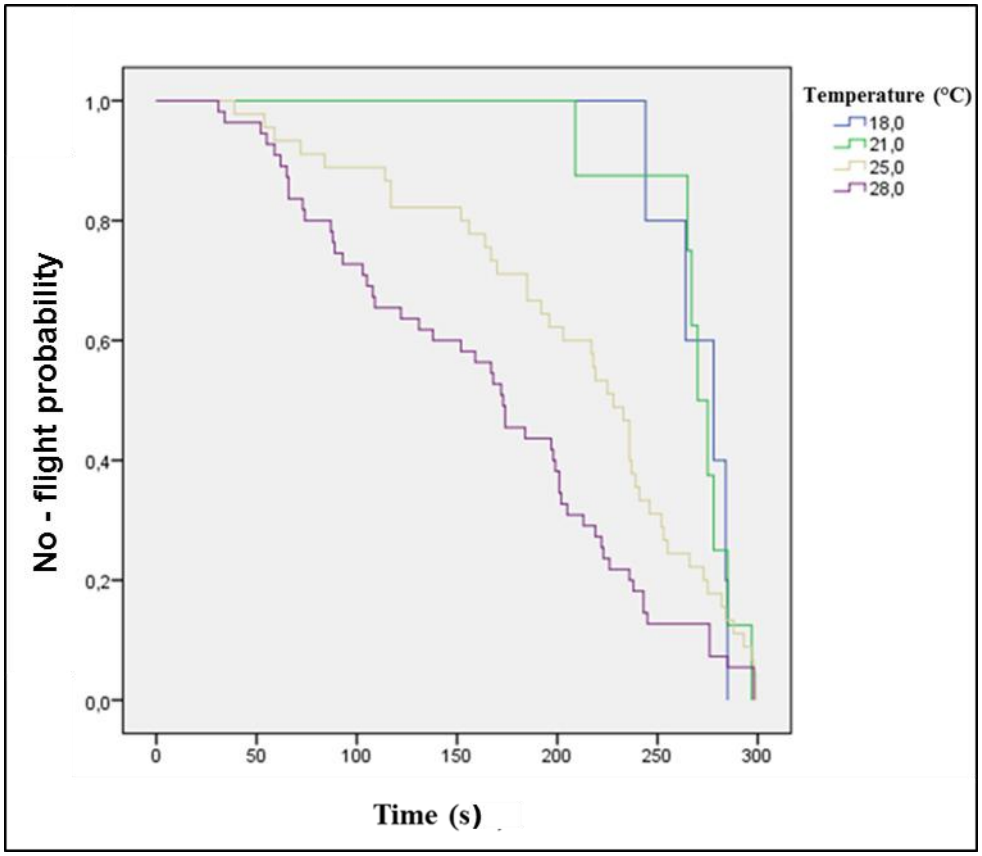


Fig. 6 - Kaplan-Meier flight starting time curves for the four considered temperatures at five minutes. It is a method of data analysis that allows estimating the (cumulative and not punctual) probability that a particular event will occur in an assumed time.

3.3.3 Discussion

The experiments showed that flight behaviour of *Stegobium paniceum* depended on temperatures for both sexes.

The flight percentage increased with the increasing of temperatures from 18 to 28 °C and the flight starting-time decreased with the increasing of temperature.

In addition, the results indicated that flight activity was very low around 18-21 °C, and it began to be considerable around 25 °C. These results are consistent with the flight behaviour in other species of the family. The furniture beetle (*Anobium punctatum* De Geer) does not readily fly at temperatures below 25 °C while the death watch beetle (*Xestobium rufovillosum* De Geer) flies at temperatures higher than 27 °C, in accord with Child (2007) who reports that, as insects need high body temperatures to fly, generally, temperatures below 25 °C limit the flight activity.

Considering that the results of the survey on the application of IPM in Italian cultural heritage facilities showed that storages and exhibition areas are often characterized by temperatures greater than 21 °C (reaching up to 32 °C) and that at present, in the UK, the temperatures in the centrally heated galleries and in the collections storage areas are usually 22 °C or above all year round (Child, 2007), it is possible to apply traps which capture flying insects.

However when the temperature in cultural heritage facilities are around 18 - 21 °C (see survey on IPM application), the traps which capture the insects during their fly, could not be efficient due to insects behaviour and not their absence. In fact, a low fly activity doesn't mean a low density of infestation, females can oviposit from 10 °C on while larvae can survive also at 10-15 °C (Kashef, 1955).

Taking into account these aspects, in order to apply "flight traps" at unfavourable flight temperatures, it will be necessary to increase the scientific research so as to enhance the attractiveness of cues, or to correctly correlate the fewer capture to risk thresholds, comparing a known level of population with the number of catches at a given temperature (for example, could be equally hazardous to capture two adults at 21 °C and 10 adults at 25 ° C).

Moreover, at temperatures that allow the flight, these traps used for monitoring could also have a control activity. In fact, since it has been demonstrated that it is not until 24 h after copulation that the great parts of females begin to fly (Ward, 1980), oviposition starts at least 16 hours after the copulation (Ward, 1980) and the number of flying females increases after oviposition had started (Ward, 1980), it is possible to capture females before and while they oviposit.

3.4 PHEROMONE TEST

Females of *S. paniceum* produce a sex pheromone that attracts males. The sex pheromone consists of two compounds: stegobinone [2,3-dihydro-2,3,5-trimethyl-6-(1-methyl-2-oxobutyl)-4Hpyran-4-one] and stegobiol (Kodama et al. 1987).

Even though, at present, laboratory synthesis of stegobinone and stegobiol are obtained with more efficient methods than those used in the past (Kalaitzakis & Smonou, 2012), commercial productions of *S. paniceum* pheromones are not yet available because of the lack of high-quality procedure for the synthesis of this molecules (Mahroof & Phillips, 2008).

At the moment, commercial pheromone traps for Anobiids ("Lasiotrap", "Serricotrap"), are commonly sold to be used in food productions factories, alimentary shops and warehouses in order to monitor the presence of both *L. serricorne* and *S. paniceum* (Trematerra & Suss, 2006).

Some practical trials in which pheromone traps were used to monitor *S. paniceum* in museums, archives and libraries, showed that these traps did not capture this Anobiid, even if it was present by sure in these environments as signs of infestations, such as frass, exit holes, were visible on the conserved objects and adults were observed on the sticky panel of light traps.

The pheromone effectiveness (in addition to its chemical nature) is affected by many environmental factors as well as the conditions in which it had been preserved, even when the instructions reported on the packaging and provided by the producing industry are complied.

Therefore, preliminary tests to verify the attractiveness of pheromone traps in indoor controlled environments were realized.

3.4.1 Materials and Methods

The tests were performed using insects from laboratory-breeding blocks, as already described.

All the trials were performed in a 14.5 m^2 rectangular darkened chamber, completely devoid of furniture except for a plastic table on which the insects were placed, maintained at 24 °C and 55% R.H..

Pheromone sticky traps differently shaped and baited with commercial pheromone were compared with light sticky traps, exactly of the same shape and capture methods. The shapes of these traps were two. The first one is a long bowl triangular in section with a sticky board on one of the internal sides while the other is a flat square glass covered with transparent glue. The pheromone dispenser was positioned in the middle of the sticky boards while the lights were constituted by light fluorescent lamps with a power of 8W or 13W and a colour temperature of 2700 K or 4100 K, mounted at the upper edge of the bowl or behind the square glass.

The traps were used one at a time and were hanged on in the middle of one of the chamber narrowest walls, at 2.5 m height.

Twenty adults (ten males and ten females) of *Stegobium paniceum* were introduced at the opposite end of the room, in an opened box positioned on the table, at 1 meter height.

The control of captures was made after 24 h, 48 h, and 72 h. Afterword the room was thoroughly cleaned.

3.4.2 Results and Discussion

Insects were caught by light traps but none with traps of the same shape, baited with pheromone. The higher captures were obtained using the bowl trap equipped with a fluorescent lamp of 13 W and colour temperature of 2700K.

The experiments conducted with *Stegobium paniceum* comparing light traps and pheromone traps, confirmed that commercial Anobiids pheromone does not catch *Stegobium* and, therefore, cannot be used to monitor the presence of this insect.

Anobiid commercial pheromone is constituted by serricornin [(4S,6S,7S)-4,6-dimethyl-7-hydroxy-3-nonanone], which is attractive to *L. serricorne*, but not to *S. paniceum*.

Furthermore, some studies demonstrated that traps baited with a prototype stegobinone lure were not attractive to *S. paniceum* (Mahroof & Phillips, 2008).

A japanese company in comparison tests on the attractive activity of syntetic molecules towards *Stegobium paniceum* verified that stegobinone is very instable, while its isomer stegobiene it is not. In fact, if stegobinone attractive capability feall drastically after one week, stegobiene efficiency was permanent for all the four weeks of the experiment

(Fuji Flavor Ltd. Ecomone, 2011 – pers. comm.). They tested also serricornin and they confirmed that it is not efficient to attract *S. paniceum*.
Unfortunately stegobiene is not yet available for commercial use.
Therefore it could be interesting and very useful to explore some of the behavioural aspects of *S. paniceum* attraction towards light.

3.5 LIGHT TESTS

3.5.1 Introduction - Insect behaviour towards the light

Light affects insect behaviour and development in a variety of ways that can be divided into several categories. One of the most typical responses to light is phototaxis which is considered to be one of the basic orientations in insects (Jander, 1963). Insects show principally two different phototactic behaviours: attraction and repulsion. The first, making them move toward a light source (positive phototaxis), can be used to trap pests, but the effective wavelengths and intensities vary among species (Menzel & Greggers, 1985; Hardie, 1989; Kinoshita & Arikawa, 2000; Yang et al., 2003). Repulsion (moving away from light) can be used to prevent pests from entering a cultivation area by presenting light at wavelengths and intensities that repel them (Reisenman et al., 1998; Kim et al., 2013; Shimoda & Honda, 2013). The other possible responses to light beyond phototaxis are well summarized by Shimoda and Honda (2013). They talk about photoperiodicity (the physiological response of insects to the length of exposure to light in a 24-hour period), light adaptation (insect (nocturnal) species becoming light-adapted within several minutes of exposure to light), circadian rhythms (behavioural rhythms including flight, locomotion, feeding, courtship, mating), light toxicity (if an insect is exposed to UV and blue light radiation their compound eyes can suffer damages and structurally degenerated) and finally, some free-flying insects show a dorsal light reaction, where they stabilize their horizontal orientation (attitude) by perceiving light that shines on their dorsal side as sunlight does during flight (Jander, 1963).
Generally, different species within the orders, respond to light in different way (Menzel & Backhaus, 1991). Three principal characteristics of light may influence insect behaviour: specific wavelength (or combination of wavelength), light intensity, and light exposure time. But other factors as the light source (light bulb or light-emitting

diode LED), direction of light source, and the contrast of light source intensity and colour to that of ambient light, are not to be underestimated (Antignus 2000; Honda, 2011; Johansen et al., 2011).

The behavioural response of insects to colour (reflected or emitted light) has been mentioned as 'colour sensation', governed by physical stimuli and sensorial receptors and an integrative system or 'spectral sensitivity' that depends on sensory cells or sensory organs (Dueh, 2011). Visual cells may be sensitive to all wavelengths, but it is the integration of the sensorial inputs to the central nervous system that results in the specific phototactic response of a given insect species (Antignus 2000; Duehl, 2011).

Most insects have two types of photoreceptive organs, compound eyes and ocelli. Compound eyes are made up of a large number of light-sensitive units termed *ommatidia*. An *ommatidium* contains an elongated bundle of photoreceptor cells, each having specific spectral sensitivities. The *ommatidia* are packed in a hexagonal array so as to cover a large visual field with certain spatial resolution and to perceive the motion of objects (Land & Nilsson, 2002).

In a single insect species, different parts of the eyes are often equipped with receptors of different spectral sensitivity, and sexual dimorphisms are not uncommon. Moreover, the shape of the spectral sensitivity functions and their maximum sensitivity values can differ between species (Briscoe & Chittka, 2001).

The spectral range covered by these photoreceptors widely differs between species. The insects can see colours because of visual retinal-based pigments with light-sensitive chromophores (Briscoe & Chittka, 2001).

The spectral sensitivities of photoreceptors determine the visible wavelengths for the insects, which often expands into the ultraviolet (UV) region that is invisible to humans. A compound eye generally contains three types of photoreceptor cells with spectral sensitivity peaks in the UV (generally maximal sensitivity at 350 nm), blue (max 440 nm), and green (max 530 nm), as verified in honeybees (Menzel & Blakers, 1976). At any rate, it is likely that many insects can perceive UV light as a unique colour (Koshitaka et al., 2008). In the Hymenoptera, some sawflies were shown to have red receptors as well (Peitsch et al., 1992). True colour vision depends on the ability to discriminate visual stimuli on the basis of chromatic content, irrespective of differences in brightness sensitivity (Kelber et al., 2003).

Colour and colours contrast are used by phytophagous insects to distinguish between the plants and the surrounding environment. The evolutionary history and physiological needs of individual species determine the wavelengths that are attractive or repellent (Duehl et al., 2011).

In this way, the main areas of research include basic research on reaction behaviour, colour perception, and polarized light perception with light of different wavelengths, and the development of pest control technology using new light sources (LEDs).

In most cases, electrophysiological techniques are used to measure the sensitivity of different pests towards a wide range of light wavelengths. In addition, the responses to LEDs and other light sources are studied by means of behavioural tests in order to clarify the relationship between the light wavelength and the ethology of the pest.

These studies are aimed to determine the wavelengths that are effective for attracting or repelling pests as well as those that affect behavioural activity and orientation to the light (Hironaka & Hariyama, 2009). The results of most recent researches show that LEDs can be used as a direct tool for controlling pests (Chu et al., 2004) or attracting predators (Chu et al., 2003; El-Waha & Abouhata, 2014). Repellent effects of specific blue (470 nm) light-emitting diodes against adults of *Lasioderma serricorne* were demonstrated, suggesting that blue LEDs could be used for environmental friendly control against insects (Min-Gi Kim et al., 2013).

Several researches on the West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) show that attractiveness of light LED colours increase in the order from red to yellow, blue, and green (Nakamoto & Kuba, 2004). For *Tribolium castaneum*, wavelengths slightly shorter than 400 nm are particularly attractive and new LED technology improves the efficiency of light and pheromone traps (Zandomeneghi et al., 2000).

In most of the Anobiids species of cultural heritage facilities, the adults evidence a positive phototropism, as they may be observed at the windows or around the artificial light sources. Regarding *S. paniceum*, empiric experiences such as monitoring in the diocesan archive of Lodi (for four year), in the library and galleries of Alberoni College – Piacenza (for two year), and in curial library of Piacenza (for three year) using light neon traps (unpubl. data) and trials performed by Alex Roach (pers. comm, 2014) have confirmed the phototactic behaviour of *Stegobium paniceum*. At beginning of 2013,

Alex Roach found several hundred of drugstore beetles (*Stegobium paniceum*) in a commercial light trap (UV). It is also reported that before mating the *S. paniceum* adults show a positive phototropism (Koestler et al., 2000), but no scientific studies about its behaviour towards different coloured lights, LEDs in particular, are known.
Since there are no scientific data on *S. paniceum* visual preferences towards coloured light and on its behaviour towards LEDs, series of experiments with different coloured LEDs were performed.
The main goal is to investigate the preferences of *S. paniceum* towards different LEDs wavelengths in order to find an alternative to UV, and to examine if these preferences depend on sexual features. In addition, the studies were focused on the individuation of an alternative to UV light in order to utilize entomological light traps in cultural heritage facilities, where UV are not appreciated, as reported by European standard "Control of Damage to Museum Objects by Optical Radiation", causing damages to the artefacts (CIE157: 2004).

The visual response of an insect may be investigated by its behaviour and by electroretinogram studies, but as Hausmann et al. (2004) wrote, spectral sensitivity does not imply discrimination of dominant wavelengths, so that behavioural evidence is necessary to prove a real attraction to a certain colour. Therefore, it has been decided to focus on behavioural experiments.

3.5.2 Materials and Methods

3.5.2.1 Experimental devices

3.5.2.1.1 Y arena

The Y arena is made of a Plexiglas plate 5 mm thick, with a Y shaped cavity (stem 50 mm long, arms 40 mm long at 130° angle) sandwiched between two glass plates.
The two arms of the Y could be illuminated with emitting diodes (LED) of different wavelengths.
To exclude reflected light, the arena was illuminated from below by infrared light.

3.5.2.1.2 Cross arena

The arena consisted of a cross road modified after Hausmann et al. (2004). It comprised two perpendicular corridors (length 36 cm, width 7 cm, height 14 cm) made of wood walls (5 mm thick) coated with a black antireflective paint.
The two corridors bisected each other forming a cross with an open square chamber at the centre.
Emitting diodes (LED) of different wavelengths could be inserted at both ends of each arm. The use of corridors avoided that the LED beams reflected by the walls of the arena, producing blends of different colours (Otálora-Luna & Dickens, 2010).

3.5.2.1.3 Circular arena

The arena consisted of a circle (diameter of 36 cm) made of metal walls (5 mm thick) coated with a black antireflective paint.
Emitting diodes (LED) of different wavelengths were inserted on the walls at two points at 180°C one from the other.

3.5.2.1.4 XBug

This is a video tracking and a motion analysis system developed for the Linux operating system (Colazza et al., 1999), working with analogical video signals from a camera (monochrome CCD camera - Sony SK-B141P model) digitalized by a video frame grabber.

3.5.2.1.5 Servosphere

The servosphere is a locomotion compensator (Syntech LC-300; Syntech, Hilversum, The Netherlands) already described by Kramer (1976), Hammock et al. (2007) and Otálora-Luna & Dickens (2010). This tracking system allowed the insect to walk unimpeded in all directions on the apex of a 30 cm diameter white sphere. A displacement detector based on an active pixel sensor technology integrated with a near infrared 8-LED lamp (wavelength peak at 940 nm) was positioned 22 cm above the insect. The signal from the displacement detector was processed and sent to servomotors that drove the sphere in the opposite direction of the insect's movement in order to maintain the insect's location at the apex of the sphere. Displacements of the sphere were transmitted to a computer by two pulse-generator encoders positioned

orthogonally at the equator of the ball, allowing the reconstruction of the tracks of the insect's movements. The displacements were measured at a rate of 0.1/s with an accuracy of 0.1 mm. The near-infrared lamp, as integrated with the sensor, illuminated a field of 7 cm in diameter on the apex of the sphere.

The x–y co-ordinates of displacements in the cross arena provided by the servosphere at intervals of 0.1 s were merged in step-sizes of 10 units for efficient summary of the tracks (Otálora-Luna et al., 2004).

3.5.2.2 Tests

All these light tests were realised in a dark room at 25 °C and 50% R.H.

The light utilized were emitting diodes (LED) (McMantom- XLed) of different wavelengths that had emission peaks at:

- 450 nm and 540±5 nm (white),
- 470 nm (blue),
- 400±5 nm (ultraviolet),
- 585 nm (yellow),
- 660 nm (red), and
- 535±5 nm (green).

Colour names correspond to the subjective visual sensation produced on humans by these wavelengths.

Light emitting diodes were the only source of visible light in the experimental room, so they were perceived as spots of light in the dark.

Experiments always considered virgin females, mated females and males, unless differently specified.

Each test was carried out with a different specimen at a time and was replicated at least 20 times.

3.5.2.2.1 Y arena

These trials were performed in the Entomology Department laboratories of University of Piacenza.

The experiments were conducted in a Y arena and a monochrome CCD camera (Sony SK-B141P model) was used to record specimens movements in the arena. In the light

experiments video tracking equipment was used to monitor insects behaviour in order to quantify *S. paniceum* movements in a Y arena.

The specimen was inserted in the arena at the end of the stem (single arm) and could choose between the two arms.

At first, the light sources were tested according to the following experimental protocol: (1) blue vs. dark; (2) red vs. dark; (3) green vs. dark; (4) yellow vs. dark; (5) white vs. dark; (6) UV vs. dark.

Secondly, comparisons between two different wavelengths were accomplished simultaneously comparing LEDs which reported significant statistical differences when exhibited in competition with darkness, according to the following experimental protocol: (7) UV vs. white; (8) UV vs. yellow; (9) UV vs. blue; (10) yellow vs. blue; (11) white vs. blue.

Obviously the control tests dark vs. dark were performed to verify that no other stimuli (except for light) existed in the Y arena that could influence insect behaviour.

Every 5 trials the light position in the Y arms was reversed.

Walking pattern in the Y arena was recorded for 10 minutes. XBug was used to process digital data.

The behavioural response to light was measured as the residence time spent in either arm and the first choice between the two arms.

Statistical analyses

The residence time was analysed with Wilcoxon test for paired comparisons and the first choice with Chi-squared test. The alpha level was set at 0.05. All tests were performed by SPSS Statistics 22.0.

3.5.2.2.2 Servosphere with cross arena

The tests were performed at the Venezuelan Institute for Scientific Research (IVIC) – Laboratory of Sensorial Ecology in Merida.

Experiments were performed in a cross arena positioned on the servosphere top so that its central chamber was exactly at the apex of the sphere overlapped the field of view of the video sensor.

The specimen was introduced in the centre of the arena and (thanks to the servomotors) even when walking, it always stayed in the open square where it was exposed to the light stimuli, unable to reach any of the arena four arms.

Within the cross arena, different pairs of monochromatic lights were offered. At the end of each corridor was positioned a LED (the same utilized in the experiment in the Y arena). The optical axes of LEDs were directed to the insect that walked on the apex of the sphere.

Opposite arms were equipped with the same colour cue at the end of each corridor to guarantee a more symmetrical stimulation environment. By using two opposite LEDs for each colour in the same corridor, the insect was able to see two similar stimuli on each opposite side. The use of corridors avoided that the LED beams reflected by the walls of the arena, producing blends of different colours (Otálora-Luna & Dickens, 2010). Corridors not supplied with LEDs provided a dark cue.

In the series of bioassays, to each insect was given a choice between different coloured light pairs, including darkness as a choice. Locomotion of each insect was recorded for 6 min: the first 2 min only darkness in the room – called "control 1", the next 2 min light (sometimes compered to dark) – called "test", the last 2 minutes still darkness – called "control 2".

To exclude unexpected influences from asymmetries in the arena and room, the colours were changed between the corridors after testing half of the insects. Insects that failed to walk after 4 min were discarded (Otálora-Luna & Dickens, 2010).

In the servosphere with cross arena the combinations which, in the previous experiments in Y arena, indicated a clear behaviour of *S. paniceum* were tested: (1) yellow vs. dark - tested on virgin females, mated females and males; (2) UV vs. dark - tested on virgin females, mated females and males; (3) UV vs. yellow- tested on virgin females, mated females and males; (4) UV vs. white – tested on mated females; (5) white vs. blue - tested on males. Obviously the control tests dark vs. dark were performed.

In these experiments were analysed two parameters (1) displacement in the 'x' corridor and in the 'y' corridor and (2) path straightness.

The last parameter is an index of tortuosity or directionality of the track based on the relative directions of an insect at each time move from the first to last move. The path

straightness estimates the concentration of the distribution of angles around the track mean direction and it is computed as the length of the mean vector of the track, ranging from 0 to 1. The parameter equals 1 if all instantaneous moves are in the same direction and 0 if all possible instantaneous directions are equally represented. The comparison between displacements in 'x' and 'y' perpendicular corridors served to measure the alternative preferred by the insect (Otálora-Luna & Dickens, 2010).

Statistical analysis

The 'x' and 'y' displacements were compared using the Wilcoxon test for paired comparisons. The path straightness was processed applying Kruskal–Wallis test. Post-hoc analyses were done when Kruskal–Wallis test was significant. Results were considered significant at the 5% level. All the post-hoc tests (Mann-Whitney – two independent samples) were adjusted for multiple comparisons using Bonferroni correction. Statistical comparisons were carried out using SPSS Statistics 22.0.

3.5.2.2.3 Cross arena

In this test the cross arena was positioned on a table characterized by an antireflective surface and its corridors were illuminated by the same LEDs used in the previous experiments.

The experiments in cross arena (without servosphere) were made to understand if the insects behaviour in servosphere with cross arena, which was not consistent with the responses in the Y arena, was due to the experimental apparatus. Only the tests in which it was possible to see a clear behaviour of the insects in the two choice Y arena were repeated in the cross arena.

Different pairs of monochromatic lights were offered to the insects: (1) yellow vs. dark - tested on virgin females, mated females and males; (2) white vs. dark – tested with males; (3) UV vs. dark - tested with virgin females, mated females and males; (4) UV vs. white – tested with mated females; (5) UV vs. yellow- tested with virgin females and mated females; (6) yellow vs. blue tests with virgin females; (7) white vs. blue – tested with males.

The specimen was introduced in the arena and observed for 5 minutes to verify its first choice.

Statistical analysis

Bivariate analysis including cross-tabulation with Chi square-statistics was used to evaluate the differences between insects responses. Results were considered significant at the 5% level. Results of statistical analysis were obtained by SPSS Statistics 22.0.

3.5.2.2.4 Servosphere with circular arena

In this case a circular arena was placed on the top of the servosphere as it was done with the cross arena.

In the circular arena all the combinations tried in the Y arena were tested: (1) blue vs. dark; (2) red vs. dark; (3) green vs. dark; (4) yellow vs. dark; (5) white vs. dark; (6) UV vs. dark. (7) UV vs. white; (8) UV vs. yellow; (9) UV vs. blue; (10) yellow vs. blue; (11) white vs. blue.

Statistical analyses

Mean direction was calculated (the mean vector). Rayleigh test was applied to assess the goodness-of-fit of the uniform distribution and consequently to determine whether the distributions of angles in a given period differed from uniformity.

All statistical analyses relating test in circular arena were performed using NCSS statistic software.

3.5.3 Results

3.5.3.1 Y arena

The comparison tests between different wavelength of LED light towards dark (Figures 9A and 10A) showed that there are not statistically significant differences between **green and dark** for both females (virgin and mated) and males, regarding both the time spent in the two arms (virgin females Wilcoxon paired test: P = 0.765; mated females Wilcoxon paired test: P = 0.455; males Wilcoxon paired test: P = 0.563) and the first choice (virgin females X^2_1= 1.80, P = 0.180; mated females X^2_1= 0.80, P = 0.371; males X^2_1= 1.80, P = 0.180).

Also the time spent in the two arms was similar when they had to choose between **blue and dark** (virgin females Wilcoxon paired test: P = 0.313; mated females Wilcoxon paired test: P = 0.179; males Wilcoxon paired test: P = 0.478), but, in this case, the first choices results confirmed this behaviour for virgin females (X^2_1= 0.20, P = 0.655) and

males (X^2_1= 1.80, P = 0.180) (for which blue and dark did not differ), while mated females chose blue arm first (X^2_1= 5.00, P < 0.05).

There are not statistically significant differences between **red and dark** for both females (virgin females Wilcoxon paired test: P = 0.823; mated females Wilcoxon paired test: P = 0.940) and males (Wilcoxon paired test: P = 0.823) regarding the time spent in the two arms. The results of the first choices indicated that mated females preferred dark arm instead of red (X^2_1= 7.20, P < 0.05) as well as males (X^2_1= 7.20, P < 0.05), while virgin females chose the illuminated and dark arm without showing any particular preferences (X^2_1= 3.20, P = 0.074).

When the insects had to choose between **white and dark,** only males preferred white both regarding the first choice (X^2_1= 9.80, P < 0.05) and spent time (Wilcoxon paired test: P < 0.05). Females did not express any preferences for the white as proved statistical analysis of the times spent in the arms (virgin females Wilcoxon paired test: P = 0.390; mated females Wilcoxon paired test: P = 0.156) or the first choices (virgin females X^2_1= 0.80, P = 0.371; mated females X^2_1= 1.80, P = 0.180).

Both **yellow** and **UV**, were preferred to **dark** when compared with this one. When the specimens were inserted in the arena at the end of the stem, in most cases they chose the yellow arm (virgin females X^2_1= 5.00, P < 0.05; mated females X^2_1= 12.80, P < 0.001; males X^2_1= 7.20, P < 0.05) or the UV arm (virgin females X^2_1= 7.20, P < 0.05; mated females X^2_1= 9.80, P < 0.05; males X^2_1= 9.80, P < 0.05) with respect to dark.

With regard to the residence time, virgin females did not show any preferences for yellow or dark (Wilcoxon paired test: P = 0.433) and for UV or dark (Wilcoxon paired test: P = 0.079). The differences were statistically significant only for mated females and males which preferred both the yellow (mated females Wilcoxon paired test: P < 0.05; males Wilcoxon paired test: P < 0.05) and the UV (mated females Wilcoxon paired test: P < 0.001; males Wilcoxon paired test: P = 0.001).

When **UV and yellow** were simultaneously compared (Figures 9B and 10B), the residence time in the two arms was not significantly different for virgin females (Wilcoxon paired test: P = 0,179), mated females (Wilcoxon paired test: P = 0.716), nor males (Wilcoxon paired test: P = 0.391). Consistently, there was no statistical difference in the number of choices made by virgin females (X^2_1= 3.20, P = 0.074), mated females (X^2_1= 0.00, P = 1.00), nor males (X^2_1= 0.80, P = 0.371).

In the **UV against white** tests, residence time spent by mated females was higher in the arm illuminated by UV (Wilcoxon paired test: $P < 0.05$) and also the choice was significantly in favour of the UV ($X^2_1 = 7.20$, $P < 0.05$). On the opposite, both virgin female and males did not show any attraction to one of these lights in particular, as confirmed by the residence time analysis (virgin females Wilcoxon paired test: $P = 0.204$; males Wilcoxon paired test: $P = 0.411$) and first choice analysis (virgin females $X^2_1 = 1.80$, $P = 0.180$; males $X^2_1 = 0.80$, $P = 0.371$).

Similarly, virgin females were not differently attracted by one of the arms when **UV** was **compared with blue,** regarding both the residence time (Wilcoxon paired test: $P = 0.232$) and the first choice ($X^2_1 = 0.80$, $P = 0.371$). Also mated females did not show any preferences in the first choice ($X^2_1 = 3.20$, $P = 0.074$) as well as in the residence time (Wilcoxon paired test: $P = 0.263$). Differently, males were more stimulated by UV than blue, as the residence time in the UV arm was significantly higher than that in the blue arm (Wilcoxon paired test: $P < 0.05$) as well as the first choice more frequently was towards UV arm with respect to blue one ($X^2_1 = 9.80$, $P < 0.05$).

The results of **yellow and blue** comparison, showed that males preferred yellow with respect to both residence time (Wilcoxon paired test: $P = 0.001$) and first choice ($X^2_1 = 7.20$, $P < 0.05$). Also females chose yellow first (mated females $X^2_1 = 7.20$, $P < 0.05$; virgin females $X^2_1 = 5.00$, $P < 0.05$), but, when mated, they spent more time in the yellow arm (as males) (Wilcoxon paired test: $P < 0.05$), while, if virgin, they did not show any preferences regarding residence time (Wilcoxon paired test: $P = 0.940$).

In the comparison between **white and blue,** males preferred white (residence time Wilcoxon paired test: $P < 0.05$; first choice $X^2_1 = 12.80$, $P < 0.001$), while females did not show any preferences with regard to both residence time (Wilcoxon paired test for mated females, $P = 0.494$ and for virgin females $P = 0.654$) and first choice (mated females $X^2_1 = 1.80$, $P = 0.180$ and virgin $X^2_1 = 0.00$, $P = 1.00$).

The results regarding the control (**dark vs. dark**) showed no difference at all. In fact, analysing the time spent in the arms (virgin females Wilcoxon paired test: $P = 0.641$; mated females Wilcoxon paired test: $P = 0.852$; males, Wilcoxon paired test: $P = 0.627$) and the first choices (virgin females, $X^2_1 = 0.20$, $P = 0.655$; mated females, $X^2_1 = 0.00$, $P = 1.00$; males, $X^2_1 = 0.20$, $P = 0.655$) there were not significant differences between right and left arm.

3.5.3.2 Servosphere with cross arena

Displacement

The results (Figure 11), which showed the percentage displacement in the x and y corridors of the cross arena, were very different, sometime opposite, from those previously obtained in the Y arena (Figures 9 and 10).

In the **yellow vs. dark** trials, virgin females preferred dark to yellow, moving significantly more in the obscure corridor than in that illuminated (Wilcoxon paired test: P= 0.001). Instead, mated females (Wilcoxon paired test: P= 0.001) and males (Wilcoxon paired test: P= 0.001) walked considerably more in the dark corridor than in that yellow one.

In the **UV vs. dark** tests, virgin females (Wilcoxon paired test: P= 0.001), mated females (Wilcoxon paired test: P < 0.001) and males (Wilcoxon paired test: P= 0.001) moved more in the dark than in the UV corridor.

In the **UV vs. yellow** comparisons, mated females (Wilcoxon paired test: P < 0.05) and males (Wilcoxon paired test: P < 0.001) preferred yellow, while the displacement percentage of virgin females in the two arms was the same (Wilcoxon paired test: P= 0.223).

In **UV vs. white** tests, mated females displacement was similar in the two corridors (Wilcoxon paired test: P = 0.949).

In the **white vs. blue** comparison, males showed a preference for white light (Wilcoxon paired test: P < 0.05).

The control tests **dark** vs. **dark**, obviously performed in complete darkness, confirmed that there was no preference for either corridor (x or y of the cross arena) for virgin females (Wilcoxon paired test: P= 0.647) as well as mated females (Wilcoxon paired test: P= 0.823) as well as males (Wilcoxon paired test: P= 0.546).

Observing insects tracks, it was possible hypothesize that insects did not move directly to the light source, but towards the corners between two corridors, at 45° from light source.

Path straightness

The results showed that, independently from the colour of the light and from the displacement in the corridors, there were significant differences of path straightness between the three conditions of the trials - control 1 (the first two minutes of darkness), test (the next two minutes in which light was on) and control 2 (the last two minutes of darkness), for virgin females ($H=7.622$, $P < 0.05$), mated females ($H=83.004$, $P < 0.001$), and males ($H=36.690$, $P < 0.001$). For mated females and males the differences were more significant than for virgin females.

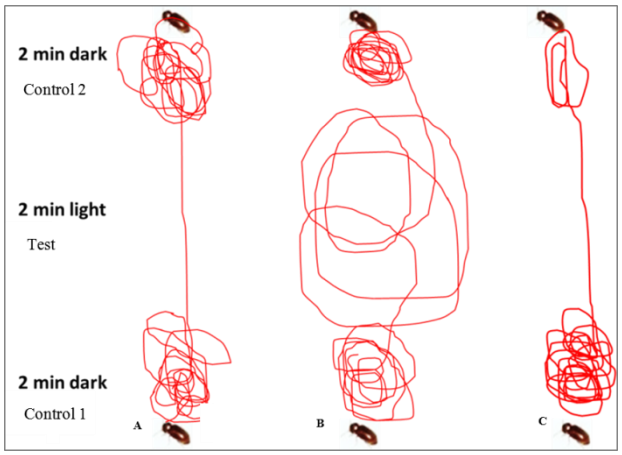


Fig. 7 - Three different paths plotted by *Stegobium paniceum* adults during servosphere tests. It is shown that in light conditions and the path tortuosity decreases. The insects can exploit the light to orientate (A) or, despite straightness increasing, they can keep walking in ample circles, proving no good orientation (B), or they can orientate (like in A) keeping in mind the direction also during the last 2 minutes of dark (B - Control 2).

At the beginning of the trials, in complete darkness the insects turned on themselves designing virtual circles, instead, when the light was on, they appeared less confused and they walked following a straighter path than in dark conditions (Figure7).

This was confirmed by the statistical analysis results which showed that there were differences between control 1 and test for virgin females ($U= 2778$, $Z= -2.62$, $P < 0.01$), mated females ($U=1321$, $Z= -8.06$, $P < 0.001$) and males ($U=1478$, $Z= -5.54$, $P < 0.001$), with a higher difference in mated females and males than in virgin females.

Also the difference on path straightness between test and control 2 was statistically high for mated females ($U=1498.50$, $Z= - 7.57$, $P < 0.001$) and males ($U=1663$, $Z= - 4.88$, $P < 0.001$) while less high for virgin females ($U= 2366$, $Z= - 1.92$, $P = 0.05$).

In general, there were no statistically significant differences in the path straightness between the dark conditions at the beginning of the trial (control 1) and the dark conditions at the end of the test (control 2) for virgin ($U=2633$, $Z= -0.94$, $P=0.347$), mated females ($U=3665$, $Z= -1.57$, $P=0.116$), and males ($U=2887$, $Z= -0.55$ $P=0.583$).

Analysing the path straightness of virgin females, mated females and males, during the light condition, significant differences were found (H=22.39, P < 0.001); mated females track was less tortuous compared to both virgin females (U=2095, Z= - 4.46, P < 0.001) and males (U= 2514, Z= - 3.36, P=0.001), while no differences were found comparing virgin females and males (U= 2624, Z= - 1.23, P=0.219). There were also no differences comparing virgin females, mated females and males with respect to path straightness in control 1 (H=4.13, P=0.127) and in control 2 (H=6.09, P=0.06) (Figure 8).

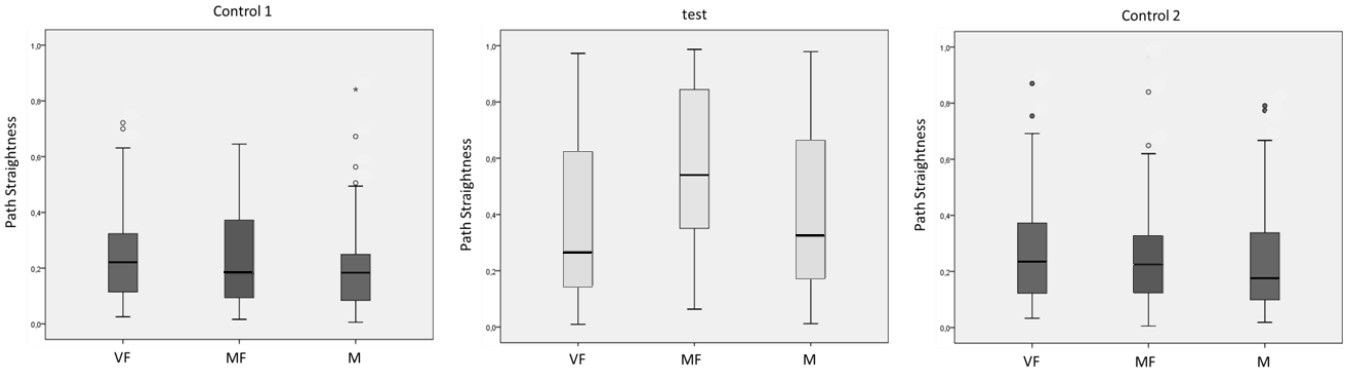


Fig. 8 – Path straightness of *Stegobium paniceum* virgin females (VF), mated females (MF) and males (M) in servosphere with cross arena tests were compared considering the three different moments in the test time (six minutes): first two minutes of dark (control 1), two minutes of light (test), and the latter two minutes of dark (control two). In the box plots, bold lines indicate the medians, lower and upper boundaries of a box indicate the 25 and 75% quartiles, respectively, whiskers below and above the box indicate the 10th and 90th percentiles, respectively, and circles indicate outliers (extreme values). Far outliers are marked by stars. With path straightness = 1 all instantaneous movements are in the same direction, and when it is 0 all possible instantaneous directions are equally represented. It is shown that in light conditions the path tortuosity decreases and that at light conditions, mated females walked straighter than virgin females (P < 0.001) and males (P=0.001).

The single statistical analysis was made for virgin females, mated females and males. Virgin females often seemed to be confused and were not particularly attracted or repelled by light; they often seemed to be indifferent to light, as they continued to move in the same way as in the dark. In fact in the yellow vs. dark (H=1.14 P=0.57) and UV vs. yellow (H=3.93 P=0.14) tests there were no differences between control 1, test and control 2. The results revealed that for the virgin females the behaviour differences were only in the UV- dark comparison (H=5.98 P=0.055).

Differently, mated females always showed walking modifications in light moment with respect to control 1 and control 2, and so path straightness was significantly different in all the comparisons: yellow vs. dark (H=28.58, P<0.001), UV vs. dark (H=30.82 P<0.001), UV vs. yellow (H=9.31 P<0.05) and UV vs. white (H=19.85, P<0.001). The differences always were between tests and controls, never between control 1 and control 2. For example, in yellow against dark the differences of path straightness between control 1 and test (U=48, Z= -4.55 P < 0.001), and test and control 2 (U=47, Z= - 4.58, P < 0.001) were highly significant while between control 1 and control 2 were not significant (U=193, Z= -1.15 P=0.250). The same happened in UV vs. dark test (H=30.82 P<0.001) in which highly significant differences were found between control 1 and test (U=45, Z= -5.01 P < 0.001), and between test and control 2 (U=67, Z= - 4.56, P < 0.001), but no differences were present between control 1 and control 2 (U=278.5, Z= -0.19 P=0.845).

Before and after the exposition to the light in the UV against white test, path straightness of mated females was very different (H=19.85, P < 0.001). In fact, the results showed high differences between control 1 and test (U=149, Z= -3.46 P=0.001) and between test and control 2 (U=107, Z= - 4.23, P < 0.001). As before, the path straightness in control 1 and control 2 were similar (U=303, Z= -0.64 P=0.522).

In the comparison between UV and yellow (H=9.31 P < 0.05) mated females did not show any preferences so, due to their indecision, they walked in all directions turning around the central space of the arena. Nevertheless, there were again differences between control 1 and test (U=93, Z= -2.89 P < 0.01) and test and control 2 (U=129, Z= - 1.92, P= 0.05), even if less significant than those detected in the UV vs. dark and UV vs. white tests. There were no differences between the two controls (U=150, Z= -1.35 P= 0.176).

Sometimes the insects, during the “control 2” phase, walked maintaining a similar trajectory to that displayed when they moved into the light, just as if they could “remember” (Figure 7c). In the test, in which yellow was compared with dark, males showed differences among the three moments of trials (control 1, test, control 2) (H=10.59, P < 0.01). The path straightness of males in control 1 was different from the test (U=75, Z= -3.08 P < 0.01), but their path straightness in these two minutes of light was very similar to that in control 2 (U=154, Z= - 0.77, P= 0.439). There were also

differences between the two controls (U=99, Z= -2.38 P < 0.05), because in the second one the walking of males was less circular than in the first.

Instead in the UV vs. dark comparison (H=7.06, P < 0.05) the differences were, as for mated females, between control 1 and test (U=84, Z= -2.08 P < 0.05), test and control 2 (U=76, Z= - 2.35, P < 0.05), and not between control 1 - control 2 (U=120, Z= -0.84 P=0.390). The same considerations are valid for UV vs. yellow comparison (H=12.53 P < 0.01) in which path straightness in the test was different from control 1 (U=80, Z= -3.24 P= 0.01) and control 2 (U=101, Z= - 2.68, P < 0.01) but there were no differences between control 1 and control 2 (U=163, Z= -1.00 P= 0.317).

This tendency was confirmed also when white and blue were compared, and the differences found among control 1, test and control 2 (H=14.11, P= 0.001) were allocated as in the previous test: path straightness in the test was different from control 1 (U=130, Z= -2.63 P < 0.01) and control 2 (U=89, Z= - 3.59, P < 0.001) but there were no differences between control 1 and control 2 (U=195, Z= -1.10 P= 0.270).

3.5.3.3 Cross arena

Both mated females and males confirmed the first choices made in the Y arena while virgin females in some cases only (Figures 12, 10A and 10B).

In the **yellow vs. dark** tests, mated females chose yellow (X^2_1=7.2 P < 0.01), in **UV vs. dark** trials they chose UV (X^2_1=12.8, P < 0.001), in the **UV vs. yellow** comparisons they did not show any preferences (X^2_1=0.00, P=1), and in the **UV vs. white**, they chose UV (X^2_1=5.00, P < 0.05).

In the **yellow vs. dark**, **white vs. dark**, **and UV vs. dark** tests, males chose yellow (X^2_1=9.8, P < 0.01), white (X^2_1=16.2, P < 0.001), and UV (X^2_1=7.2, P < 0.01), while in the **white vs. blue**, they chose white (X^2_1=9.8, P < 0.01).

Virgin females behaviour was ambiguous and uncertain. In **yellow vs. dark** tests (X^2_1= 0.80, P= 0.371), **UV vs. dark** (X^2_1= 3.2, P= 0.07), **UV vs. yellow** (X^2_1=0.80, P=0.371), and **yellow vs. blue** (X^2_1=3.2, P=0.07), they did not show any preferences.

The results showed that, irrespective of the wavelength compared, when mated females (X^2_1=7.2, P<0.01) and especially males (X^2_1=20, P < 0.001) were positioned in the open square, they immediately reached the corner and then walked in the corridor they choose, moving along its wall and not in the middle of it. On the opposite, virgin

females did not immediately make their way to the corner (X^2_1= 3.2, P= 0.74), when released into the arena.

These results in the cross arena without servosphere confirmed that the tested insects did not walk directly toward the light or in the opposite direction, but at a nearly 45° angle to the corridors, as hypothesised after the servosphere with cross arena results.

3.5.3.4 Servosphere with circular arena

In the **green vs. dark** comparison there were no significant differences. The insects walked in the circular arena in all possible direction, in fact in the virgin females (Rayleigh test: Z=4.04, P=0.133), the mated females (Rayleigh test: Z=2.28, P=0.320) and the males (Rayleigh test: Z=3.38, P=0.184) trials the distribution of the directional choices resulted to be random (Figure 13).

In the **blue vs. dark** tests, the distribution of the directional choices was not random for mated females (Rayleigh test: Z=8.20, P < 0.05) and males (Rayleigh test: Z=16.25, P < 0.01) which oriented to blue light. On the opposite, virgin females were not oriented to a favourite direction (Rayleigh test: Z=0.28, P=0.868).

In the **red vs. dark** tests, virgin females (Rayleigh test: Z=7.14, P < 0.05) were oriented to the dark, whereas mated females (Rayleigh test: Z=0.69, P=0.70) and males (Rayleigh test: Z=0.23, P=0.889) did not prefer any particular direction.

In the comparison **white vs. dark**, virgin females (Rayleigh test: Z=1.71, P=0.424) and mated females (Rayleigh test: Z=5.05, P=0.079) did not walk in a particular direction, while the distribution of male directional choices was concentrated in the white region (Rayleigh test: Z=7.94, P < 0.05).

In this experimental setup, yellow and UV were the favoured colours for mated females and males. In fact in the comparison **yellow vs. dark**, mated females (Rayleigh test: Z=13.22, P=0.001) and males (Rayleigh test: Z=9.16, P=0.010) significantly oriented in the yellow region. On the opposite, directional choices of virgin females were significantly concentrated in the dark area of servosphere (Rayleigh test: Z=7.52, P < 0.05). In the **UV vs. dark** tests, mated females (Rayleigh test: Z=12.36, P < 0.01) and males (Rayleigh test: Z=9.65, P < 0.01) directional choices were in favour of UV.

Virgin females did not show any favourite directions (Rayleigh test: Z=2.04, P=0.359).

In the **UV vs. yellow** tests, there were no differences between directional choices of

virgin females (Rayleigh test: Z=2.98, P=0.225), mated females (Rayleigh test: Z=1.83, P=0.400) and males (Rayleigh test: Z=2.07, P=0.354).

In the **UV vs. white** tests virgin females directional choices were in favour of white (Rayleigh test: Z=7.34, P=0.025), while mated females preferred UV (Rayleigh test: Z=12.64, P=0.001), and males did not show any preferences (Rayleigh test: Z=0.07, P=0.926).

In the **UV vs. blue** tests, virgin females (Rayleigh test: Z=0.27, P=0.872) and males (Rayleigh test: Z=2.59, P=0.272) did not show any preferences, while mated females (Rayleigh test: Z=13.87, P=0.001) preferred UV.

In the **yellow vs. blue** tests mated females (Rayleigh test: Z=9.30, $P < 0.01$) and males (Rayleigh test: Z=7.68, $P < 0.05$) showed a strong preference for **yellow** on the opposite, virgin females did not display any preferences (Rayleigh test: Z=0.46, P=0.794).

In the **white vs. blue** comparison the directional choices were not concentrated in a particular region of the sphere for virgin females (Rayleigh test: Z=1.33, P=0.513) mated females (Rayleigh test: Z=0.28, P=0.865) and males (Rayleigh test: Z=2.75, P=0.258).

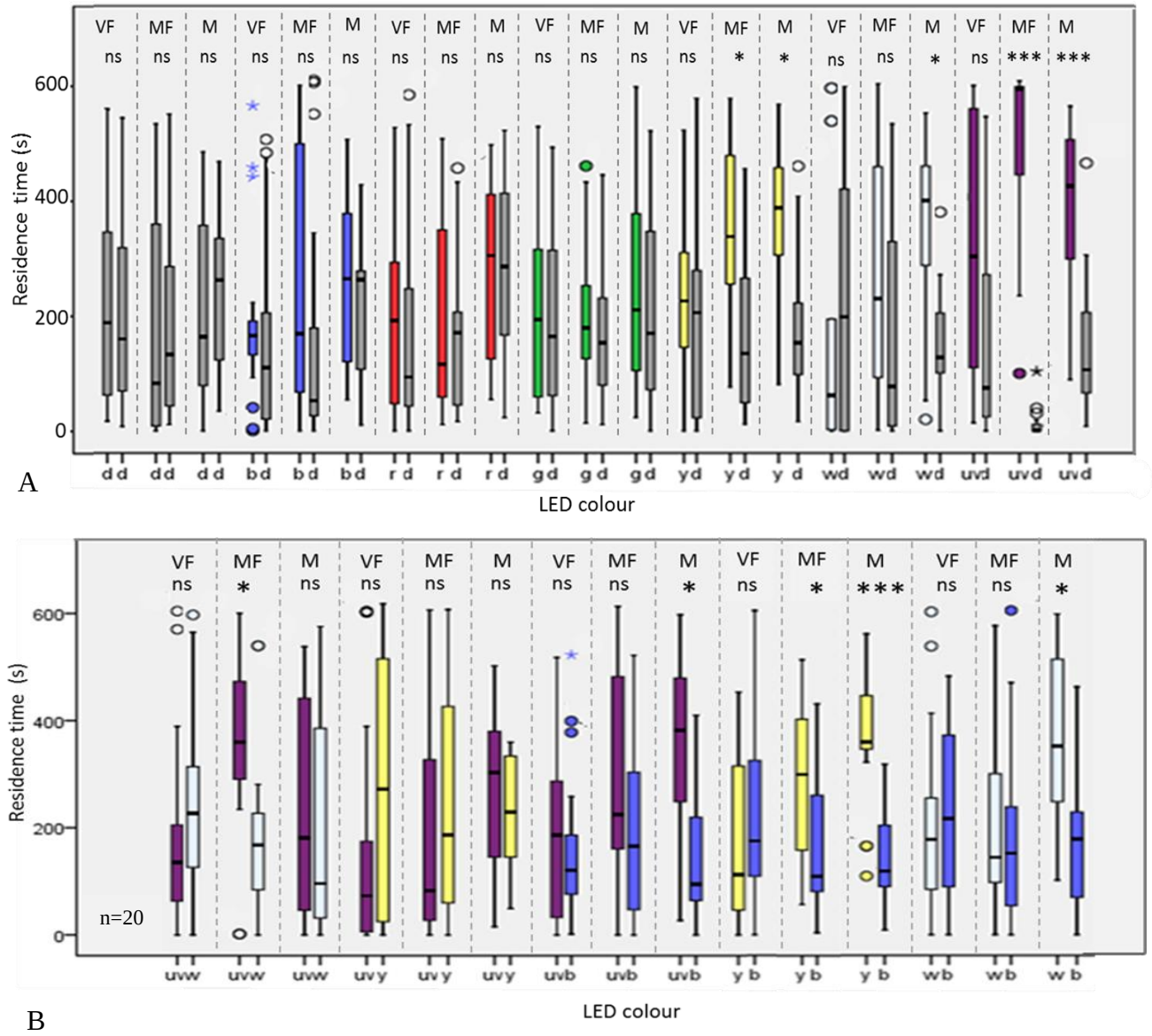
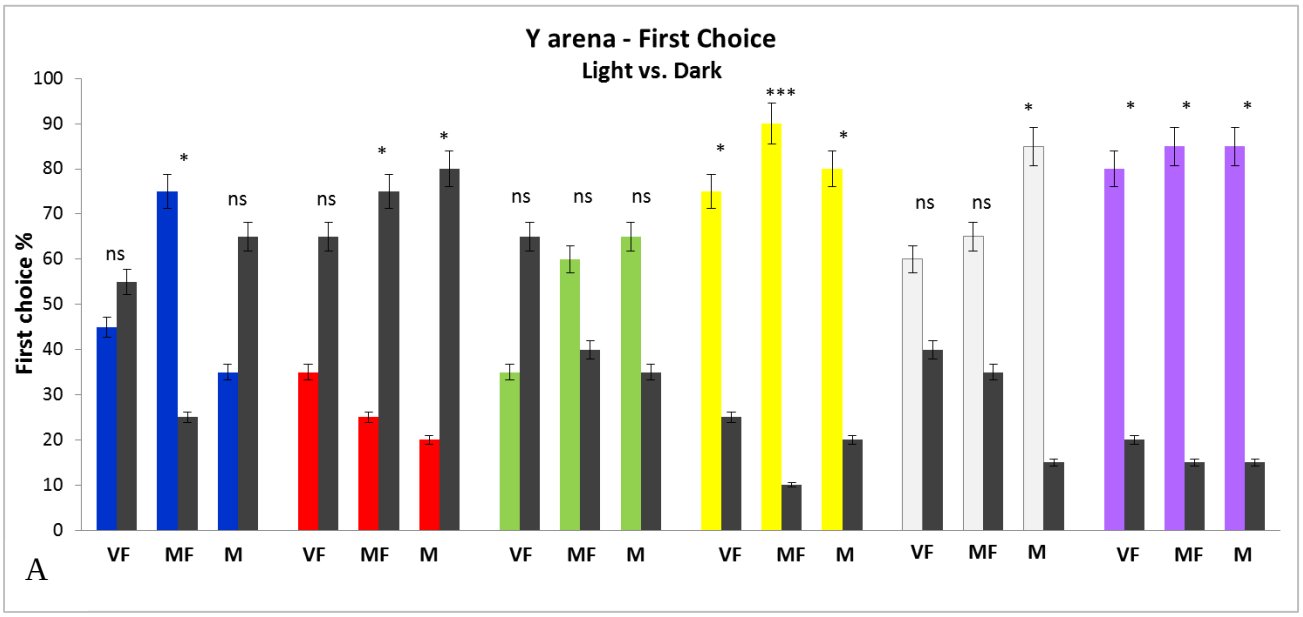


Fig. 9: Residence time spent by Stegobium paniceum virgin females (VF), mated females (MF) and males (M) in the two arms of a Y arena, when different colour pairs were presented. (A) Responses of light vs. dark comparison (d=dark; b=blue; r=red; g=green; y=yellow; w=white; uv= uv LED). (B) Responses of S. paniceum in simultaneous comparisons between the lights which gave significant results in the comparison with dark. The duration time of the trials was 10 minutes. Twenty insects were tested in all comparison (n=20). Asterisks and 'ns' indicate significant and not significant differences, respectively (see Results for statistics). In the box plots, bold lines indicate the medians, lower and upper boundaries of a box indicate the 25 and 75% quartiles, respectively, whiskers below and above the box indicate the 10th and 90th percentiles, respectively, and circles indicate outliers (extreme values). Far outliers are marked by coloured stars.



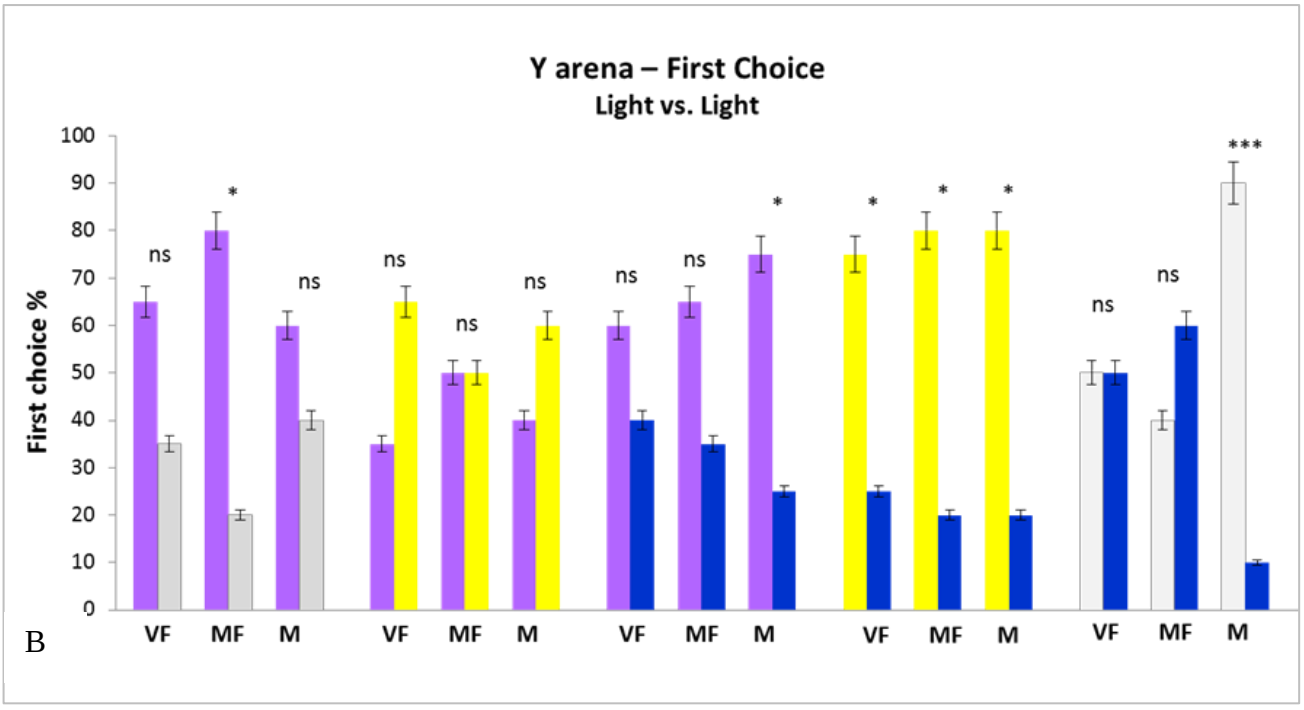


Fig. 10: Percentage of virgin females (VF), mated females (MF) and males (M) which chose (first choice test) a specific wavelength, compared with dark (A) or with another one (B) in the Y arena. Vertical lines indicate 95%CIs. The coloured bars represent coloured LEDs. Asterisks and 'ns' indicate significant and not significant differences, respectively (Chi-squared test; the alpha level was set at 0.05).

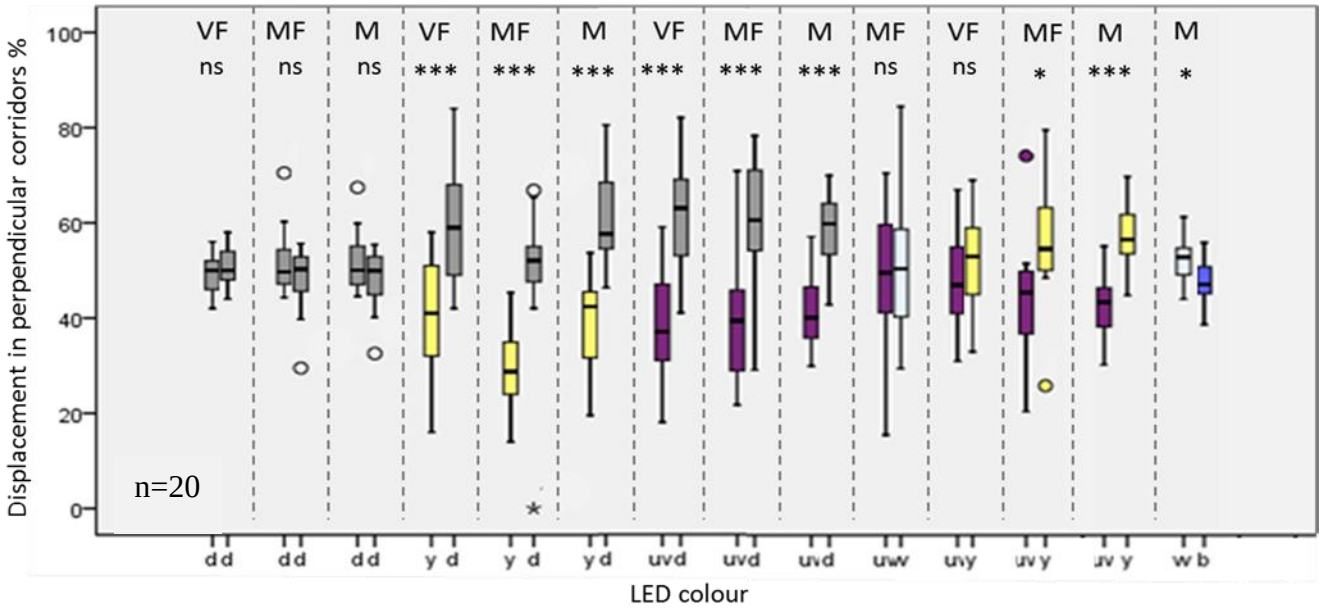


Fig. 11: Light preferences of *S. paniceum* virgin females (VF), mated females (MF) and males (M) in the servosphere with cross arena are expressed as percentage displacement in perpendicular corridors. Results are very different, sometimes opposite, to those obtained in Y arena, probably due to the different experimental setup.

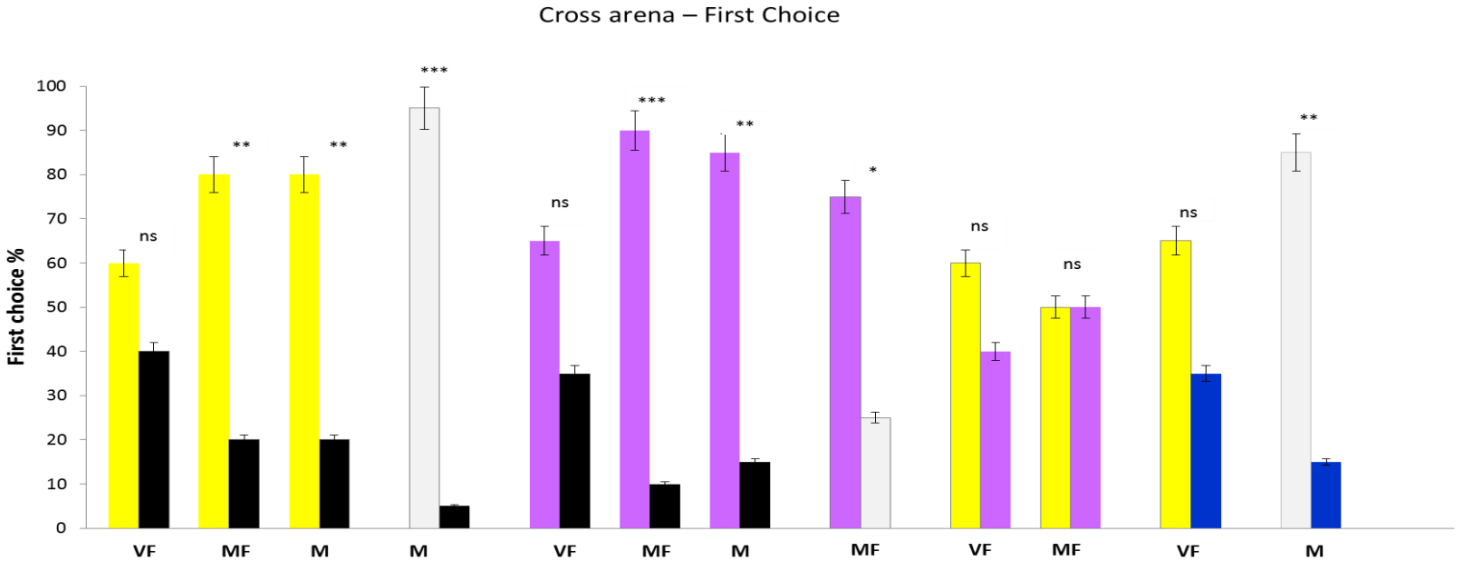
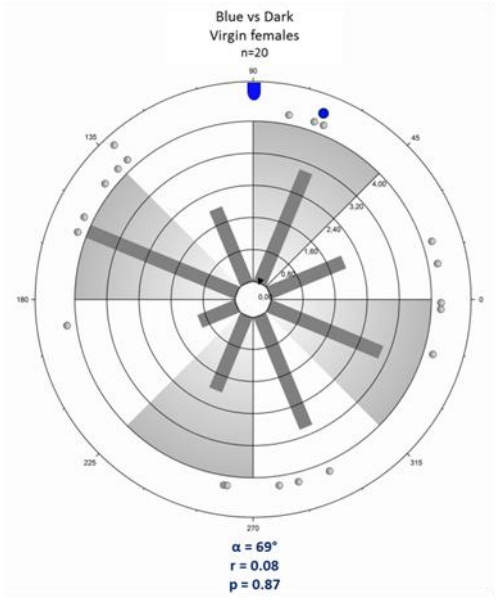
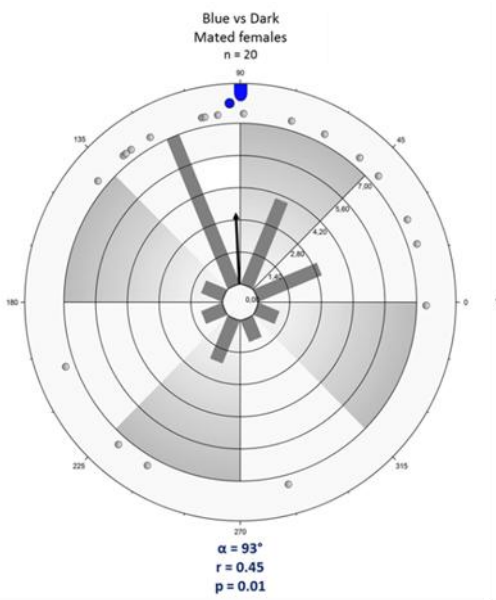


Fig. 12: Preference of virgin females (VF), mated females (MF) and males (M) are expressed as percentage of their choices, observed in light comparisons tests in the cross arena (without servosphere). Only the trials in which *S. paniceum*, when tested in the Y arena, reported a well-defined behaviour (Figure 10) are made with this device. Vertical lines indicate 95%CIs. The coloured bars represent coloured LEDs. Asterisks and 'ns' indicate significant and not significant differences, respectively (Chi-squared test; the alpha level was set at 0.05).


Blue vs Dark
Virgin females
n=20
α = 69°
r = 0.08
p = 0.87


Blue vs Dark
Mated females
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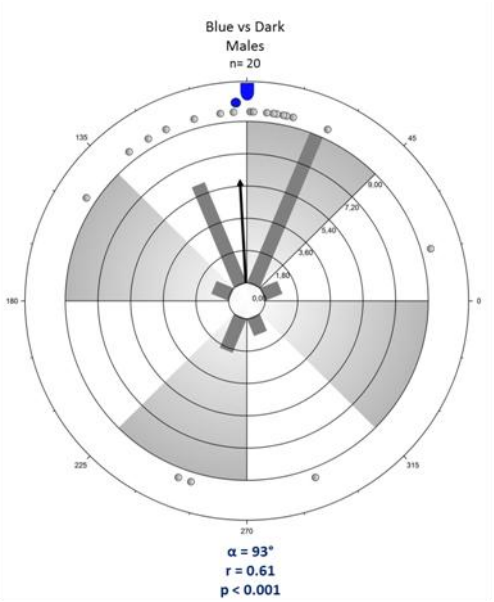
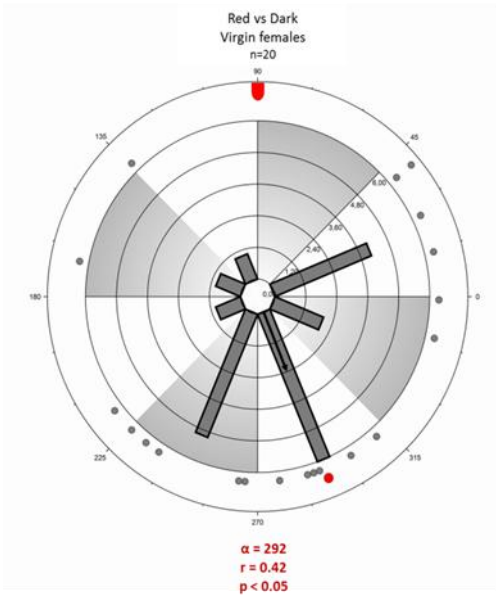
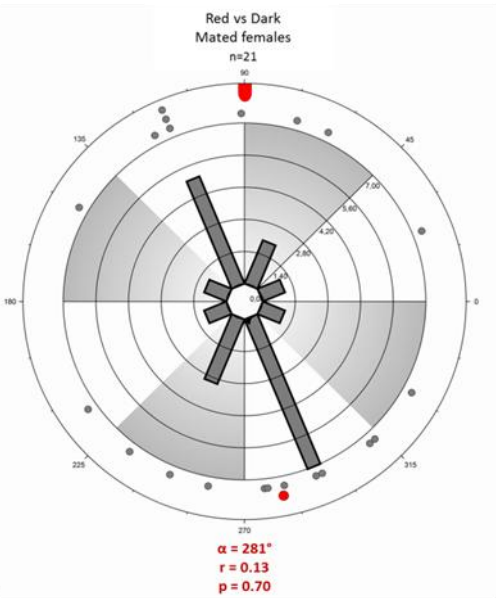
r = 0.45
p = 0.01


Blue vs Dark
Males
n= 20
α = 93°
r = 0.61
p < 0.001


Red vs Dark
Virgin females
n=20
α = 292
r = 0.42
p < 0.05


Red vs Dark
Mated females
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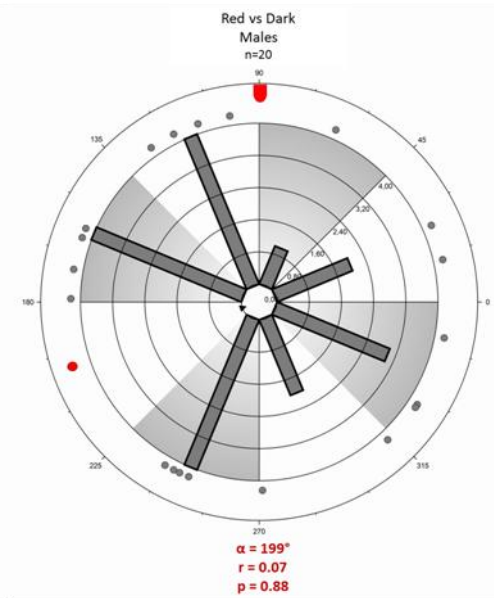
p = 0.70


Red vs Dark
Males
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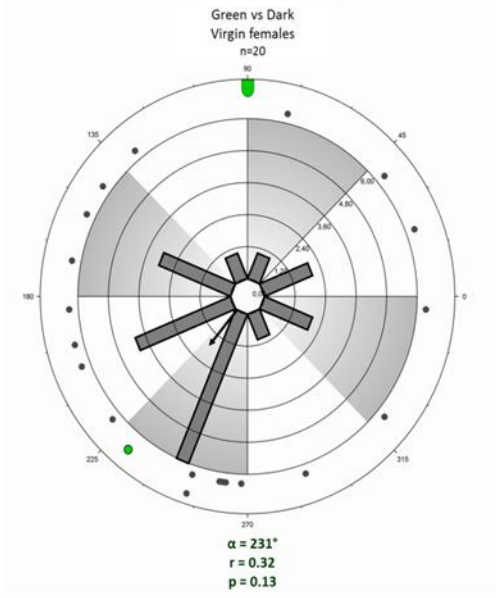
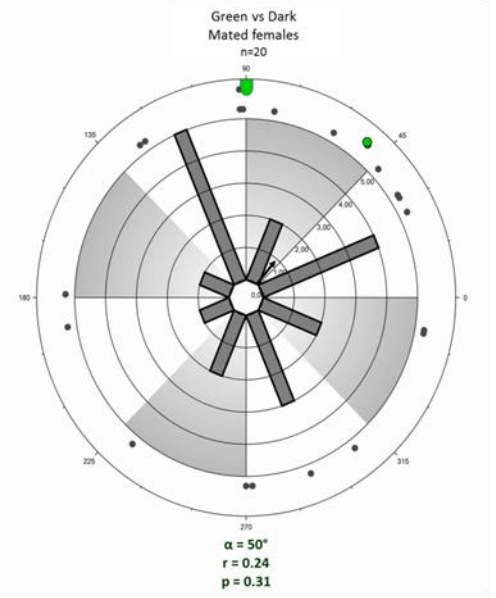
p = 0.88


Green vs Dark
Virgin females
n=20
α = 231°
r = 0.32
p = 0.13


Green vs Dark
Mated females
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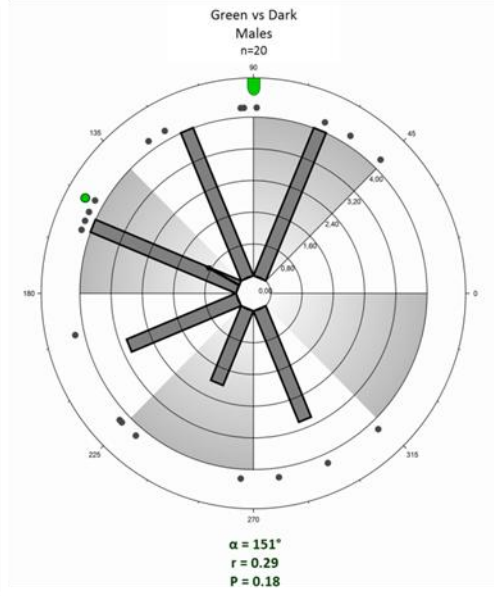
α = 50°
r = 0.24
p = 0.31


Green vs Dark
Males
n=20
α = 151°
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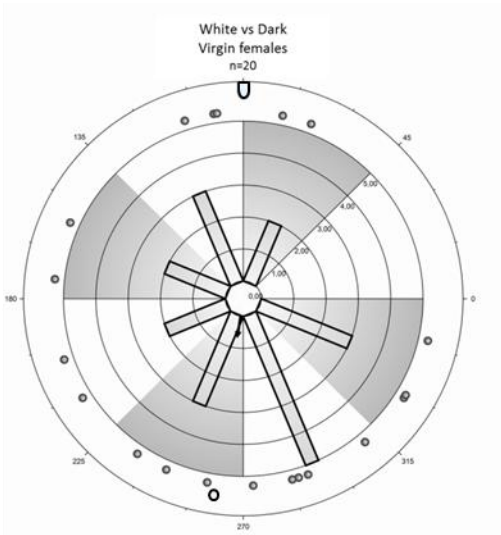
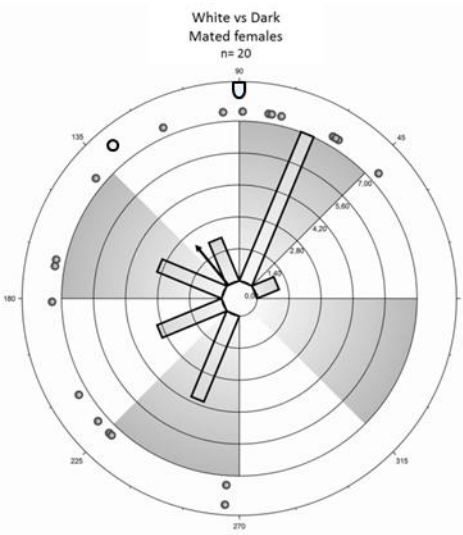
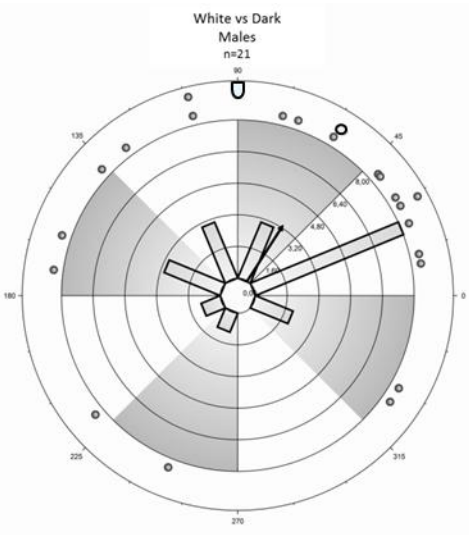
P = 0.18



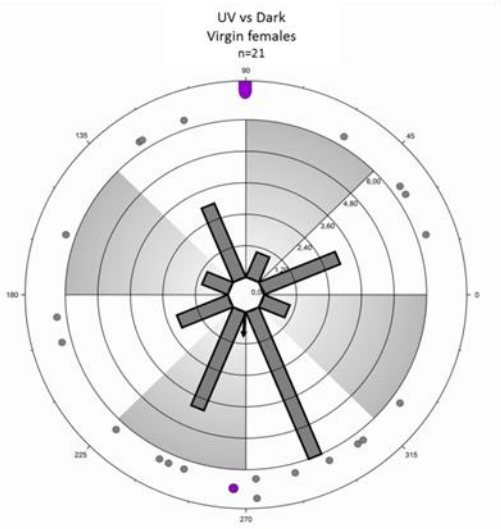
α = 261°
r = 0.21
p = 0.42



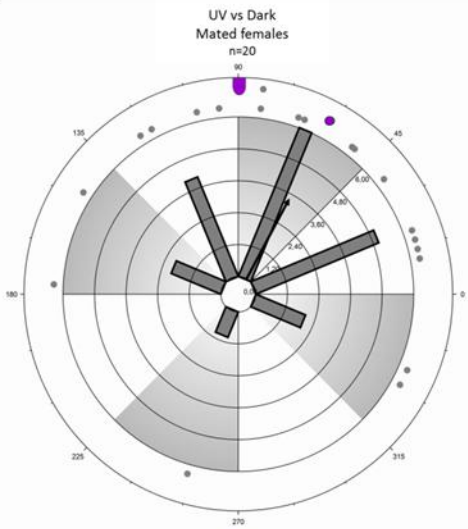
α = 129°
r = 0.35
p = 0.07



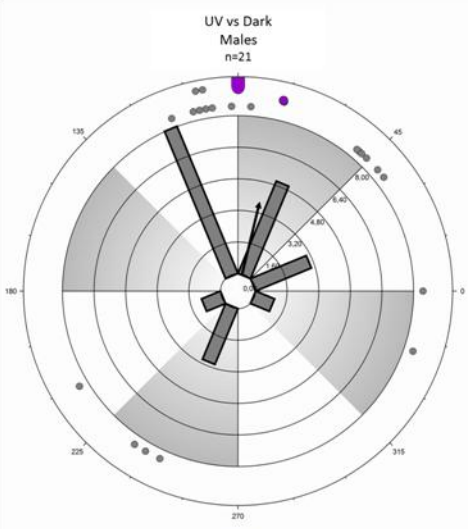
α = 57°
r = 0.43
p < 0.05



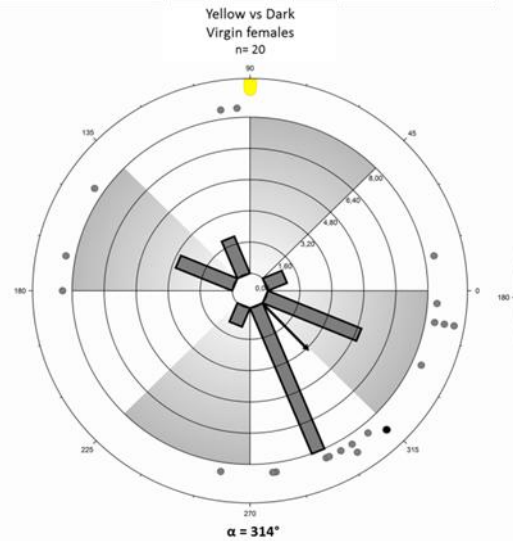
α = 266
r = 0.22
p = 0.36



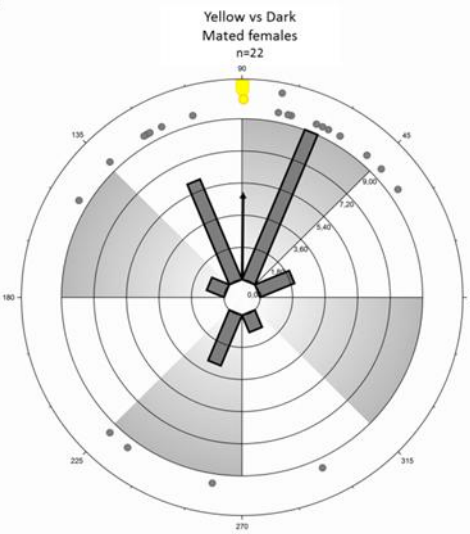
α = 62°
r = 0.54
p< 0.01



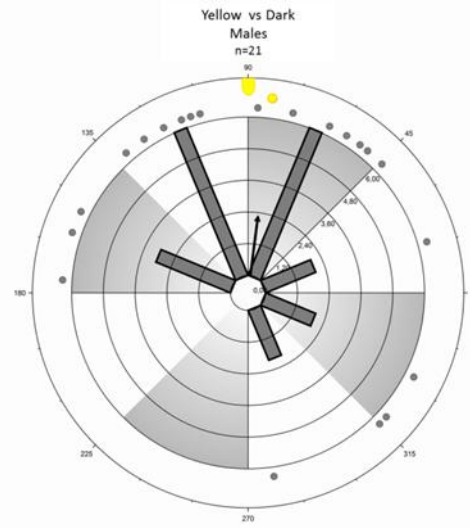
α = 76°
r = 0.47
p < 0.01



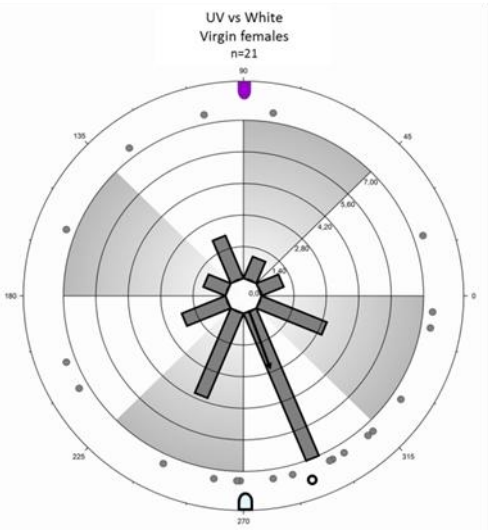
α = 314°
r = 0.43
p < 0.05



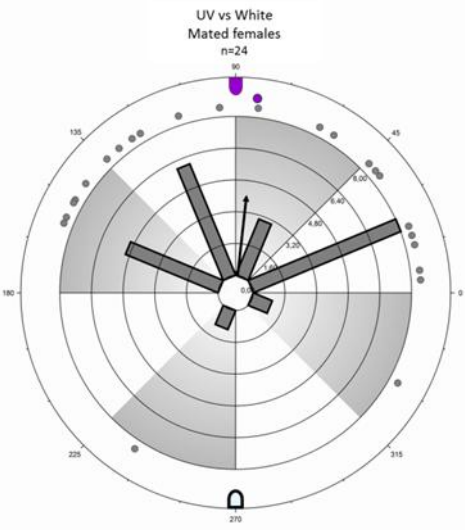
α = 89°
r = 0.53
p = 0.001



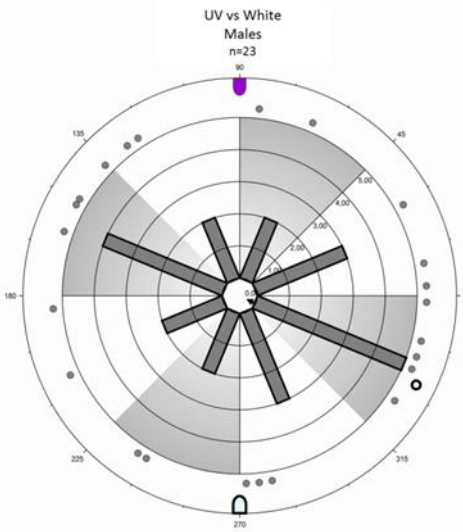
α = 83°
r = 0.46
p = 0.01



α = 290°
r = 0.41
p < 0.05



α = 84°
r = 0.50
p = 0.001



α = 333°
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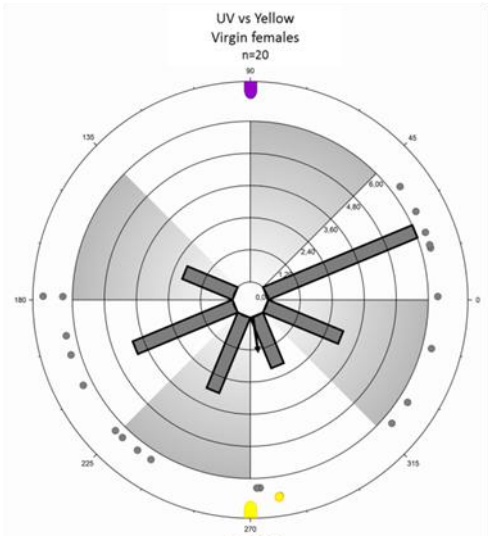
p = 0.96



α = 278°
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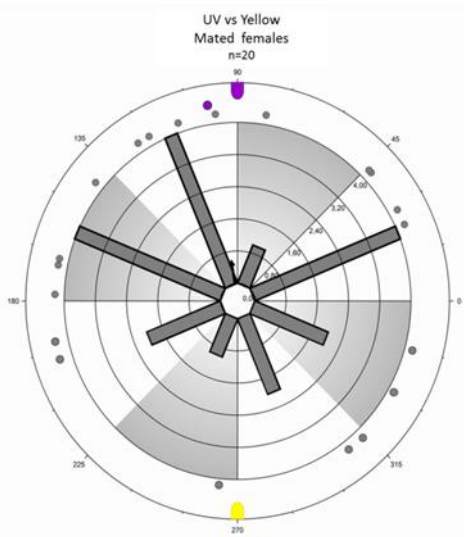
p = 0.22



α = 99°
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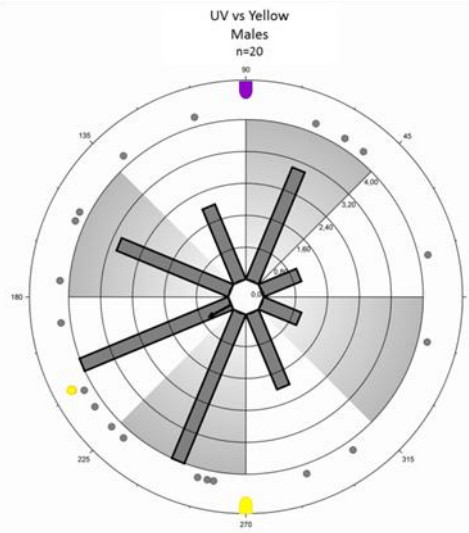
p = 0.40



α = 208°
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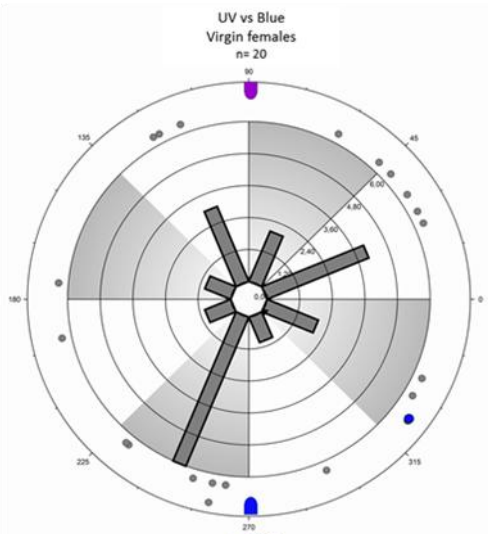
p = 0.35



α = 322°
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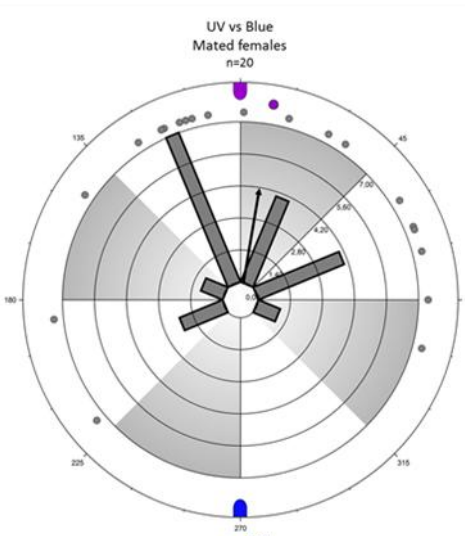
p = 0.87



α = 80°
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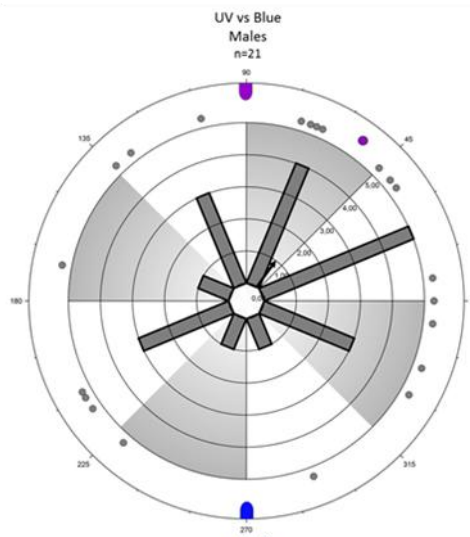
p = 0.001



α = 54°
r = 0.24
p = 0.27

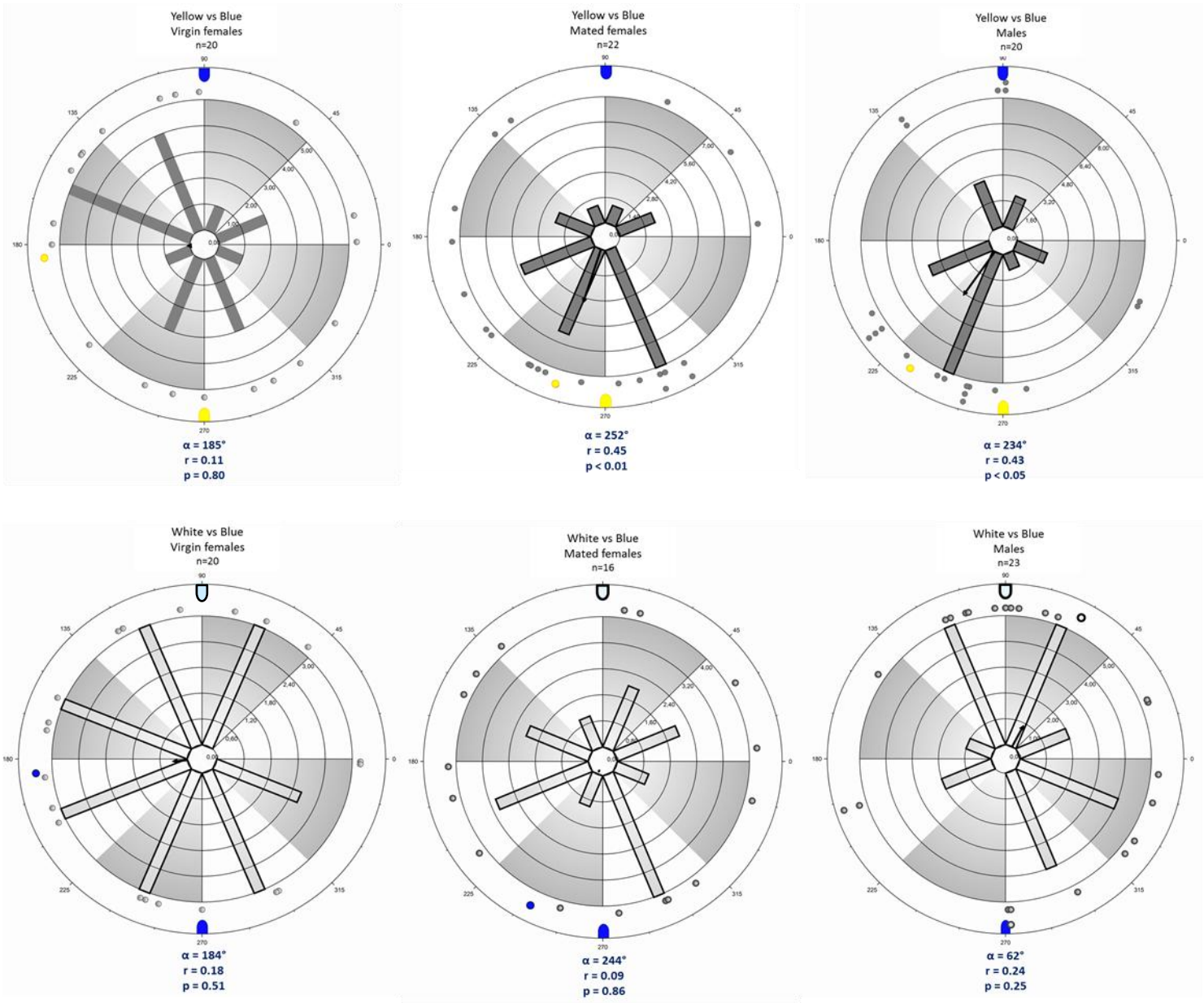


Fig. 13: The circular histograms show the direction of *Stegobium paniceum* virgin females, mated females and males in a circular arena, stimulated by different coloured LEDs. Relative positions of the light emitting diodes (LEDs) in the dual choice arena are shown. Mean angles of orientation (α) are indicated by the coloured spot and mean vectors (r) (ranging between 0 -the angles are not concentrated in the same direction and 1 – all the data are concentrated in a specific direction) are represented by the black arrow. Values of α and r are also reported below the circular histograms. The orientation of the bars indicates mean direction values at their respective positions on the sphere sectors. Significance levels (p) are according to the Rayleigh test.

3.5.4 Discussion

Light experiments are very useful in order to increase the efficacy and use of light traps to optimize entomological monitoring in conservation structures. Light traps using light emitting diodes (LED) sources with wavelengths different from UV, could represent a smart solution to verify the presence of dangerous insects and prevent their damages.

Regarding *S. paniceum*, empiric experiences (unpubl. data; Alex Roach pers. comm, 2014; Koestler et al., 2000; Gămălie & Mustață, 2006) show its phototactic behaviour towards fluorescent sources, but, up to now, there were no scientific data on its visual preferences comparing different coloured light and, in particular, towards LED.

In these light tests, we investigated the responses of *S. paniceum* adults (virgin females, mated females and males) to emissive colours produced by LEDs to evaluate preferences and orientation of *S. paniceum* towards light. At the same time, using different experimental setups, we could validate a protocol which might be applicable to other insects.

The first trials, conducted in the Y arena (Figures 9 and 10), provided both the immediate preference of the insect positioned in front of the two different lights (first choice), and the total residence time in the two arms, serving to establish if the insect is attracted by a certain colour and how much it likes to stay in that specific light condition. This aspect is important as it is related to the traps capture efficacy as, even when an insect is attracted to a certain wavelength, it might not be induced to enter the trap. The fact that it likes to stay in a specific light condition could encourage it to land on the illuminated surfaces and so to be caught by the trap capture system. In other experiments with different species, for example, an additional attractant, such as a pheromone, that seemed not to be effective at a longer distance in an indoor environment, was needed to encourage the insects to go in (McQuate, 2014).

The obtained data indicate that, for mated females and males, the results of the first choice correspond with those of the residence time (Figures 9 and 10). In fact, when they chose a colour, either they returned again in the stem or remained still close to the LED, the total time they passed in the arm they chose first is the highest. On the contrary, virgin females, even when at first they chose a colour, spent an equal time in the two arms (either dark or illuminated). This proves that the probability to be captured

is higher for mated females and males than for virgin females which, even when responding to a light (rarely), do not undergo an attraction strong enough to hold them.
The experiments carried out in the servosphere with the cross arena did not provide reliable information about insects preferences to specific wavelength, because of their tendency to move not directly towards the light stimulus, but at an angle of about 45° to both sources. In fact, in most cases, they walked along a virtual line situated at 45° from both stimuli, tottering on its left and right side, as if they were pointing to the corner of arena between the two corridors. In this way, depending on how much they walked on the left or right side of the line, the system recorded their behaviour as a choice or a non-choice, giving unaffordable results different or even opposite from those previously obtained in the Y arena. So, it was with the check experiments in the cross arena without servosphere that the misleading results obtained in the servosphere with cross arena were understood. The tests in the cross arena (without servosphere) showed that *S. paniceum*, an instant before choosing between the two light sources, went to the corner (confirming the "corner effect" previously hypothesized) and, subsequently, walked to the light moving close to the wall of corridor. A similar behaviour was discussed by Camhi and Johnson (1999) about the cockroaches but, in this case, these insects utilize mechanoreceptors on the antennae to "follow surfaces with remarkable consistency". It was not possible to verify this aspect with *S. paniceum* in the performed tests due to the size of the species and the kind of the experiments.
Yet, the experiments carried out in the servosphere with the cross arena were useful to investigate another aspect of the orientation of the insects towards the light.
S. paniceum mated females and males may be able to orientate towards a light stimulus, while it is not so for virgin females. The results of path straightness analysis show that *S. paniceum* adults in complete darkness turned on themselves designing virtual circles. At light condition, both mated females and males walked following a straighter path, thus appearing less confused while virgin females continued to move in a similar way as in the dark, appearing confused and not particularly attracted or repelled by light of any colours. Mated females, at light conditions, walked even straighter than males; the latter being able, after having walked in the light, to maintain a trajectory similar to that displayed when moving at light conditions, just as if they could "remember" it.

With regard to the preferred wavelength, the results obtained in the Y arena for mated females and males were almost always confirmed by the tests in the cross arena without servosphere and by the tests in the servosphere with circular arena.

S. paniceum mated females showed a positive phototaxis towards blue, yellow and UV lights, while white, green and red were not preferred when compared to dark (Figures 9, 10, 13). In addition, while the results with the first two lights are consistent in the three devices, those obtained with red suggest an even repellent effect in the first choice test in the Y arena. Among blue, yellow and UV, the most attractive colours, in all the experiments, were **UV** and **yellow**, both when compared with dark or with other colours, and without any significant differences between the two, when compared to each other. Their attitude towards blue is less clear as in the blue vs. dark tests, the residence time in the Y arena is not significantly different between the two while it is so both as to the first choice and the mean direction in the circular arena. Also in the UV vs. blue tests, the mated females did not distinguish between the two in the Y arena, while in the circular arena, the UV was again preferred to blue.

S. paniceum males showed a positive phototaxis towards **yellow** and **UV** lights, as for mated females, but also towards **white**. When compared to dark, blue was not chosen in the Y arena (both as first choice and residence time) (Figures 9 and 10) but it was so in the circular one (Figure 13), with a high significant level. This result could have probably biased also those obtained in the circular arena when comparing blue to UV, white, and yellow. In fact, in the comparisons with the first two colours, which were slightly preferred to blue in the Y arena, the results in the circular arena where not significant, while in the comparison with the third, which was highly preferred to blue in the Y arena, the result still indicates a preference towards yellow. An explanation was not found for the different behaviour towards blue in the Y and circular *arenae*.

These data are unexpected because it was demonstrated that *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), a very near species to *S. paniceum*, is repelled by yellow LED light whereas in this case yellow is one of the favourite lights (Min-Gi Kim et al., 2013).

The confused behaviour of virgin females towards light, verified in the Y arena and the cross arena without servosphere, was also confirmed in the servosphere with circular arena. Only in the first choice tests in the Y arena virgin females showed a significant

preference for UV and yellow with respect to dark and for yellow with respect to blue. In the servosphere with circular arena chose UV when compared to white. Nevertheless, these results were never confirmed in the other tests, while, on the contrary, the results were sometimes opposite.

These results also pointed out that behavioural differences between virgin females and both mated females and males are present. These differences have not previously reported.

4 CONCLUSIONS

The results of the survey about IPM application in the Italian cultural heritage facilities show that pest management is not considered as a daily practice and is not included in the ordinary conservative actions, even if, for 75% of cultural heritage Italian facilities pests are a problem. However, these are perceived as a problem only when the damages on the objects are visible and often irreparable. Specific preventive pest control programmes are not adopted and IPM is not known by the museum, archive and library operators. The lack of prevention is partly due to the lack of funds, but, above all, to the lack of knowledge (of pests, methods to locate their presence and to eradicate or avoid them). Also the lack of reference points (public organizations) to consult in order to define and solve the problem is a cause of incorrect approaches. In fact, when a cultural heritage facility is in need of pest eradication, it consults the superintendence to find pest control companies. Therefore, a register which includes disinfestation companies (ranging from food storages or other public environments), certified by the government institutions (superintendence) and qualified through accredited and specific courses on cultural heritage facilities pests (which implicate different conditions from those in food storages or other public environments), could be extremely useful.

The results show that notwithstanding the fact that the directors of cultural heritage facilities are conscious of the importance of pests and biodeteriogens knowledge, they are not educated and trained on pest management. The necessity of staff training, at all levels (managers, librarians, curators, housekeeping staff), is evident. It is possible that the pest problems are not often solved in the correct way because the people responsible for cultural heritage facilities have not the knowledge instruments to prevent, identify and manage this kind of problems. In fact they think a standard could be useful to ensure correct pest management.

The operators awareness of sustainability of pests prevention, inevitably would lead to consistent savings regarding pest issues. In fact, a recent comparison between the costs of normal protection management, which rely on disinfestation treatments, and those of a preventive management, including staff training, showed that the latter were extremely reduced with respects to the first, not to mention the fact that there is no loss

of artistically and historically precious objects and/or documents (unpubl. data) of inestimable value. The fact that the value of cultural heritage items tends to be considered “immeasurable” and the real costs related to pest prevention are unknown, is often the most important reason why museum, archive, library and historic house managers do not invest in prevention. In fact, attempting to define the “inestimable” value of something instead of giving it a true value, gives it “no value” at all. Also for this reason, it would be very helpful that insurance companies, which must include an economic estimate of the objects in their contracts, could not only consider theft and fire risk, but include also pest risk.

To estimate a pest risk, it is necessary to consider the environmental conditions, often not recorded (as shown in the survey results), the building characteristics, the material of the conserved objects. Nevertheless, these data only enable to speculate on which insects could be present. To verify which dangerous species are really present in a particular environment, entomological monitoring must be used. At present, entomological monitoring is usually not applied as a prevention action and, in the rare cases in which it is employed, it is used after the damage has already become visible (and the risks for artefacts high), as an exceptional instrument to surrender to after verifying the destructive pests action.

Moreover, it is usually planned and supervised by external pest control operators that frequently do not plan controls at the right times, and do not know the species captured (as they usually work in food premises), particularly for what concerns the hazard they pose for the conserved objects.

In any way, these monitoring application limits, are not the only ones that make it difficult to efficiently use it. Researches are needed to acquire data that will make monitoring a valid and easy instrument for cultural heritage conservation. In fact, monitoring researches are usually focused on agriculture or food pests and it is necessary to increase the studies on the insects that damage cultural heritage items.

Therefore, the results of this work, regarding the photoresponse and flight behaviour of one of the most dangerous insect in cultural heritage environments (the test species *Stegobium paniceum*), have basic implication on monitoring pest trapping programmes in infested buildings. These data represent a first step towards a more efficient trap design and use. In addition, a deeper knowledge of pest habits and ethology, will

guarantee a more affordable analysis of the catches data, in relation to the conditions of the environment in which the capture devices are placed.

Trap placement, in dimmer or shadowed areas and along corridors, ensuring sufficient contrast between the light trap and the background, should increase trap efficiency (Duehl et al., 2011).

The results show that *S. paniceum* adults are capable to perceive and discriminate the light wavelengths of the surrounding environment. The attraction of *S. paniceum* to specific wavelengths could be caused by adaptive responses to ovipositing substrate that will represent the nourishment of the larvae. For example, the attraction of deathwatch beetles, *Xestobium rufovillosum* DeGeer (Coleoptera: Anobiidae), to white coloured card traps was interpreted as an adaptive response to whitish decaying timber (Belmain et al., 1998), even if the white cards could also be attractive as a result of their strong contrast with the surrounding timber (Conlon & Bell, 1991; Kostal, 1991; Hughes, 1992). Other experiments with the small hive beetle, *Aethina tumida* Murray, show that attraction to UV wavelengths may be related to finding open spaces so that would be particularly useful for dispersal (Belmain, 1999). Other insect species are also attracted to near UV and UV wavelengths for similar reasons. The red flour beetle lives in the flour which has a high reflectance at 390 nm, thus its attraction to this wavelength should be advantageous for foraging (Cohnstaedt et al., 2008).

S. paniceum occurs in several habitats, therefore its light preferences in the UV could be connected to the reflectance of its food (wheat flour and derived products) which is in this wavelength range.

Nevertheless, the results obtained by behavioural tests, permitted to find alternative wavelengths to UV light, which is not recommended in cultural heritage conservation environments.

The more discriminating response of the mated female with respect to the virgin females in the visual tests may reflect a higher visual responsiveness in mated females than in virgin females, possibly because they have to select oviposition sites and this choice is final as the larvae cannot abandon the substrate on which the eggs are laid (Hausmann et al., 2004). This behaviour differs from that of other beetles in which the females, especially the mated ones, prefer the dark when they have to deposit their eggs (Belmain, 1999; Gămălie & Mustață, 2006; Duhel et al., 2011).

Up to now, nothing, was known on the differences in *S. paniceum* males and females behaviour, in particular between mated and virgin females.

Laboratory trials indicate that the light traps efficiency could be improved using specific wavelengths that will increase insect capture. Also the use of LEDs, as those utilized for these experiments and also recommended in the Standard for Control of Damage to Museum Objects by Optical Radiation (CIE 157:2004), will improve trap efficiency (Duhel et al., 2011) and reduce power consumption.

Another important result obtained in this research is the validation of the protocol utilized that will be useful to test other species behaviour towards light.

The results achieved from the survey and the behavioural tests, highlighted the need of a new monitoring system that will guarantee objective data, speed of response, and low costs.

The main technological aspects required for its design are:

- a vision subsystem, represented by high tech optical devices capable to detect the insects in a large variety of configurations as, for example, the new technique of linear CMOS sensors (very fast, with high resolution and low cost), sensitive from the visible range to NIR range;
- a recording apparatus;
- a new device (that will support the electronic apparatus for detecting and recording) characterised by a capture system that will not damage the insect so that, if necessary, a specialist check / validation could be provided;
- a database of the most common insects present in cultural heritage commodities in all their possible shapes in order to ensure their automatic identification;
- a software that will manage the acquired data in order to compare them with risk thresholds and alarm in case these are exceeded.

Certainly, in the case of museums that have not yet adopted integrated pest management, the transition to such a complex planning will not be easy. In fact, the decision-making process in IPM requires a high level of organization, constant updating of the operators, continuous and appropriate collection and exchange of information. Initially, it may need support tools such as networks / monitoring protocols, consulting systems, decision support in real time, but, in the long run, it will ensure best results, money saving, and higher staff motivation. To achieve this goal it is necessary to

change the way of thinking; as De Guichen (1995) states "we must begin to think big, not considering the single object, but the collection, not the single environment, but the entire building as a whole".

The possibility to use a smart system, that includes a service operated by a control centre where the results of monitoring would be recorded and processed and technical assistance provided for the identification of dubious insect species by skilled entomologists and advice in case of exceeded risk threshold, surely could help in this process.

Such a project, designed for cultural heritage conservation facilities, could also have positive applications in food premises (industries, storages, markets, restaurants, canteens etc.) and other productive realities or services such as, for example, pharmaceutical companies, hospitals.

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