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Factors influencing the mobility of Red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) adults

DOCTORAL THESIS
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*Als meus pares, per
fer de mi qui sóc...*

SOBRE LA FORQUILLA (Sueca, 14 de Juliol de 1963)

També la forquilla forma part de la civilització, no cal dir-ho: és exactament, i per qualificar-la amb paraules tòpiques, una vertadera «conquesta de la civilització». I de les importants. No exagero. En general, quan de «civilització» es tracta, els especialistes tendeixen a referir-se a una altra mena de coses: al Partenó, a Sòcrates, a la física nuclear, a les novel·les de Proust, a l'aspirina, a la lògica matemàtica, a Albinoni, al Mantegna, al «telstar». Em sembla molt bé. No hi ha dubte que les supremes creacions de les arts, de la ciència i del pensament són altament representatives de l'esforç humà al llarg de la història. I tant com ho són! Però també s'ha de tenir en compte que en el nivell, més humil, de la vida quotidiana i de l'home comú, es produeixen «superacions» no gens espectaculars i que, per la seva índole i pel seu arrelament, constitueixen guanys ben significatius. Ningú no discutirà el valor de les aportacions d'un filòsof, d'un biòleg, d'un astrònom, d'un poeta, d'un pintor—d'un «geni» qualsevol. Ara: les seves seran sempre aportacions excepcionals. La societat en viu, naturalment, i la nostra gratitud no ha de faltar-los, als qui ens les proporcionen. Això no obstant, també el «progrés» s'acompleix en la modèstia de les accions diàries de la gent, i potser s'hi acompleix amb l'única i més autèntica eficàcia. Perquè en convertir-se en hàbit regular i multitudinari, és quan allò que anomenem «civilització» obté un primer aval raonable: la convalidació pragmàtica a escala decisiva. Tal és el cas de la forquilla.

Joan Fuster i Ortells

(Sueca, 1922-1992)

"Causar-se d'esperar"

Agraïments

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Resum

Rhynchophorus ferrugineus Oliv. (Coleoptera: Dryophthoridae) és una de les pitjors amenaces per a les palmeres en tot el món. Aquest insecte, natiu del sud est d'Àsia i Melanèsia, s'ha dispersat en els últims 25 anys a través d'Orient Mitjà i la conca del Mediterrani, detectant-se també la seua presència en el continent Americà i a Austràlia. *R. ferrugineus* pot instal·lar-se en un gran nombre d'espècies vegetals, la majoria d'elles pertanyents a la família Arecaceae. A les seues zones d'origen la plaga ataca principalment al Cocoter, *Cocos nucifera* L. No obstant, durant la seu dispersió a altres continents ha ampliat el seu rang d'hostes, mostrant gran preferència per espècies del gènere *Phoenix*, com *P. canariensis* Hort. ex Chabaud o *P. dactylifera* L. El maneig d'aquesta plaga es porta a terme mitjançant l'aplicació de diverses tècniques preventives i curatives: detecció precoç, tractaments químics o biològics, destrucció del material vegetal infestat, sanejament mecànic i trampeig amb atraients olfactius. Degut a la baixa eficàcia de les mesures de control citades, *R. ferrugineus* continua causant importants pèrdues econòmiques i paisatgístiques. Amb la finalitat de definir els aspectes que han intervingut en la seua ràpida dispersió i contribuir a la millora del seu maneig, en aquesta tesi doctoral s'ha analitzat l'efecte que tenen diversos factors, com la visió i el vol, en la mobilitat dels adults de *R. ferrugineus*.

El present treball ha estudiat la preferència cromàtica dels adults de *R. ferrugineus* mitjançant l'anàlisi de les seues captures en trampes poal acolorides. Les trampes negres són les que capturen el major nombre d'insectes en comparació amb la resta de colors estudiats. Aquests resultats han sigut similars tant en assajos realitzats amb trampes encebades amb atraients olfactius, com en els portats a terme amb trampes que no contenen aquest tipus de compostos. Açò demostra que el color per ell mateix és un factor important d'atracció. A més, s'ha analitzat l'espectre de longitud d'ona dels colors estudiats i de diversos teixits de *P. canariensis*. L'espectre de longitud d'ona del color negre mostra gran similitud amb el de les fibres de *P. canariensis*, és per açò pel que *R. ferrugineus* podria presentar preferència per aquest color. En relació a la proporció de sexes d'aquest insecte a les trampes, s'observa que a les que estan encebades amb atraients olfactius les captures de femelles són significativament més elevades. En canvi, en absència d'aquests compostos no sempre es produeix un major nombre de captures d'aquest sexe. Per altra banda, l'estudi de la proporció de sexes en poblacions naturals de l'insecte demostra que la ràtio és d'una femella per cada mascle. Per tant, les majors captures de femelles en trampes comercials

es deuen exclusivament a una major atracció d'aquestes cap als atraients olfactius utilitzats.

Altre dels aspectes estudiats ha sigut el potencial de vol dels adults de *R. ferrugineus* sota condicions de laboratori. Amb la finalitat de conèixer amb més detall la mobilitat d'aquest insecte s'han analitzar diversos paràmetres mitjançant la utilització d'un molinet de vol computeritzat. Els paràmetres estudiats i utilitzats per a definir el seu potencial de vol han sigut: nombre de vols, distància total volada, vol més llarg, duració del vol i velocitats mitjana i màxima dels vols. A més, s'ha analitzat com influeixen el sexe, el tamany del cos i l'edat dels adults en dits paràmetres de vol. El sexe de *R. ferrugineus* no mostra un efecte significatiu sobre els paràmetres de vol comparats. El tamany del cos de les femelles és significativament major, però tot i això, no influeix estadísticament en el seu potencial de vol. En adults amb edats compreses entre 8-23 dies s'observa un major percentatge de vol que en aquells amb 1-7 dies d'edat. En canvi, l'edat no influeix significativament sobre el seu potencial de vol. En analitzar el vol més llarg portat a terme per cadascun dels adults, obtenim que més del 63 % dels insectes es classifiquen com voladors de curta distància (menys de 500 m), el 27.3 % com voladors de mitja distància (entre 500 i 5000 m), i per damunt del 9 % com voladors de llarga distància (més de 5000 m).

Finalment, per a complementar els resultats obtinguts en l'estudi del potencial de vol en laboratori, s'ha dut a terme un assaig de camp en condicions naturals. En ell s'ha analitzat el comportament de vol i dispersió de *R. ferrugineus* mitjançant la tècnica de marcatge-solta-recaptura. Aquest assaig ha permès estudiar la influència de diferents factors biòtics i abiòtics, com són el sexe, la temperatura, la humitat relativa i la radiació solar, en l'enlairament i dispersió dels adults. La probabilitat d'enlairament dels adults de *R. ferrugineus* és significativament major en mascles. A més, aquesta probabilitat s'incrementa quan la temperatura i la radiació solar augmenten. Respecte a la dispersió de l'insecte mitjançant el vol, el nombre de recaptures es veu influenciat per la temperatura, augmentant significativament quan aquesta s'incrementa. De la mateixa manera, les distàncies de dispersió de *R. ferrugineus* també s'incrementen significativament a mesura que ho fa la temperatura. L'insecte tendeix a volar distàncies inferiors a 500 m (77.1 % dels adults recapturats), seguint la mateixa tendència observada en els estudis realitzats amb molinet de vol. No obstant, *R. ferrugineus* és capaç de recórrer fins a 7 km, arribant-se a registrar vols potencials en laboratori de fins a 20 km. Per últim, el temps de dispersió dels adults és molt curt (més del 90 % dels adults es recapturaren durant

els set primers dies), reduint-se significativament quan la humitat relativa s'incrementa.

Com a conclusió general, a partir de l'anàlisi de factors que intervenen en la mobilitat de *R. ferrugineus*, es demostra la influència que tenen sobre el vol, diversos factors biòtic i abiòtics, com són el sexe, la temperatura, la humitat relativa i la radiació solar. A més, tot i que els adults tendeixen a volar distàncies curtes, són capaços de volar varis kilòmetres, dispersant-se de manera eficaç. Per tant, es pot concloure que aquest insecte presenta un elevat potencial de dispersió, demostrat tant en experiments de laboratori com de camp. Per altra banda, l'anàlisi de la visió de l'insecte mitjançant l'estudi de preferència cromàtica, indica que els adults de *R. ferrugineus* són capaços de distingir diferents colors, preferint aquells que més es pareixen als colors dels seus hostes. Aquesta habilitat, junt amb l'elevat potencial de dispersió i la capacitat per a orientar-se i comunicar-se mitjançant compostos feromonals i kairomonals, incrementen la probabilitat d'èxit de l'insecte durant el procés de colonització. Els factors analitzats en la present tesi doctoral poden contribuir a la millora de les tècniques de maneig de *R. ferrugineus*, tot i que encara és necessari continuar investigant el comportament d'aquesta plaga per a minimitzar els seus efectes perjudicials.

Resumen

Rhynchophorus ferrugineus Oliv. (Coleoptera: Dryophthoridae) es una de las peores amenazas para las palmeras en todo el mundo. Este insecto, nativo del sudeste de Asia y Melanesia, se ha dispersado en los últimos 25 años a través de Oriente Medio y la cuenca del Mediterráneo, detectándose también su presencia en el continente Americano y en Australia. *R. ferrugineus* puede instalarse en un gran número de especies vegetales, la mayoría de ellas pertenecientes a la familia Arecaceae. En sus zonas de origen la plaga ataca principalmente al Cocotero, *Cocos nucifera* L. Sin embargo, durante su dispersión a otros continentes ha ampliado su rango de hospedantes, mostrando gran preferencia por especies del género *Phoenix*, como *P. canariensis* Hort. ex Chabaud o *P. dactylifera* L. El manejo de esta plaga se lleva a cabo mediante la aplicación de diversas técnicas de prevención y control: detección precoz, tratamientos químicos o biológicos, destrucción del material vegetal infestado, saneamiento mecánico y trampeo con atrayentes olfativos. Debido a la baja eficacia de las medidas de control citadas, *R. ferrugineus* continúa causando importantes pérdidas económicas y paisajísticas. Con la finalidad de definir los aspectos que han intervenido en su rápida dispersión y contribuir a la mejora de su manejo, en esta tesis doctoral se ha analizado el efecto que tienen diversos factores, como la visión y el vuelo, en la movilidad de los adultos de *R. ferrugineus*.

El presente trabajo ha estudiado la preferencia cromática de los adultos de *R. ferrugineus* mediante el análisis de sus capturas en trampas cubo coloreadas. Las trampas negras son las que capturan el mayor número de insectos en comparación con el resto de colores estudiados. Estos resultados han sido similares tanto en ensayos realizados con trampas cebadas con atrayentes olfativos, como en los llevados a cabo con trampas que no contienen este tipo de compuestos. Esto demuestra que el color por sí solo es un factor importante de atracción. Además, se ha analizado el espectro de longitud de onda de los colores estudiados y de diversos tejidos de *P. canariensis*. El espectro de longitud de onda del color negro muestra gran similitud con el de las fibras de *P. canariensis*, es por esto por lo que *R. ferrugineus* podría presentar preferencia por este color. En relación a la proporción de sexos de este insecto en las trampas, se observa que en las que están cebadas con atrayentes olfativos las capturas de hembras son significativamente más elevadas. En cambio, en ausencia de estos compuestos no siempre se produce un mayor número de capturas de este sexo. Por otra parte, el estudio de la proporción de sexos en poblaciones naturales del insecto demuestra que el ratio es de una hembra por cada macho. Por tanto, las mayores capturas de

hembras en trampas comerciales se deben exclusivamente a una mayor atracción de éstas hacia los atrayentes olfativos utilizados.

Otro de los aspectos estudiados ha sido el potencial de vuelo de los adultos de *R. ferrugineus* bajo condiciones de laboratorio. Con el fin de conocer con más detalle la movilidad de este insecto se han analizado diversos parámetros mediante la utilización de un molinillo de vuelo computerizado. Los parámetros estudiados y utilizados para definir su potencial de vuelo han sido: número de vuelos, distancia total volada, vuelo más largo, duración del vuelo y velocidades media y máxima de los vuelos. Además, se ha analizado cómo influyen el sexo, el tamaño del cuerpo y la edad de los adultos en dichos parámetros de vuelo. El sexo de *R. ferrugineus* no muestra un efecto significativo sobre los parámetros de vuelo comparados. El tamaño del cuerpo de las hembras es significativamente mayor, pero a pesar de ello, no influye significativamente en su potencial de vuelo. En adultos con edades comprendidas entre 8-23 días se observa un mayor porcentaje de vuelo que en aquellos con 1-7 días de edad. Sin embargo, la edad no influye estadísticamente sobre su potencial de vuelo. Al analizar el vuelo más largo llevado a cabo por cada uno de los adultos, obtenemos que más del 63 % de los insectos se clasifican como voladores de corta distancia (menos de 500 m), el 27.3 % como voladores de media distancia (entre 500 y 5000 m), y por encima del 9 % como voladores de larga distancia (más de 5000 m).

Finalmente, para complementar los resultados obtenidos en el estudio del potencial de vuelo en laboratorio, se ha llevado a cabo un ensayo de campo en condiciones naturales. En él se ha analizado el comportamiento de vuelo y dispersión de *R. ferrugineus* mediante la técnica de marcaje-suelta-recaptura. Este ensayo ha permitido estudiar la influencia de diversos factores bióticos y abióticos, como son el sexo, la temperatura, la humedad relativa y la radiación solar, en el despegue y dispersión de los adultos. La probabilidad de despegue de los adultos de *R. ferrugineus* es significativamente mayor en machos. Además, esta probabilidad se incrementa cuando la temperatura y la radiación solar aumentan. Respecto a la dispersión del insecto mediante el vuelo, el número de recapturas se ve influenciado por la temperatura, aumentando significativamente cuando ésta se incrementa. De la misma manera, las distancias de dispersión de *R. ferrugineus* también se incrementan significativamente a medida que lo hace la temperatura. El insecto tiende a volar distancias inferiores a 500 m (77.1 % de los adultos recapturados), siguiendo la misma tendencia observada en los estudios realizados con molinillo de vuelo. No obstante, *R. ferrugineus* es capaz de recorrer hasta 7 km, llegándose a registrar vuelos potenciales en laboratorio de hasta 20 km. Por último, el tiempo de dispersión de los adultos es muy corto (más

del 90 % de los adultos se recapturaron durante los siete primeros días), reduciéndose significativamente cuando la humedad relativa se incrementa.

Como conclusión general, a partir del análisis de factores que intervienen en la movilidad de *R. ferrugineus*, se demuestra la influencia que tienen sobre el vuelo diversos factores bióticos y abióticos, como son el sexo, la temperatura, la humedad relativa y la radiación solar. Además, a pesar de que los adultos tienden a volar distancias cortas, son capaces de volar varios kilómetros, dispersándose de manera eficaz. Por tanto, se puede concluir que este insecto presenta un elevado potencial de dispersión, demostrado tanto en experimentos de laboratorio como de campo. Por otro lado, el análisis de la visión del insecto, mediante el estudio de preferencia cromática, indica que los adultos de *R. ferrugineus* son capaces de distinguir diferentes colores, prefiriendo aquellos que se asemejan más a los colores de sus hospedantes. Esta habilidad, junto con el elevado potencial de dispersión y la capacidad para orientarse y comunicarse mediante compuestos feromonales y kairomonales, incrementan la probabilidad de éxito del insecto durante el proceso de colonización. Los factores analizados en la presente tesis doctoral pueden contribuir a la mejora de las técnicas de manejo de *R. ferrugineus*, aunque todavía es necesario continuar investigando el comportamiento de esta plaga para minimizar sus efectos perjudiciales.

Abstract

Rhynchophorus ferrugineus Oliv. (Coleoptera: Dryophthoridae) is still the worst threat for palm trees worldwide. This weevil, native to Southeast Asia and Melanesia, has spread throughout the Middle East and the Mediterranean basin over the last 25 years, being detected as well in America and Australia. *R. ferrugineus* can affect a large number of species, most belonging to the Arecaceae family. In their native areas the pest mainly affects the Coconut palm, *Cocos nucifera* L. However, in its spread to other continents, it has increased its host range, showing a strong preference for species of the *Phoenix* genera, such as *P. canariensis* Hort. ex Chabaud and *P. dactylifera* L. The management of this pest consists in several preventive and curative techniques: early detection, application of chemical or biological insecticides, destruction of infested plant material, mechanical sanitation, and a trapping system based on olfactory attractants. Because of the low efficacy of the aforementioned control measures, *R. ferrugineus* still causes major economic and landscape losses. In order to define aspects that have facilitated its rapid dispersal and contribute to improving its management, in the present thesis the effects of key aspects, such as vision and flight, which influence the mobility of *R. ferrugineus,* have been analysed.

In the present research, the chromatic preference of *R. ferrugineus* adults has been studied by analysing their captures in coloured bucket traps. Black traps capture the highest number of insects compared with the other colours studied. The results were similar in tests carried out using traps baited with olfactory attractants, and in those performed with traps without this kind of compounds. This demonstrates that colour by itself is a crucial attraction factor. Moreover, the spectral reflectance of studied colours and of some *P. canariensis* tissues has been analysed. The wavelength spectrum of black shows great similarity to that of fibres of *P. canariensis*, so this may be the reason why *R. ferrugineus* prefers this colour. Regarding sex ratio of the insect in traps, female captures are significantly greater in those baited with olfactory attractants. However, a higher number of female captures does not always occur when these compounds are not used. On the other hand, the study of sex ratio in natural populations of the insect demonstrates that the proportion is one female per male. Therefore, the greater number of captures of females in commercial traps is due exclusively to their greater attraction towards the olfactory attractants used.

Another of the analysed aspects has been the flight potential of *R. ferrugineus* adults under laboratory conditions. In order to know more about the mobility of this insect, different parameters have been studied using a computer-

monitored flight mill. The selected parameters used to define the *R. ferrugineus* flight potential have been the number of flights, total distance flown, longest single flight, flight duration, and average and maximum speed. Moreover, the influence of sex, body size, and age of the adults on the aforementioned parameters has been examined. *R. ferrugineus* sex does not have a significant effect on the compared flight parameters. The body size in females is significantly greater, but this does not influence their flight potential. A higher percentage of flight is observed for adults with an age range of 8-23 days old, compared to the 1-7 day old adults. However, age does not significantly influence their flight potential. By analysing the longest single flight undertaken by each adult, up to 63 % of the insects can be classified as short-distance flyers (under 500 m), 27.3 % as medium-distance flyers (between 500 and 5000 m), and up to 9 % as long-distance flyers (above 5000 m).

Finally, in order to complement the data obtained in the flight potential study under laboratory conditions, a test has been performed in field under natural conditions. The flight behaviour and dispersal of *R. ferrugineus* has been analysed using the mark-release-recapture method. This test focused on detecting the influence of different biotic and abiotic factors, such as sex, temperature, relative humidity, and solar radiation, in the take-off and dispersal of the adult insects. Take-off probability of *R. ferrugineus* adults is significantly greater in males. Moreover, this probability increases when temperatures and solar radiation rise. Concerning the insect dispersal by flying, the number of recaptures is influenced by temperature, increasing significantly when this factor increases. Likewise, dispersal distances of *R. ferrugineus* also increase significantly as temperatures rise. The insect tends to fly distances below 500 m (77.1 % of recaptured adults), following the same tendency observed in studies performed using the flight mill. However, *R. ferrugineus* is able to travel up to 7 km, being recorded under laboratory conditions a potential flight up to 20 km. To conclude, dispersal time of the adults is very short (more than 90 % of the adults were recaptured during the first seven days), being significantly lower when relative humidity increases.

As a general conclusion of the analysis of factors influencing the mobility of *R. ferrugineus* adults, flight is influenced by different biotic and abiotic factors, such as sex, temperature, relative humidity, and solar radiation. Moreover, even though adults tend to fly short distances, they are able to travel several kilometres, which allows them to carry out an efficient dispersal. For this reason we can conclude that this weevil presents a high dispersal potential, demonstrated both in laboratory and field studies. On the other hand, the analysis of the insect vision,

through the study of chromatic preference, indicates that *R. ferrugineus* adults are able to distinguish different colours, preferring colours very similar to those of their host plants. This ability, together with the high dispersal potential, and the orientation and communication capacity through pheromonal and kairomonal compounds, increase the likelihood of success of the insect during the colonization process. The factors analysed in the present doctoral thesis may contribute to the improvement of management techniques of *R. ferrugineus*, but it is still necessary to continue research into the behaviour of this pest to minimize their damaging effects.

Introduction

1.1. The Arecaceae family

1.1.1. General characteristics

Palm trees are monocotyledonous plants that belong to the Arecaceae family, formerly known as Palmae, being the only family of the Arecales order. The Arecaceae family is composed of some 2300 to 2500 species (variation due to the constant revisions in this botanical family), and classified in around 180 genera (Del Cañizo 2011).

The plants of the Arecaceae family are mainly distributed throughout tropical areas of the planet, occupying many different biotopes, such as mangroves, tropical rainforests, mountaintops, or deserts. However, there are palm species adapted to temperate zones, such as the Mediterranean dwarf palm, *Chamaerops humilis* L., or the Cretan date palm, *Phoenix theophrasti* Greuter, both endemic to the Mediterranean basin (Moya *et al.* 2005). The Windmill palm, *Trachycarpus fortunei* (Hook.) H. Wendl., originally from China and Myanmar, is another palm tree species with high tolerance to cold temperatures (Del Cañizo 2011).

1.1.2. Uses and economic importance

Since ancient times, palm trees have been used by humans worldwide. Among other applications, palm trees have been used as a feed source, construction material, in the production of biofuels, as a writing material, for manufacturing household utensils, in religious celebrations, or even for their chemical properties to be applied as medications (Plumed & Costa 2014).

Palm species with the greatest economic value worldwide are the Coconut palm, *Cocos nucifera* L., the Oil palm, *Elaeis guineensis* Jacq., and the Date palm, *Phoenix dactylifera* L., of which approximately 17.2, 12.1, and 1.1 million hectares, are grown respectively (Del Cañizo 2011; FAOSTAT 2014).

1.1.3. Major diseases and pests

There are many harmful agents for palm trees, both diseases and pests, and their occurrence varies depending on the species and the area.

Among the main pathological agents of palm trees are the phytoplasms responsible for Lethal yellowing (LY) or Texas phoenix palm decline (TPPD). Other most harmful diseases are produced by fungi, such as *Fusarium oxysporum* f. sp. *albedinis* (Kill & Maire) Malençon and *F. oxysporum* f. sp. *canariensis* Mercier & Louvet, *Phytophthora palmivora* Butler which causes the Bud rot, and *Ceratocystis paradoxa* (Dade) C. Moreau that produces Stem bleeding (Harrison

& Elliott 2005; Moya *et al.* 2005; Harrison & Elliott 2007). Regarding nematodes, one of the most harmful is the Coconut nematode, *Bursaphelenchus cocophilus* (Cobb.) Baujard which causes Red ring disease (Kranz *et al.* 1982).

Palm trees are also affected by a large number of pests that attack different parts of the plant. The most common species of defoliator insects belong to the orders Lepidoptera, Coleoptera, and Orthoptera (Howard *et al.* 2001). Sucking insects also create serious problems, because they weaken the plant, and some are vectors of diseases, such as *Myndus crudus* Van Duzee (Hemiptera: Cixiidae), vector of the phytoplasm that causes LY (Harrison & Elliott 2005). The fruits of the Coconut palm can be infested by the mite *Aceria guerreronis* Keifer (Acari: Eriophyidae), and the fruits of *P. dactylifera* by *Batrachedra amydraula* Meyrick (Lepidoptera: Batrachedridae), among others. Finally, there is a large number of stem borers, such as *Paysandisia archon* Burmeister (Lepidoptera: Castniidae), or different species of *Rhynchophorus,* like *R. palmarum* L., *R. phoenicis* Fab. (Coleoptera: Curculionidae), or *R. ferrugineus* Oliv. (Coleoptera: Dryophthoridae), which cause serious problems in many hosts worldwide (Barranco *et al.* 1995; Howard *et al.* 2001).

1.2. The Red palm weevil, *Rhynchophorus ferrugineus*

1.2.1. Systematic classification

The Red palm weevil, *Rhynchophorus ferrugineus* Oliv. (1790), is classified as follows (Alonso-Zarazaga & Lyal 2004):

Kingdom: Animalia
    Subkingdom: Eumetazoa
        Phylum: Arthropoda
            Subphylum: Hexapoda
                Class: Insecta
                    Order: Coleoptera
                        Suborder: Polyphaga
                            Infraorder: Cucujiformia
                                Superfamily: Curculionoidea
                                  Family: Dryophthoridae
                                    Subfamily: Rhynchophorinae
                                      Tribe: Rhynchophorini
                                        Genus: *Rhynchophorus*
                                          Species: *ferrugineus* (Olivier, 1790)

The confusion that has historically accompanied its systematic classification lead Wattanapongsiri (1966) to revise the genus *Rhynchophorus* and conclude that most of the species described previously belonged to the *R. ferrugineus* species, such as *Curculio ferrugineus* Herbst (1795), *Calandra ferruginea* Fab. (1801), or *Cordyle sexmaculatus* Thunberg (1797), among others (Rugman-Jones *et al.* 2013).

Common names for *R. ferrugineus* are "Red palm weevil" or "Asian palm weevil" in English, "Picudo rojo de las palmeras" in Spanish, "Morrut roig de les palmeres" or "Becut vermell de les palmeres" in Catalan/Valencian, "Charançon rouge des palmiers" in French, or "Punteruolo rosso delle palme" in Italian.

1.2.2. Origin, distribution, and economic importance

The Red palm weevil is originally from Southeast Asia and Melanesia countries, such as India, Sri Lanka, Philippines, Indonesia, or Papua New Guinea, among others (Wattanapongsiri 1966; Faleiro 2006a).

The massive transport and trade of palm trees infested by *R. ferrugineus* to countries with no presence of the pest, together with their considerable ability to adapt to a broad range of climatic conditions, have caused their global spread (Fig. 1.1) and therefore considerable economic losses (Abraham *et al.* 1998; Murphy & Briscoe 1999). In the mid-1980s the pest was detected in the Middle East and in 1992 the weevil reached North Africa, some of the main date producer areas worldwide (Cox 1993; El-Sabea *et al.* 2009). From 1992 to 2000, over 261,000 infested palm trees were detected in Egypt, of which approximately the 23 % were removed (El-Sebay 2007). In the Persian Gulf region, El-Sabea *et al.* (2009) estimated a cost of over US$31 million to eradicate over 300,000 palm trees with an infestation level between 1 and 5 %. In Europe, Spain was the first country where the pest was detected, in the region of Granada in 1993 (Barranco *et al.* 1995), being reported 50,000 infested palm trees, mainly *P. canariensis*, from 1996 to 2009 (Giblin-Davis *et al.* 2013). During the first decade of the 21st century, the pest has spread rapidly throughout the Mediterranean basin, including countries on the Black Sea coast and in Portugal (Table 1.1), and also in Australia (EPPO/OEPP 2008; Chebbi 2011; EPPO/OEPP 2013; Masten Milek & Šimala 2013; Karpun *et al.* 2014). *R. ferrugineus* was first detected in North Asia, concretely in China and Japan, in 1999 and 2000, respectively (Li *et al.* 2000; Yoshitake *et al.* 2001). In 2009 this insect was detected on the American continent, on the Curaçao Island, located in the southern Caribbean Sea (EPPO/OEPP 2009).
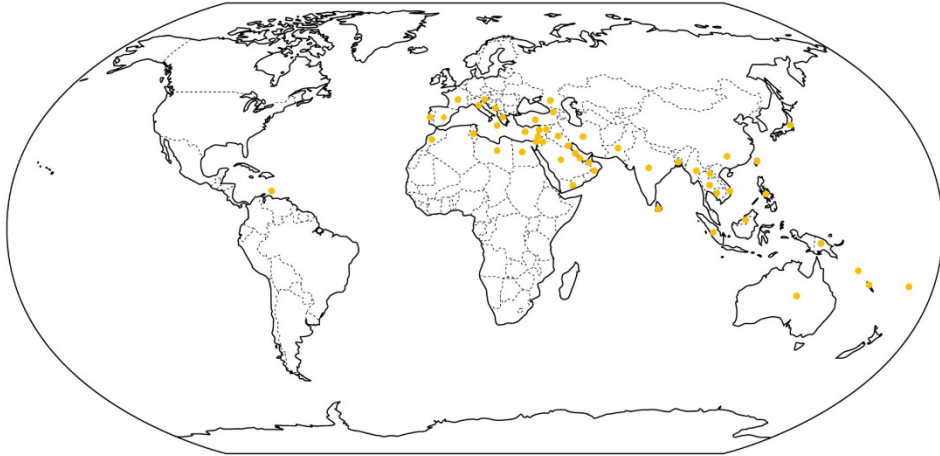
Fig. 1.1. Countries with presence of *Rhynchophorus ferrugineus*

Table 1.1. List of countries in the Mediterranean basin (including Black Sea coast countries* and Portugal) with presence of *Rhynchophorus ferrugineus* and year of detection

Country	Year of detection
Egypt	1992
Spain	1993
Israel	1999
Palestinian Auth.	1999
Syria	2001
Italy	2004
Turkey	2005
Greece	2006
France	2006
Cyprus	2006
Malta	2007
Portugal	2007
Morocco	2008
Albania	2009
Georgia*	2009
Libya	2009
Slovenia	2009
Lebanon	2010
Croatia	2011
Tunisia	2011
Russia*	2014

1.2.3. Host plants

As *R. ferrugineus* has spread worldwide, it has been able to adapt to new hosts, mostly belonging to the Arecaceae family, thus attacks a wide range of plant species (Table 1.2) (EPPO/OEPP 2013). The Canary Island date palm, *Phoenix canariensis* Hort. ex Chabaud, the Date palm, *P. dactylifera*, and the Coconut palm, *C. nucifera*, are three of its main hosts (Wattanapongsiri 1966; Murphy & Briscoe 1999). Less frequent infestations occur in other palm tree species (EPPO/OEPP 2013). Moreover, the pest has also been detected in hosts of other families, such as *Agave americana* L. (Agavaceae) and *Saccharum officinarum* L. (Poaceae), although there are no reports of its complete cycle development under field conditions (EPPO/OEPP 2008; Bertone *et al.* 2010).

Table 1.2. Hosts of *Rhynchophorus ferrugineus*

Family	Species
Agavaceae	*Agave americana* L.
Arecaceae	*Areca catechu* L.
Arecaceae	*Arenga pinnata* (Wurmb) Merr.
Arecaceae	*Borassus flabellifer* L.
Arecaceae	*Butia capitata* (Mart.) Becc.
Arecaceae	*Calamus merrillii* Becc.
Arecaceae	*Caryota cumingii* Lord. ex Mart.
Arecaceae	*Caryota maxima* Blume ex Mart.
Arecaceae	*Cocos nucifera* L.
Arecaceae	*Corypha utan* Lam.
Arecaceae	*Elaeis guineensis* Jacq.
Arecaceae	*Howea forsteriana* (F. Muell.) Becc.
Arecaceae	*Livistona decora* (W. Bull) Dowe
Arecaceae	*Metroxylon sagu* Rottb.
Arecaceae	*Phoenix canariensis* Hort. ex Chabaud
Arecaceae	*Phoenix dactylifera* L.
Arecaceae	*Phoenix sylvestris* L.
Arecaceae	*Roystonea regia* (Kunth) O. F. Cook
Arecaceae	*Sabal palmetto* (Walt.) Lodd.
Poaceae	*Saccharum officinarum* L.
Arecaceae	*Trachycarpus fortunei* (Hook.) H. Wendl
Arecaceae	*Washingtonia filifera* (Lindl.) H. Wendl
Arecaceae	*Washingtonia robusta* H. Wendl

1.2.4. Life cycle, morphology and bio-ecology

Like the other coleopteran species, *R. ferrugineus* presents a holometabolous development, going through the egg, larva, pupa, and adult instars. The morphological and biological characteristics of each one of the aforementioned developmental instars are specified below. Table 1.3 lists the different biological parameters, established by diverse authors for each one of the developmental instars of *R. ferrugineus*.

Table 1.3. Development time (days) of the different instars, number of larval instars, longevity (days), and fecundity (Number of eggs/♀) of *Rhynchophorus ferrugineus* adults, defined in the literature. Adapted from Dembilio & Jacas (2010)

Reference	Development time (d)			No. larval instars	Adult longev. (d)		Fecundity (No. eggs/♀)
	Egg	Larva	Pupa		Male	Female	
Ghosh (1912)	3-4	25-61	18-33	-	50-90	50-90	127-276
Leefmans (1920)	3	60-105	13-15	-	107	107	531
Dammerman (1929)	3	60-120	14	-	100	100	-
Lepesme (1947)	3	60	15	-	-	-	30-300
Viado & Bigornia (1949)	3	25-48	11-17	9	63-109	39-72	162-350
Nirula (1956)	3	55	15	-	60-90	60-90	204
Rahalkar *et al.* (1972)	3	36-72	16-22	-	142-176	95-109	136-158
Butani (1975)	2-5	24-61	18-34	-	60-90	60-90	76-355
Kranz *et al.* (1982)	2-3	60	14-21	-	-	90	200
Rahalkar *et al.* (1985)	2-4	38-75	-	-	-	-	150-400
Avand-Faghig (1996)	1-6	41-78	15-27	11	-	20-120	3-186
Esteban-Durán *et al.* (1998)	-	76-102	19-45	-	16-96	16-96	109-208
Jaya *et al.* (2000)	-	-	-	7	-	-	-
Salama *et al.* (2002)	-	-	13-26	-	-	-	-
Martín-Molina & Cabello (2004)	3-4	88-93	25-30	7-17	121-163	70-137	71-348
Kaakeh (2005)	3-4	71-102	16-23	-	49-96	45-96	68-185
Abe *et al.* (2009)	-	-	-	12	-	-	-
Prabhu & Patil (2009)	2-4	32-65	10-21	8	62-78	59-75	211-380
Salama *et al.* (2009)	-	69-128	16-29	5	27-47	33-48	99-375
Shahina *et al.* (2009)	4-5	50-80	20-30	9	55-75	55-80	125-200
Dembilio & Jacas (2010)	-	-	-	13	-	-	-
Ju *et al.* (2010)	3-4	30-67	23-36	9	-	38-192	92-323
El-Shafie *et al.* (2013)	3-5	33-46	20-36	8	82-94	73-85	174-221

Egg

Ranging in size from 0.98 to 2.96 mm, the egg of *R. ferrugineus* has a cylindrical shape with rounded ends and a slightly narrowed anterior end. The whitish-yellowish chorion is reticulated and bright (Fig. 1.2A) (Lepesme 1947; Wattanapongsiri 1966). According to different studies listed in Table 1.3, the middle range of eggs per female is approximately 110-285. The egg takes between 1 and 6 days to hatch (Table 1.3), and has a lower lethal threshold temperature of 10 °C, according to Martín-Molina & Cabello (2005) while Dembilio *et al.* (2012) reported a treshold of 13.95 °C. El-Ezaby (1997) established an upper lethal threshold temperature of 40 °C, and Dembilio & Jacas (2010) reported a thermal constant of 40.4 ± 2 degree-days.
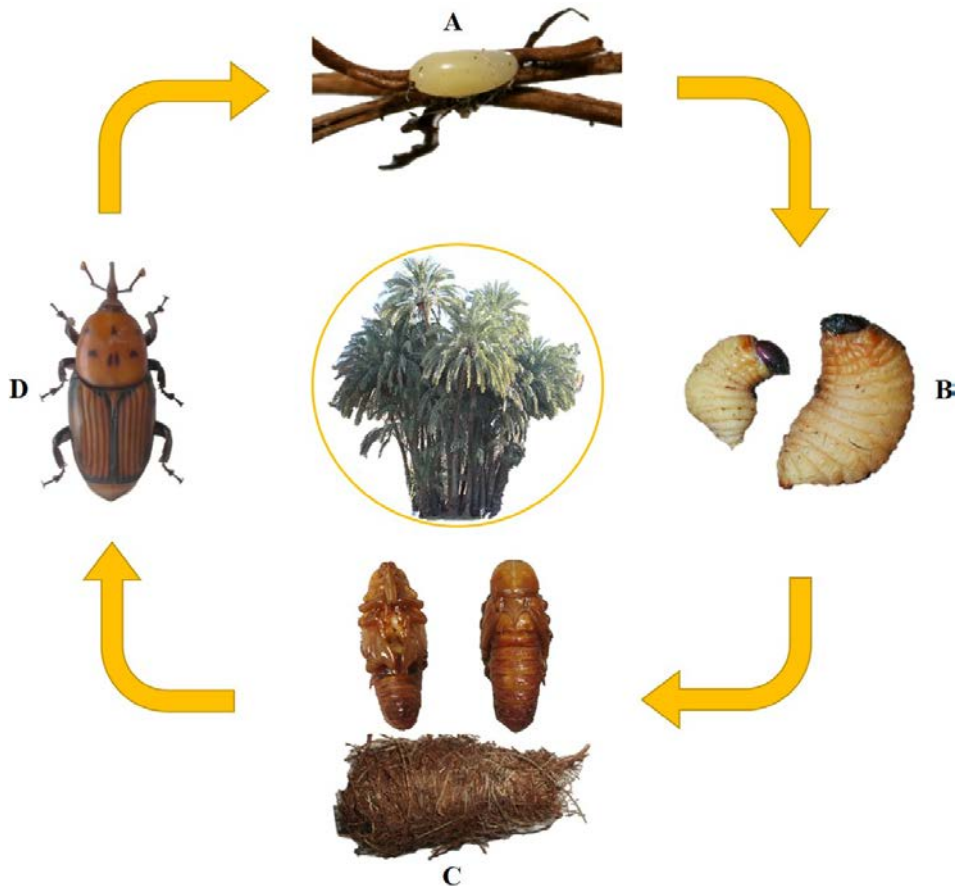
Fig. 1.2. Life cycle of *Rhynchophorus ferrugineus*. **A**: Egg on palm fibres; **B**: Different larvae instars; **C**: Pupal case and ventral and dorsal view of the pupa; **D**: Adult

Larva

The size of a fully-grown *R. ferrugineus* larva can range from 36-47 mm in length and 15-19 mm in width, showing whitish-cream or ivory colouring. The cephalic capsule is body differentiated and has a size between 8-9 mm length and 7-8 mm width, with reddish-brown or blackish-brown colouring. Legless, the larva of this insect has powerful mandibles strongly chitinized, with which it feeds on the palm tissues (Fig. 1.2B) (Lepesme 1947; Wattanapongsiri 1966; EPPO/OEPP 2007; EPPO/OEPP 2008). Depending on the author, from 5 to 17 larval instars have been described, and its developmental time varies between 24 and 128 days (Table 1.3). When the eggs have hatched, the larvae drill tunnels towards the internal part of the palm, feeding on the soft tissues of the plant (Viado & Bigornia 1949). Dembilio & Jacas (2010) pointed out a lower lethal threshold temperature of 10.3 °C for neonate larvae, and 4.5 °C for older instars. Martín-Molina & Cabello (2005) obtained lower and upper lethal threshold temperatures of 5 and 40 °C, respectively, and established the developmental threshold between 15 and 38 °C. For a complete larval development in *P. canariensis*, Dembilio & Jacas (2010) obtained a thermal constant of 666.5 degree-days. On the other hand, in an artificial diet under laboratory conditions, Martín-Molina & Cabello (2006) reported 1106 degree-days.

Pupa

The size of the *R. ferrugineus* pupa ranges 27-40 mm in length and 13-16 mm in width, showing a creamy white colour that darkens as development proceeds to adulthood (Fig. 1.2C). When the *R. ferrugineus* larva reaches its maximum development, it begins to intertwine palm fibres to construct a pupal case of around 50-95 mm in length and 25-40 mm in width (Fig. 1.2C), in which it will be protected to carry out metamorphosis (Wattanapongsiri 1966; EPPO/OEPP 2008). Pupation takes place approximately two days after the cocoon has been completed (Nirula 1956), and according to several authors, the pupal period varies between 11 and 45 days (Table 1.3). Martín-Molina & Cabello (2006) reported a developmental threshold temperature between 13 and 40 °C, while Salama *et al.* (2002) obtained values between -2.3 and 44-45 °C. According to Salama *et al.* (2002), Martín-Molina & Cabello (2005), and Dembilio & Jacas (2010), the thermal constant is about 423, 328, and 282.5 degree-days, respectively.

Adult

The size of the *R. ferrugineus* males is slightly less than females, 19-42 and 26-40 mm in length, respectively. The body colour can vary from

ferruginous-red to black, and it may be glossy or matte. Moreover, in the pronotum of both males and females there are a variable number of black spots (Wattanapongsiri 1966) (Fig. 1.2D). Another remarkable characteristic of this developmental stage is the elongation of the facial region, called the rostrum. Broad at base, the male rostrum has setae in the dorsal part, while in females it is longer and thinner, and with no setae (Wattanapongsiri 1966). The rostrum, the shape of the final abdomen segment, more sharply-pointed in females than in males, as well as the medial margins of protibiae, which features long setae in males and sparse and small in females, are morphologic characteristics used to distinguish sex (Fig. 1.3) (Wattanapongsiri 1966; Longo 2007). The longevity for males varies between 16 and 176 days, while in females it fluctuates between 16 and 192 days (Table 1.3). When females are ready to oviposit, generally one day after mating, they select a suitable place to lay the egg, then drill a hole to deposit it and finally seal the hole with a pinkish coloured plug to protect it (Nirula 1956). Dammerman (1929) reported that Corbett observed a maximum oviposition of 832 eggs/female. To feed, adults choose suitable tissues, generally on the soft portions of the palm, making a hole with the rostrum to feed on the juice of the plant (Nirula 1956).
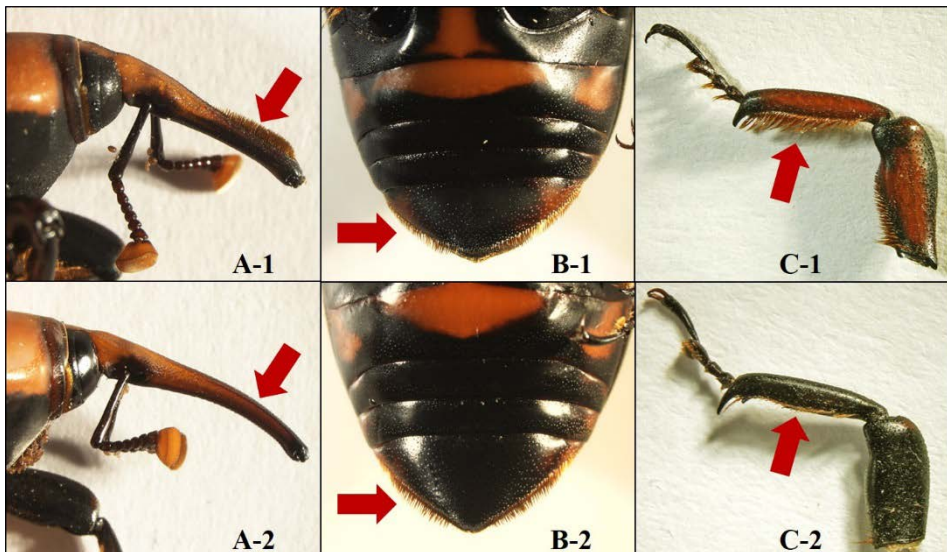
Fig. 1.3. Differences between *Rhynchophorus ferrugineus* males and females (Red arrows indicate location of the described differences). **A**: Detail of rostrum in males (A-1) and females (A-2). **B**: Final abdomen segment of males (B-1) and females (B-2). **C**: Detail of medial margins of protibiae in males (C-1) and females (C-2)

1.2.5. Number of generations and rate of multiplication

R. ferrugineus is a multivoltine species, but the number of generations per year reported in the literature varies depending on the conditions and feeding substrate with which each study is performed. Under laboratory conditions, Nirula (1956) and Rahalkar *et al.* (1972) reported 3 to 4 generations per year rearing the insect in Coconut leaf petioles and Sugarcane, respectively. Avand-Faghig (1996) and Abe *et al.* (2009) found the same number of generations in the Saravan Region (Iran) and in southern Japan, respectively. Salama *et al.* (2002), obtain an uncommonly high number of generations using banana fruits to rear the insect, predicting around 21 generations per year in Egypt. Finally, Dembilio & Jacas (2010) indicated that in *P. canariensis* with mean annual temperatures below 15 °C, this weevil can complete one generation per year, and more than two when this climatic factor reaches 19 °C. Besides, inside a palm tree infested by *R. ferrugineus* several overlapping generations comprised of different developmental instars can be found (Faleiro 2006a).

According to Nirula (1956), the rate of multiplication of this insect is high, and in the absence of controlling factors, a single female is able to produce more than five million weevils in four generations, within 14.4 months. However, other studies show that the multiplication rate estimated in the aforementioned work is uncommonly high (Rahalkar *et al.* 1972; Avand-Faghig 1996; Esteban-Durán *et al.* 1998; Cabello 2006). The high multiplication rate is a consequence of the continuous egg-laying throughout the year, although there are differences in the oviposition intensity depending on the period (Faleiro 2006a). Even when air temperatures are low, inside an infested palm tree, temperatures can reach values between 30 and 40 °C, allowing for the non-stop development and propagation of the insect (Abe *et al.* 2010). The heating of the internal tissues of an infested palm tree is a consequence of the fermentation of microbes, including yeasts (Abe *et al.* 2010).

1.2.6. Damage and symptomatology

Damage is directly caused by larvae and its feeding on the soft tissues of the palm tree, which destroys the vascular system of the plant (Ghosh 1912). Generally, the attack of *R. ferrugineus* occurs in the tree crown or the upper part of the stem, including the base of petioles, but in certain species that produce offshoots, such as *P. dactylifera*, the attack frequently occurs at the stem base, where the offshoots emerge (Faleiro 2006b). The pest affects both dying and undamaged palm trees (Blumberg 2008). The symptomatology of a palm tree

infested by *R. ferrugineus* varies depending on the palm tree species, infestation level, and attack area. It is frequent to observe withered and yellowish palms (Fig. 1.4A), and holes and galleries in the stem and in the base of petioles. Sawdust or gummy exudate with a characteristic odor may also be found (Fig. 1.4B), and even the sound of larvae feeding can be heard (Abraham *et al.* 1966; Kranz *et al.* 1982; Abraham *et al.* 1998; Esteban-Durán *et al.* 1998). Furthermore, high infestation levels can cause the death of the bud and the collapse of the palm tree (Justin *et al.* 2008).

Fig. 1.4. Some examples of the symptomatology caused by *Rhynchophorus ferrugineus* in *Phoenix canariensis*. **A**: Collapse of lateral palms. **B**: Hole with sawdust exudation in the palm base. Red arrows indicate location of the described symptoms

1.2.7. Management

The biology and ethology of *R. ferrugineus* make its management extremely difficult, being necessary the establishment of an integrated control strategy that brings together a battery of measures, both preventive and curative, thus allowing for a reduction in their effect on palm trees.

Early detection

The nature of the attacks of *R. ferrugineus* and the cryptic habits of the insect make early detection of the infestation very difficult (Murphy & Briscoe 1999). There are different methodologies to detect this pest quickly and accurately*,* such as periodic and intensive visual inspections, which require the knowledge of the symptomatology of an infested palm tree (Blumberg 2008). It is also possible to detect signs of infestation by analysing different physiological

parameters such as diffusive resistance or transpiration rate, that vary as a consequence of a *R. ferrugineus* attack (Bokhari & Abuzuhira 1992). Additionally, during the last decade several methods have been developed to detect the presence of the insect inside a palm tree, such as bio-acoustic detection (Pinhas *et al.* 2008; Potamitis *et al.* 2009) or x-rays (Ma *et al.* 2011). Finally, sniffing dogs are also being trained to detect the pest through its powerful sense of smell (Nakash *et al.* 2000; Suma *et al.* 2014).

Cultural methods

These practices can be used to prevent or reduce the *R. ferrugineus* attack. A quick removal of the infested plant material is very important (Kurian & Mathen 1971). It is also recommended pruning dry or senescent fronds, to avoid the emission of odours that may attract *R. ferrugineus* adults. Moreover, the pruning should be performed during cold months, because the number of adults in flight is lower, and this must be followed by an insecticidal treatment (Gómez & Ferry 2007). Azam & Razvi (2001) recommended the removal of the *P. dactylifera* offshoots and the subsequent application of a phytosanitary treatment. On the other hand, Sallam *et al.* (2012) pointed out that a close planting density and open flood irrigation system favours the attack of *R. ferrugineus*.

Chemical and biological control

Chemical control against *R. ferrugineus* is carried out by the preventive or curative application of insecticides (Murphy & Briscoe 1999). Numerous studies, both under laboratory and field conditions, have been performed to check the efficacy of different active ingredients against this pest (Abraham *et al.* 1975; Cabello *et al.* 1997; Barranco & Peña 1998; El-Sebay 2004; Dembilio *et al.* 2014). Chemical insecticides should be applied by pulverization with systemic or contact compounds, either to the palm crown or the basal area, where offshoots appear, and also through stem injection or soil treatments with systemic insecticides (Faleiro 2006b).

Generally, biological control of *R. ferrugineus* is carried out using entomopathogenic nematodes of different species, mainly *Steinernema carpocapsae* Weiser (Abbas *et al.* 2001). Furthermore, the use of some species of entomopathogenic fungi, such as *Beauveria bassiana* Bals. and *Metarhizium anisopliae* Metsch. is being developed (Gindin *et al.* 2006). Other organisms are indicated as biological control agents, such as different species of insects belonging to Dermaptera, Heteroptera, Coleoptera and Hymenoptera, mites, viruses, and even some birds and mammals, but their presence can be considered

anecdotal and their efficiency very low (Murphy & Briscoe 1999; Mazza *et al.* 2014).

Olfactory trapping

In the management of this insect olfactory trapping with pheromones and kairomones can be effective both to monitor and to reduce *R. ferrugineus* populations (Abraham *et al.* 1998; Vidyasagar *et al.* 2000; Soroker *et al.* 2005; Abbas *et al.* 2006). Buckets placed above-ground have been reported as the most effective traps, because they facilitate the entry of the weevil adults (Oehlschlager 2006a), and the addition of a funnel inside the trap prevents their escaping (Faleiro 2006a). Moreover, Ávalos & Soto (2013) pointed out that bucket traps captured a significantly higher number of *R. ferrugineus* adults when compared with pyramidal traps. Trap densities vary for monitoring and mass trapping, between 1 trap/ha to 1 trap/100 ha, and between 1 and 10 traps/ha, respectively (Giblin-Davis *et al.* 2013). To bait traps an aggregation pheromone produced by *R. ferrugineus* males is used (Hallett *et al.* 1993a; Hallett *et al.* 1993b). The addition of ethyl acetate and different fresh plant material improved trap effectiveness, being possible the addition of a contact insecticide to avoid the escape of the adults (Hallett *et al.* 1993b; El-Sebay 2003; Oehlschlager 2006a).

Mechanical sanitation

This technique consists in the mechanical elimination of infested parts of the plant, removing the maximum possible number of insects attacking the palm tree. If meristematic tissues have not yet been damaged by the insect, the palm tree can produce new fronds and recover (Giblin-Davis *et al.* 2013). This technique is based on the extraction of palm syrup from the *P. canariensis*, called "guarapo", typical of the Canary Islands (Spain) (La Mantia *et al.* 2008).

Other measures and techniques

The application of quarantine regulations is vital to prevent the spread of *R. ferrugineus* (Blumberg 2008). Because of the uncontrollable expansion of the pest, and to ensure that production and movement of plants follow the regulations established by the European Commission and by the member states, specific requirements for the internal movements within the EU have been established. The EU established a plant passport and an import prohibition of palm trees from non-EU countries, but this measure has now been modified to allow importation under specific requirements (Giblin-Davis *et al.* 2013). Finally, the areas where the pest is present were defined as "demarcated areas", covering the outbreak area

and a buffer zone of 10 km around it, in order to apply specific eradication measures (European Commission 2011).

To prevent and control *R. ferrugineus* damage, the male sterile technique could be used. This consists in the irradiation of adult males with gamma rays to induce sterility, and then the weevils could be released to reduce pest populations (Faleiro 2006b; El-Mergawy & Al-Ajlan 2011).

Finally, could be possible to use host plant resistance to reduce the effects of this pest. Barranco *et al.* (2000) reported resistance to this weevil in different palm species, and Farazmand (2003) found differences in susceptibility between some Date palm cultivars and a native wild palm, *Nannorrhops ritchiana* (Griff.) Aitch. Dembilio *et al.* (2009) reported resistance against *R. ferrugineus* in species *W. filifera* and *Chamaerops humilis* L., based on antibiosis and antixenosis, respectively. Therefore, in the future, selection of palm tree species or varieties with resistance to *R. ferrugineus*, both for use as ornamental plants or to obtain some product, could be used.

1.3. Ecology and behaviour of *Rhynchophorus ferrugineus*

1.3.1. Potential worldwide distribution

Depending on the environmental conditions in areas where pests are found, and using different modelling techniques and specialized software, such as bioclimatic models, species distribution models, or ecological niche modelling approaches, it is possible to predict the potential distribution of invasive species (Taylor & Kumar 2012). Ju *et al.* (2008), using the aforementioned techniques, predicted the distribution of *R. ferrugineus* in China, reporting that southern, eastern, and south-western China are the critical quarantine and control areas. On the other hand, Fiaboe *et al.* (2012) and Ge *et al.* (2015) analysed the potential worldwide distribution of this insect, successfully predicting the known distribution areas of the pest and adding regions where the insect has not yet been reported in sub-Saharan Africa, Asia, Europe, Oceania, and South, Central and North America (Fig. 1.5).
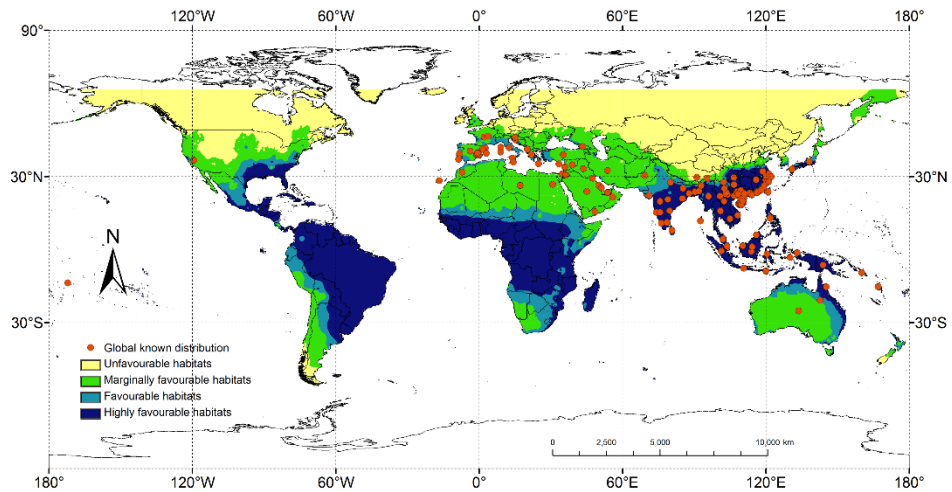
Fig. 1.5. Predicted worldwide distribution suitability of *Rhynchophorus ferrugineus* under current climatic conditions (Ge *et al.* 2015).

1.3.2. Distribution of developmental instars inside infested palm trees

Depending on the biological requirements of each developmental instar of *R. ferrugineus*, the distribution within the host varies. Wahizatul & Nurul'Izzah (2012) pointed out that in Coconut palm there is a strong association between the infested part of the plant and the abundance of the different developmental instars of *R. ferrugineus*. Oviposition occurs on soft tissues near the palm crown in holes made by females or using plant wounds, so that when eggs hatch the young larva can feed successfully (Viado & Bigornia 1949; Nirula 1956). Generally, females lay their eggs individually and close to the surface, being difficult to detect this developmental instar because of its small size (Murphy & Briscoe 1999; Wahizatul & Nurul'Izzah 2012). Regarding the larvae, Wahizatul & Nurul'Izzah (2012) also reported that its number is higher on the offshoots and cabbages, because these are the softest parts of the plant. On the other hand, Kalshoven (1981) highlighted that mature *R. ferrugineus* larvae, when ready to become pupae, are found principally in the peripheral part of the stem and in the base of the petioles of *P. canariensis*, with the aim of favour the adult exit and dispersal. Likewise, the number of cocoons is also greater on petioles and stems, where the fibres to construct the pupal case are more readily available. Wahizatul & Nurul'Izzah (2012) suggested that this variation in distribution is due to the differences in resources available (white intact tissue or fibres) among the different parts of the palm tree.

1.3.3. Seasonal and diurnal activity

The *R. ferrugineus* seasonal activity fluctuates throughout the year depending on the climatic conditions of the study area, and these seasonal variations can be detected by analysing the adult captures in pheromone traps. In areas with temperate climate, such as the Valencia Region (Spain) and Sicily (Italy), there have been reports of a low peak in *R. ferrugineus* activity during the spring, and a higher one during autumn that decreases during the winter months (Conti *et al.* 2008; Ávalos *et al.* 2011). In areas with dry climate, such as the Kingdom of Saudi Arabia, Abraham *et al.* (1999) and Vidyasagar *et al.* (2000) detected two weevil activity peaks, during April-May and September-November. Also El-Sebay (2003) detected two activity peaks, during April and November in Egypt. Likewise, in the United Arab Emirates, Abbas *et al.* (2006) pointed out that the number of *R. ferrugineus* captured in traps increases gradually from January, reaching a peak between March and May. El-Garhy (1996) and Soroker *et al.* (2005) reported that in Israel and Egypt, respectively, the highest number of weevils are captured from April to June, decreasing the number of captures as the winter months approach. In southern Japan, a subtropical climate area with humid summers and mild and cool winters, two activity peaks during summer and fall have been detected (Aman *et al.* 2000; Abe *et al.* 2009). Finally, in Goa (western India), with tropical climatic conditions, the insect activity is lower between June and July (monsoon season) and higher during October and November, and the climatic conditions have a significant influence, with a positive and negative correlation with temperature and rainfall, respectively (Faleiro 2006a). Likewise, Ajlan & Abdulsalam (2000) reported a negative significant correlation between relative humidity and the number of weevils captured per trap, but experiments were performed in areas with low infestation levels of the pest.

Like the seasonal activity, different authors have analysed the captures of *R. ferrugineus* adults in food-baited pheromone traps throughout the day, reporting fluctuations in the diurnal activity of weevil adults. Leefmans (1920) first reported that weevil adults are more active during day, then Lepesme (1947) claimed that they have purely diurnal habits. On the other hand, Faleiro & Satarkar (2003) indicated that in Goa the insect is more active from midnight to 6 am, and registered less activity from 6 pm to midnight. Finally, Gunawardena & Bandarage (1995) reported that in Sri Lanka *R. ferrugineus* adults only respond to pheromone-baited traps from 6 to 8 pm, and from 6 to 8 am. Faleiro (2006a) suggested that these differences in the diurnal activity of the weevil are due to the influence of climatic factors.

1.3.4. Locomotion, dispersal, and spatial distribution

The *R. ferrugineus* adults can move about walking as well as flying, being strong flyers able to cover long distances, and their displacement may be facilitated by winds (Nirula 1956). Leefmans (1920) reported that this insect is capable of flying at least 900 m, detecting at this distance fresh tissues of Sago palm, *Metroxylon sagu* Rottb. Abbas *et al.* (2006), using a mark-release-recapture (MRR) study, affirmed that *R. ferrugineus* adults are able to migrate at least 7 km from the Date palm plantations where they were released, most being recaptured within 3-5 days after release.

Several authors have also analysed the spatial distribution of *R. ferrugineus* considering different dispersion parameters and indices. Nirula (1956) indicated that this insect is gregarious in habits. Faleiro *et al.* (2002) concluded that in Coconut palm plantations, this weevil follows an aggregated or contagious spatial distribution pattern, during periods of both low and high insect activity. In Date palm plantations in the Kingdom of Saudi Arabia, there have been reports of contagious distribution patterns of the pest (Anonymous 1998), and an aggregated distribution of its populations that produces a continuous *R. ferrugineus* attack on plantations near gardens infested by the weevil.

1.3.5. Chemical ecology and olfactory behaviour

Semiochemicals, and concretely pheromones play vital role in the behaviour and communication of coleopterans belonging to Curculionidae and Dryophthoridae families (Giblin-Davis *et al.* 1996). Several *Rhynchophorus* species such as *R. palmarum, R. cruentatus, R. phoenicis,* or *R. ferrugineus* produce male aggregation pheromones (Rochat *et al.* 1991; Gries *et al.* 1993; Hallett *et al.* 1993a; Weissling *et al.* 1994b), so as to increase the density of conspecifics near the pheromone source, attracting individuals of both sexes (Wyatt 2003). Moreover, these insect species are able to detect odour cues emitted by their host plants, with specific olfactory receptors, which allow weevils to find them (Jaffé *et al.* 1993; Weissling *et al.* 1994b; Abdallah & Al-Khatri 2005). These olfactory compounds have been used in the development and improvement of management techniques based on olfactory trapping against *Rhynchophorus* species (Rochat *et al.* 1991; Hallett *et al.* 1993b; Giblin-Davis *et al.* 1996). Regarding *R. ferrugineus*, males produce an aggregation pheromone composed of "ferrugineone" (4-Methyl-5-nonanone) and "ferrugineol" (4-Methyl-5-nonanol) (Hallett *et al.* 1993a). As occurs in other insect species, food additives such as date fruits, and several compounds such as ethyl acetate, ethyl

propionate, ethyl butyrate or ethanol, used as pheromone synergists, increase the *R. ferrugineus* attraction (Gunawardena & Bandarage 1995; Abdallah & Al-Khatri 2005; Oehlschlager 2006a). Moreover, using pheromone/food-baited traps, both males and females are attracted, but the sex ratio of captures is approximately two females per male (Faleiro 2005; Al-Saoud 2011).

1.3.6. Visual attraction

Insect behaviour and mobility, and thus their response to the surrounding environment, are determined to a great extent by visual stimuli such as shape or colour. Insects are able to distinguish shapes through motion, perceiving the spatial correlation between structural elements of an object as a sequence of light flashes, which in turn are transformed into a sequence of stimuli. Likewise, some are influenced in a different way by different radiations. Namely, in terms of human colour vision, they are able to distinguish colours (Mazokhin-Porshnyakov 1969). Colour vision and wavelength selective behaviour are involved in visually guided behaviours of insects such as orientation, host selection, or discrimination within concrete plant structures of a large number of insect pests, allowing them to perform vital roles such as feeding, mating or ovipositioning (Prokopy & Owens 1983; Menzel & Backhaus 1991; Reeves 2011). There are a large number of insects whose visual attraction has been demonstrated, such as the Cabbage seedpod weevil, *Ceutorhynchus obstrictus* Marsh. (Coleoptera: Curculionidae) (Tansey *et al.* 2010), or the Emerald ash borer, *Agrilus planipennis* Fairm. (Coleoptera: Buprestidae) (Crook *et al.* 2009), among others. In addition, visual stimuli combined with olfactory cues often have a synergic effect that increases insect attraction (Björklund *et al.* 2005). The analysis of the visual preferences of an insect can help us in the study of their dispersal and behaviour, allowing for an improvement in their management. Regarding *R. ferrugineus*, no studies have analysed its vision physiology. On the other hand several studies have analysed their response to visual stimuli, by comparing their recaptures in pheromone/food-based traps of different colours and shapes (Hallett *et al.* 1999; Abdallah & Al-Khatri 2005; Faleiro 2005; Kalleshwaraswamy *et al.* 2006; Martínez *et al.* 2008; Al-Saoud *et al.* 2010; Al-Saoud 2010; Tapia *et al.* 2010; Abuagla & Al-Deeb 2012; Al-Saoud 2013; Ávalos & Soto 2013; Vacas *et al.* 2013; Abdel-Azim *et al.* 2014). In the aforementioned studies, visual attractants are generally analysed in combination with the action of olfactory attractants. Therefore, there is a lack of knowledge about the vision physiology of this weevil and the effect of visual stimuli in the capture efficacy of *R. ferrugineus* trapping systems.

Rationale and Objectives

Rationale and Objectives

Rhynchophorus ferrugineus is perhaps the most damaging pest of palm trees worldwide. The spread of this insect seems unstoppable, mainly due to its great adaptability to a wide range of climatic conditions and hosts. This weevil is already present across all five continents and is causing major economic and biodiversity losses in invaded areas.

To prevent and control the damage caused by the *R. ferrugineus* infestation, different control measures are used, such as the application of chemical and biological pesticides, intensive surveillance of palm trees for early detection, destruction of infested plant material, pheromone/food-based trapping systems, and mechanical sanitation. The low effectiveness of the aforementioned control techniques, along with the lack of knowledge about the weevil's mobility, make the containment of the pest extremely difficult.

Given this lack of knowledge about the mobility of *R. ferrugineus*, and in order to improve the current management strategies applied against this pest, while allowing for a better understanding of the possible reasons for its rapid spread, in the present doctoral thesis the following objectives were established:

i – To study the chromatic preference of *R. ferrugineus* adults, by analysing their captures in coloured bucket traps.

ii – To analyse the possible influence of the chromatic similarity between trap colours and different palm tree parts, in the chromatic preference of weevil adults.

iii – To compare the sex ratio of *R. ferrugineus* in natural populations and in the captures of coloured bucket traps, with and without olfactory attractants.

iv – To measure the flight performance of *R. ferrugineus* adults, using a computer-monitored flight mill, analysing different flight parameters, such as the number of flights, total distance flown, longest single flight, flight duration, and the average and maximum flight speed.

v – To examine the influence of sex, body size and age of *R. ferrugineus* adults in the aforementioned flight parameters.

vi – To characterise the flight behaviour and dispersal of *R. ferrugineus* adults, using a mark-release-recapture method, analysing their take-off and recaptures in pheromone/food-baited bucket traps.

vii – To determine the influence of the weevil sex, temperature, relative humidity and solar radiation, on the take-off, recapture, and dispersion distance and time of *R. ferrugineus* adults.

viii – To evaluate the maximum flight distance covered by *R. ferrugineus* adults in field, using a mark-release-recapture study.

Study of chromatic preference of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) adults using bucket traps

Study of chromatic preference of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) adults using bucket traps

Abstract
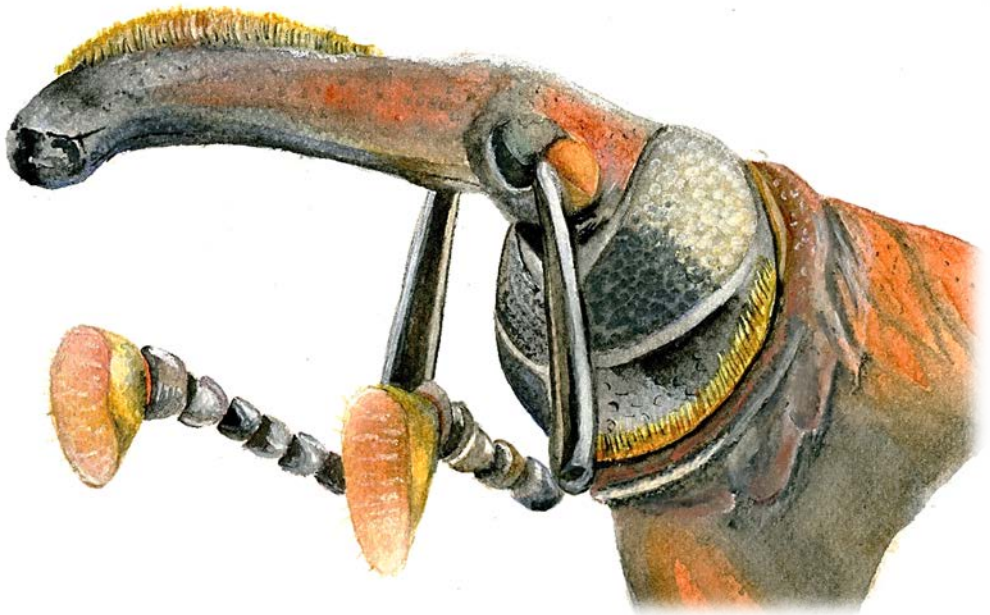
Rhynchophorus ferrugineus Oliv. (Coleoptera: Dryophthoridae) is at present considered the biggest threat to palm trees worldwide. Due to the current trend towards the use of environmentally-friendly control measures, increasing interest is being shown in trapping as a way of dealing with this pest. The present study assessed the influence of chromatic preference on the capture of *R. ferrugineus* adults and identified possible reasons for the better performance of pheromone traps. Two tests were carried out using coloured bucket traps; in the first the traps were baited with male aggregation pheromone and kairomone from *R. ferrugineus*, while the second involved traps without olfactory attractants. The spectral reflectance of the colours tested and of leaves and external stem fibres from the *Phoenix canariensis* Hort. ex Chabaud were measured by spectrophotometer. The internal climatic conditions of the coloured traps were also analysed. Black traps, both with and without olfactory attractants, captured significantly more *R. ferrugineus* adults than red and white traps. The higher efficacy of the black traps was mainly due to chromatic preference and not only to the possibly higher emission of olfactory attractants. The olfactory attractants used to bait traps are responsible for the female-biased captures, a phenomenon found to occur with all the colours tested. This study provides useful information for improving current management strategies against *R. ferrugineus*.

3.1. Introduction

The Red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae) is one of the most invasive and destructive palm tree (Arecaceae) pests worldwide (Faleiro 2006a). The larval stages feed inside the stem and frequently destroy the apical growth area, causing the death of the palm (Murphy & Briscoe 1999; EPPO/OEPP 2008). Infestations are usually detected after the palm tree has been seriously damaged (Blumberg 2008), and only well-trained technicians can detect early symptomatology, so that the numbers of plants lost and the consequent financial impact is considerable. In the Valencia Region in eastern Spain, around 20,000 palms were killed by *R. ferrugineus* between 2004 and 2009, at a cost of approximately €27 million (Dembilio & Jacas 2010). The pest has a broad range of hosts and is able to breed in a wide variety of climatic conditions (Murphy & Briscoe 1999), thus increasing its invasive ability and potential for damage.

Rhynchophorus ferrugineus is native to southern Asia and Melanesia, where it is a key pest of the Coconut, *Cocos nucifera* L. (Arecaceae), and Oil palm, *Elaeis guineensis* Jacq. (Arecaceae) (Wattanapongsiri 1966; Misra 1998). During the first decade of the 21st century, it spread all over the Mediterranean Basin, also being detected in Australia (EPPO/OEPP 2008; EPPO/OEPP 2013). The pest was recorded for the first time in some countries of Northeast Asia such as China in 1999, and Japan in 2000 (Li *et al.* 2000; Yoshitake *et al.* 2001). The massive shipments of infested palm trees to countries previously free of the pest contributed to its rapid spread (Abraham *et al.* 1998). It was first detected in the American continent in 2009, on the island of Curaçao, in the Caribbean (EPPO/OEPP 2009).

Different methods are currently being used to control *R. ferrugineus*. Preventive and curative applications of chemical pesticides (Faleiro 2006b). An extended alternative to the use of chemical insecticides involves the application of entomopathogenic nematodes, principally *Steinernema carpocapsae* Weis. (Nematoda: Steinernematidae) (Abbas *et al.* 2001; Llácer *et al.* 2009). Other natural enemies of this insect have been identified but have poor efficacy against *R. ferrugineus* (Mazza *et al.* 2014). Cultural methods are also used, such as the early detection and destruction of infested plant material (EPPO/OEPP 2008) and the removal of the damaged parts of the palm (known as mechanical sanitation) (La Mantia *et al.* 2008). Finally, a pheromone/food-based trapping system is also used for monitoring and mass trapping of the pest (Hallett *et al.* 1999; Abbas *et al.* 2006). In the European Union, pest management in urban green areas is dictated by the guidelines on pesticides issued in 2009, which restricts the use of

chemical plant protection products (European Parliament 2009). Therefore, mass trapping constitutes an environmentally-friendly method of integrated management against *R. ferrugineus*, based on the use of different semiochemicals designed to attract and capture the weevils (El-Sayed *et al.* 2006). Moreover, several studies carried out in different regions of Saudi Arabia show that pheromone traps, used as a part of an integrated pest management program, reduced infestation levels and captures rates of the pest (Abraham *et al.* 2000; Vidyasagar *et al.* 2000).

The colour of the trap is a key factor in attracting and trapping many insect species (Cross 1973; Prokopy & Owens 1983). For example, the New Guinea sugarcane weevil, *Rhabdoscelus obscurus* Boisduval (Coleoptera: Curculionidae) is attracted to russet-brown colours in field conditions (Reddy *et al.* 2011). The Apple blossom weevil, *Anthonomus pomorum* L. (Coleoptera: Curculionidae) is attracted to blue (Hausmann *et al.* 2004), while the Sweetpotato weevil, *Cylas formicarius* Fab. (Coleoptera: Brentidae) is attracted to light red (Reddy *et al.* 2012).

Many studies have examined the trap colour preferences of *R. ferrugineus*, but the results have been discordant (Hallett *et al.* 1999; Abdallah & Al-Khatri 2005; Faleiro 2005; Kalleshwaraswamy *et al.* 2006; Martínez *et al.* 2008; Al-Saoud 2010; Al-Saoud *et al.* 2010; Tapia *et al.* 2010), although others identified black as the insects' favourite colour (Abuagla & Al-Deeb 2012; Al-Saoud 2013; Vacas *et al.* 2013; Abdel-Azim *et al.* 2014). Although the aforementioned studies analysed weevil chromatic preference, there is a lack of knowledge about the effect of the colour as the main factor in their captures, and the possible reasons for this colour preference.

The aim of the present study was to analyse the importance of the chromatic factor in captures of *R. ferrugineus* adults. In order to confirm the effect of chromatic preference on the number of captures, tests were carried out on the attractiveness of traps baited with and without olfactory lures. The efficacy of coloured traps were also assessed in relation to internal trap climatic conditions (temperature and relative humidity), and the spectral reflectance of the leaves and external fibres of the stem of the Canary Island date palm, *Phoenix canariensis* Hort. ex Chabaud (Arecaceae). The sex ratio of the natural populations of this weevil was analysed and a check was made to determine whether trap colour had any influence on the female/male ratio of captured *R. ferrugineus* adults. The results obtained provide information that should be helpful in improving the control of *R. ferrugineus* by mass trapping systems.

3.2. Materials and methods

3.2.1. Trap characteristics

In both tests the traps, Rhynchonex model (Rhynchonex® trap, Econex Ltd, Murcia, Spain), consisted of: (1) a 15.6 litre plastic bucket (height = 31 cm; bottom Ø = 25 cm; upper Ø = 28 cm), with four 6 x 4 cm holes cut below the upper rim, at a distance of 21 cm from the base; (2) a funnel, placed inside the bucket to prevent the weevils from escaping; (3) a lid for the bucket, with four holes similar to those in the bucket, with a piece of steel wire to hang the chemical attractants on (pheromone and kairomone dispensers); and (4) a plastic mesh on the outside of the bucket to help the insects climb into the trap. All external surfaces were painted in a certain colour of commercial acrylic spray paints (Tkrom Spray®, Eupinca Inc., Murcia, Spain). The traps were placed on the ground, following Oehlschlager (2006a); in this position, the insects have a better landing surface, and consequently the traps capture more weevils.

3.2.2. Test with olfactory attractants

The traps baited with olfactory attractants were tested in eight 7-year-old palm tree groves highly infested by *R. ferrugineus*, near the town of Torrente in eastern Spain (39°25′37.6″N, 00°27′28.9″W; 17 m elevation) from end May to mid-July 2009. The traps were painted either red (Red fire gloss, RAL 3000), white (White gloss, RAL 9010) or black (Black gloss, RAL 9005). They contained: (1) a *R. ferrugineus* male aggregation pheromone dispenser (Ferrolure+) (Rhynchonex® Pheromone, Econex Ltd, Murcia, Spain) composed of 4-methyl-5-nonanol and 4-methyl-5-nonanone (9:1), containing 700 mg of 95 % pure active ingredients; (2) a kairomone dispenser (Rhynchonex® Kairomone, Econex Ltd, Murcia, Spain), containing 40 ml of 95 % pure ethyl acetate (El-Sebay 2003); (3) a piece of infested *P. canariensis* petiole (sized 8 x 5 cm); and (4) four litres of water containing propylene glycol to minimize its evaporation (5-10 ml/l) (Oehlschlager 2006b). The pheromone and kairomone dispensers had a usable life of 3 months (Econex 2013a; Econex 2013b). The experimental design consisted of a total number of 24 traps, distributed in eight replications with three treatments per replication (one of each colour under study). Within each replication, the distance between treatments was 1.2 m. All the treatments were similarly combined with the same olfactory compounds, so that if captures differed among treatments the colour could be identified as the contributing factor. The traps were inspected weekly during the experimental period, carrying out eight countings in which the number and sex of captured adult weevils were

registered. Trap position within a repetition was rotated clockwise after each count, to minimize the influence of trap position on the results.

A data logger (Delta® OHM, HD226-1, Padova, Italy) was placed inside the black and white traps, without affecting the insect entrance. Internal temperature and relative humidity (RH) were registered every 30 min over five consecutive days (8 to 12th July 2009), to compare the internal climatic conditions in the aforementioned traps, as these are the colours with the maximum and minimum light absorption.

3.2.3. Test without olfactory attractants

This test was carried out in semi-field conditions without olfactory attractants at the Universitat Politècnica de València (Spain), from January to March 2010. The goal was to determine whether the insect captures under field conditions in the previous trial were due to chromatic preference or to a factor related to the release of olfactory attractants. This trial was conducted inside a secure enclosure (2 x 6 x 2 m), with double entry door and constructed of iron wire mesh with a small hole size (0.5 x 0.5 cm), to prevent the adult weevils from escaping. The coloured traps were tested under semi-field conditions, according to the results obtained from the test with olfactory attractants. First we compared the black and red traps, which had the largest captures in the field assay. Next, the coloured trap with the highest captures (black) was compared with the coloured trap with the lowest number of captured adults (white) in the field test. Each pairwise comparison (black *vs.* red and black *vs.* white) consisted of six replications. The traps to be tested were arranged at a distance of 1.2 m from each other. After each replication, the position of each coloured trap was interchanged. The bucket traps were filled with water (to a height of 7 cm) to keep the insects inside the trap. The water was renewed at the end of each repetition. Mean (± SE) temperature and RH during the trial were 19.5 ± 0.07 °C and 48.4 ± 0.36 %, respectively. A total of 20 weevil adults per replication (10 males and 10 females), obtained from the field traps, were released simultaneously at a distance of 2 m from the traps. The traps were inspected between 48 and 72 hours after each release, and the number and sex of the captured adults were recorded.

3.2.4. Spectral reflectance

The spectral reflectance of the coloured traps (black, red, and white) and the leaves and fibres of the upper external part of the stem of *P. canariensis* was analysed by a Minolta CM-3500d spectrophotometer (Minolta, Tokyo, Japan). Sample reflectance was quantified using the CIE (International Commission on

Illumination) standard illuminant C (average daylight) with a field-of-view of 10°. A wavelength range from 400 to 700 nm was measured at 10 nm sampling intervals, covering most of the insect spectral sensitivity range (Menzel & Backhaus 1991).

3.2.5. Female/male ratio of natural populations

Rhynchophorus ferrugineus cocoons were collected from infested palm trees in different sites near the town of Torrente, during summer and autumn of 2008. Collected cocoons (the number depending on availability) were held in individual sterilized 100 ml plastic containers with perforated lids and maintained in a climatic chamber at 25 ± 2 °C and 65 ± 5 % RH. Adult emergence was checked once a day, determining the sex of the adult weevils.

3.2.6. Statistical analysis

In order to detect the possible effect of trap colour on the number of captures and on the female/male ratio of captured adults, we used a Generalized linear mixed model (GLMM), assuming a Poisson error distribution and a log link function for the former and a binomial error distribution and an logit link function for the latter. The field test evaluated trap colour, date, and plot. In the semi-field test the factors analysed were: trap colour, date, and trap position. Trap colour was considered as a fixed factor, and date, plot, and trap position as random. The analyses were performed using the function 'glmer' of the R package 'lme4' (Bates *et al.* 2014). The models were evaluated in terms of the Akaike's Information Criterion (AIC) (Akaike 1987), comparing the ΔAIC of the null model with respect to the aforementioned model. Models with ΔAIC ≤ 10 were considered equivalent. In order to test for differences among the different levels of the fixed factor, a Tukey's post-hoc test was used by applying the 'glht' function of the R package 'multcomp' (Hothorn *et al.* 2008). A paired *t*-test with a 95 % confidence level was used to compare the internal trap climatic conditions of the different coloured traps. As the internal trap conditions values were not normally distributed, the data were transformed by ln (x) before the analysis. To study any differences in the percentages of males and females emerged from the cocoons collected in the field, we used a *Chi-square* test with a 95 % confidence level. All these analyses were performed using the statistical software 'R' (R Core Team 2013).

3.3. Results

3.3.1. Test with olfactory attractants

A total of 935 *R. ferrugineus* adults (0.69 captures/trap/day) were captured during the field test; 589 in the black traps, 239 in the red, and 107 in the white. The trap colour influenced the level of adult weevil captures (ΔAIC = 390.1). The mean ($\pm$ SE) number of captures/trap/day was 1.31 $\pm$ 0.23 in black traps, significantly greater than those captured by red (0.53 $\pm$ 0.09) and white traps (0.24 $\pm$ 0.04) (Fig. 3.1.; Tukey's post-hoc test: black-red, $P < 0.0001$; black-white, $P < 0.0001$; red-white, $P < 0.0001$).
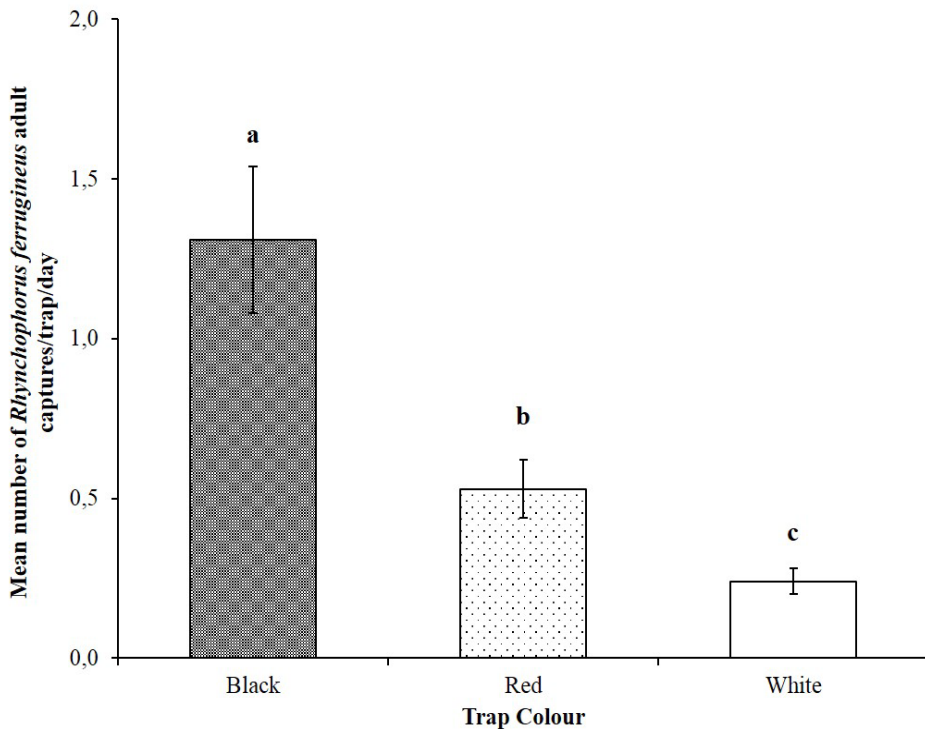
Fig. 3.1. Mean ($\pm$ SE) number of *Rhynchophorus ferrugineus* adults captured per trap and day in coloured traps, from end May to mid-July 2009, in eight palm tree groves in Torrente (eastern Spain). Different letters above the columns denote statistically significant differences at *P < 0.05* (Tukey's post-hoc test)

3.3.2. Internal trap climatic conditions

The mean (± SE) temperature in the black and white traps was 28.61 ± 0.38 °C and 28.19 ± 0.35 °C, respectively, being the mean (± SE) temperature outside traps 25.24 ± 0.54 °C. The maximum temperature recorded in the black and white traps was 45.1 °C and 43.7 °C, respectively. Statistical analysis showed significant differences of temperature between black and white coloured traps, being greater in the first one ($t = 11.9521$; df = 239; $P < 0.0001$). The average (± SE) internal temperature difference was 0.42 ± 0.04 °C, and was higher in the black trap. For 23.8 % of the recording period there was either no temperature difference between the black and white traps or the temperature was higher in the latter. During the 73.3 % of the analysed time, temperature differences were between 0 and 2 °C, and were greater than 2 °C only during 2.9 % of the test period, reaching a maximum of 2.9 °C. Mean (± SE) internal RH was 84.16 ± 1.4 % in black traps and 84.05 ± 1.33 % in the white ones. Maximum RH recorded was 100 % in both coloured traps. The average (± SE) internal RH difference was 0.11 ± 0.48 °C, and was higher in the white trap. Statistical analysis indicated that RH did not differ significantly between black and white traps ($t = 0.2351$; df = 239; $P = 0.8143$).

3.3.3. Test without olfactory attractants

In the first pairwise comparison, the black and red traps captured 54 and 16 of the 120 released adults, respectively. Mean (± SE) captures/trap/day was 4.50 ± 0.73 in the black traps and 1.33 ± 0.46 in the red ones. In the second pairwise comparison, the black and white traps captured 62 and 5 of the 120 released adults, respectively. Mean (± SE) captures/trap/day was 5.17 ± 0.70 in the black and 0.42 ± 0.15 in the white. As observed in the tests with no olfactory attractants, the colour of the trap influenced the number of captured adult weevils (black *vs.* red: ΔAIC = 19.8; black *vs.* white: ΔAIC = 55.3). Captures in black traps were significantly higher than in red (Fig. 3.2.a; Tukey's post-hoc test: $P < 0.0001$). The black traps also captured significantly more adult weevils than the white (Fig. 3.2.b; Tukey's post-hoc test: $P < 0.0001$).
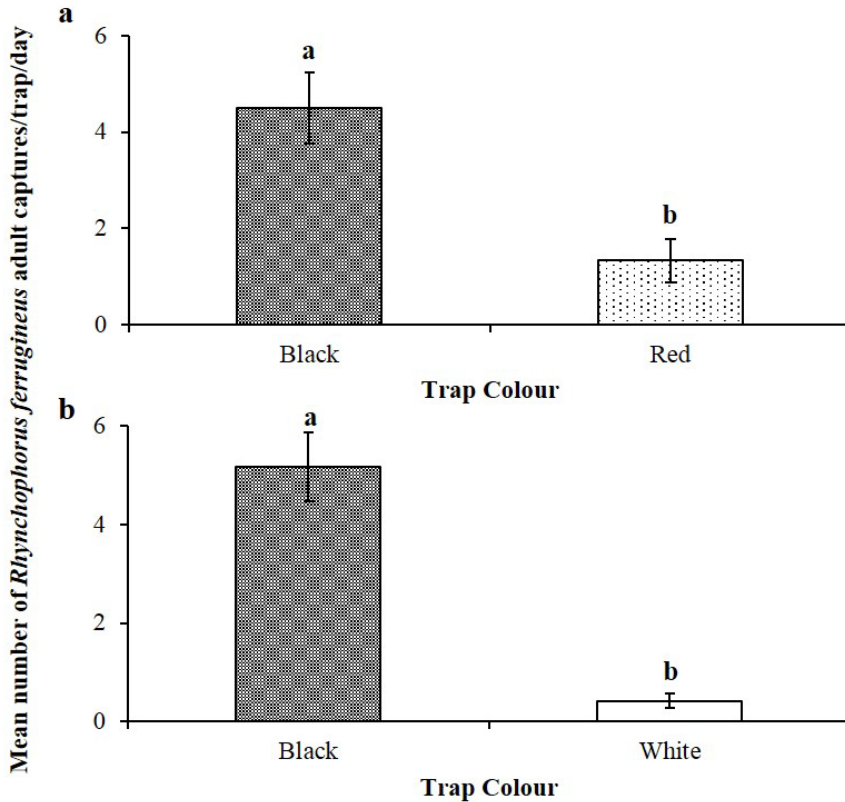
Fig. 3.2. Mean (± SE) number of *Rhynchophorus ferrugineus* adults captured per trap and day in pairwise comparisons of coloured traps, in tests without olfactory attractants: (a) black *vs.* red, and (b) black *vs.* white. Different letters above the columns denote statistically significant differences at *P < 0.05* (Tukey's post-hoc test)

3.3.4. Female/male ratio

In the test with traps baited with olfactory attractants, the mean (± SE) female/male ratio (number of ♀: number of ♂) of the captured adults was always female-biased, ranging from 1.70 ± 0.2 to 2.42 ± 0.4 ♀ per trapped ♂. Trap colour did not influence this ratio ($\Delta AIC = -3.7$).

In the tests with no olfactory attractants, the sex ratio of the captured adults was not always female-biased and, as under field conditions, was not influenced by trap colour (black *vs.* red: $\Delta AIC = -1.8$; black *vs.* white: $\Delta AIC = -1.1$).

With regard to the sex ratio of *R. ferrugineus* natural populations, the number of cocoons collected during summer and autumn were 165 and 225,

respectively. In summer, 69 ♀ and 75 ♂ emerged, with 21 non-emerged cocoons. In autumn the number of emerged ♀ and ♂ was 104 for both sexes, and 17 cocoons did not emerge. The percentage of emerged ♀ and ♂ from the collected pupal cases was 50.9 % and 49.1 %, respectively, with a ratio of 1.03:1 ♀ per ♂. There were no significant differences between the female/male insect ratio of natural populations for both seasons (*Chi-square* test: $\chi^2 = 0.24$; df = 3; $P = 0.9706$).

3.3.5. Spectral reflectance

The spectral reflectance values of the leaves and stem fibres of *P. canariensis*, as well as of the coloured traps tested, are shown in Figure 3.3. The values of the black traps were very similar to those of the palm stem fibres across all the wavelengths measured, reflecting approximately only 5 % of the light. Palm leaves and the remaining trap colours had more complex reflectance-wavelength curves. The palm leaves had a dominant peak from 540 to 580 nm and a second peak at 700 nm. Red traps had a peak from 660 to 700 nm. White had the highest reflectance, close to 90 %, for all wavelengths above 430 nm.
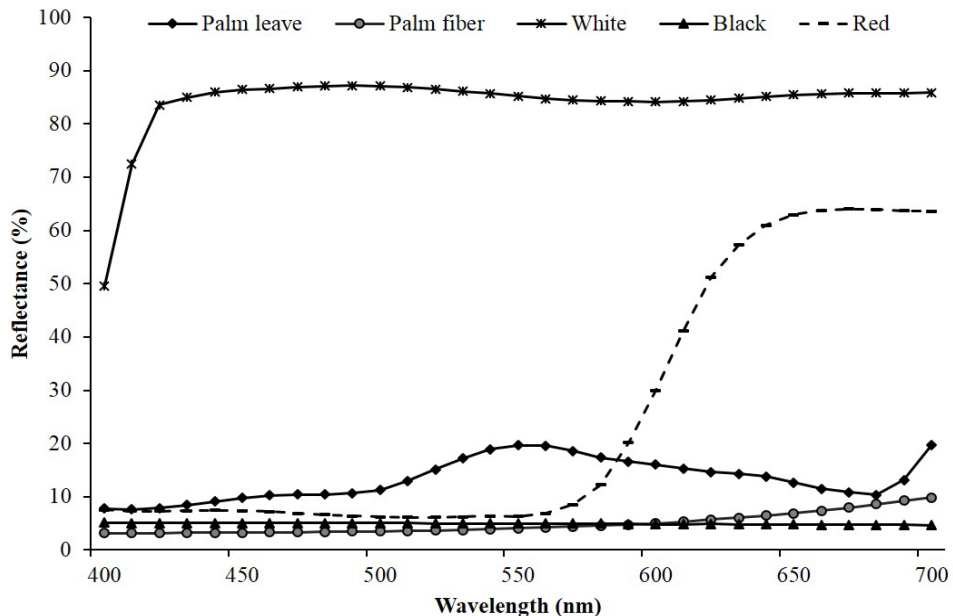
Fig. 3.3. Spectral reflectance of palm leaves, palm stem fibres, and coloured traps (black, red, and white). Reflectance values were obtained from a Minolta CM-3500d spectrophotometer

3.4. Discussion and conclusions

The tests both with and without olfactory attractants showed evidence of chromatic preference in *R. ferrugineus*. There were significant differences in the captures of the different coloured traps. This is particularly important for improving the efficacy of weevil trapping systems, especially bearing in mind the highly variable results as regards the level of trap efficacy obtained in previous studies. Sansano *et al.* (2008) reported that reddish brown traps were more attractive than white ones or traps camouflaged with palm stem fibres. On the other hand, Martínez *et al.* (2008) obtained more captures in yellow traps than in red or white ones. Kalleshwaraswamy *et al.* (2006) and Faleiro (2005) compared different trap colours but did not obtain significant differences in captures, the latter work most likely because did not include red and black, which attracted most *R. ferrugineus* adults in this study. Similarly, Tapia *et al.* (2010) did not obtain significant differences when they compared white and yellow/orange traps. Black traps were only tested in comparison with white traps by Hallett *et al.* (1999), Abuagla & Al-Deeb (2012), Al-Saoud (2013), and Abdel-Azim *et al.* (2014), confirming the significantly higher attraction of *R. ferrugineus* adults to black. Vacas *et al.* (2013) compared black pyramidal traps with white bucket traps, analysing a combination of colour and shape, but without explaining if the greatest captures in the first one were due to the colour or the shape of the trap.

The second most efficient trap colour in our study was red, which captured significantly fewer adult *R. ferrugineus* than the black traps, but significantly more than the white. Abdallah & Al-Khatri (2005), Al-Saoud *et al.* (2010), Al-Saoud (2010; 2013), Abuagla & Al-Deeb (2012), and Abdel-Azim *et al.* (2014) reported similar results for red traps. The aforementioned studies generally combined the chromatic factor with other aspects, such as trap shape, trap location, type of pheromone dispenser or type of substance used as kairomone. There is a lack of knowledge about the effect of trap colour as the only factor influencing the captures of this weevil. Moreover, none of the current papers examines the possible reasons of the colour preferences of *R. ferrugineus*.

Hallett *et al.* (1999) suggested that the higher temperatures in black traps might cause greater pheromone release, which in turn would result in higher insect captures. In the present study, we measured the internal climatic conditions of differently coloured traps in order to determine possible effects on the release of olfactory attractants. The internal temperature values in the black and white traps were significantly different. Nevertheless, it is unlikely that an average difference of 0.42 °C in internal temperature would cause a greater pheromone

release in the darker traps. According to the obtained results, we also consider that the time interval in which a temperature difference would cause a higher pheromone release in the darker traps is very short, between 2 and 3 °C during only 2.9 % of the recorded time. Furthermore, in the test without olfactory attractants, black traps captured the highest percentage of *R. ferrugineus* adults, exactly as had occurred in the field trials. These results suggest that the efficiency of the black traps was mainly determined by chromatic preference and not only by the possible higher emission of attractants. The observations in this study suggest that the attraction of *R. ferrugineus* adults to traps is the result of a two-step process: firstly, long-distance attraction to semiochemicals, and secondly, short-distance visual attraction to trap colour and semiochemicals. Both factors can have a synergic effect on the number of adults trapped. For example, Björklund *et al.* (2005) reported that traps with a combination of odour and visual stimuli captured higher numbers of Pine weevils, *Hylobius abietis* L. (Coleoptera: Curculionidae). Nevertheless, further studies are necessary to evaluate the exact effect of the internal trap climatic conditions on the emission of attractants and consequently on the number of *R. ferrugineus* captures at different temperatures and RH.

Entwistle (1963) and Timmons & Potter (1981) reported that some Coleoptera and Lepidoptera with wood-boring larvae are caught by red, brown and black traps, probably responding to dark shades and the contrast with the background rather than to any visual cue. Reddy *et al.* (2011; 2012) confirmed these results, capturing a significantly greater number of New Guinea sugarcane weevils, *R. obscurus*, and Sweetpotato weevils, *C. formicarius*, in russet-brown and light-red colours, respectively. On the other hand, Leskey (2006) and Machial *et al.* (2012), did not obtain significant differences in the captures of the Plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) and the Warren root collar weevil, *Hylobius warreni* Wood (Coleoptera: Curculionidae), for the colours tested. Entwistle (1963) speculated that the general attraction of scolytine and platypodid beetles to red-coloured traps was due to their resemblance to host bark. In the present study, the spectral reflectance of the tested colours and different palm tree components was measured. The spectral analyses revealed that black traps produced a spectral reflectance similar to the stem fibres of the *P. canariensis* palm tree. The similarity between the spectral reflectance of some palm tree components, through which the adults enter their host or lay their eggs, and the colour black could explain the stronger preference of *R. ferrugineus* by black traps. Moreover, the cryptic habits of the insect, which

generally tends to hide (Viado & Bigornia 1949) may also help to explain the chromatic affinity to this colour.

With regard to the sex ratio, the study of the natural populations of *R. ferrugineus* shows the existence of an equal number of males and females in the field. Hunsberger *et al.* (2000) pointed out the same ratio for other *Rhynchophorus* species, *R. cruentatus*. On the other hand, in the study using olfactory attractants, a mean of 2.4 females per trapped male was obtained. These results are similar to previous studies reporting a sex ratio of 2 or 2.04 females per captured male (Faleiro 2005; Al-Saoud 2011). On the other hand, in the test without olfactory attractants, the sex ratio of the captured adults was not always female-biased. Therefore, although there is a 1:1 sex ratio in the field, the captures of females were higher when traps baited with olfactory attractants were used, regardless of trap colour. The female-biased sex ratio of the trapped *R. ferrugineus* in our field study is apparently due to the fact that females are more strongly attracted than males to the semiochemicals used to bait traps, whatever their colour. The female-biased sex ratio in traps with attractants has important practical implications for the mass trapping of *R. ferrugineus*. Higher female captures mean their numbers decrease in natural population, and therefore the biological potential of the pest is reduced (Oehlschlager 2006a).

In conclusion, the present study shows the importance of the colour of the traps used to capture insects. Commercial traps have been traditionally white or red (Hallett *et al.* 1999; Guarino *et al.* 2011), but black traps substantially increase insect captures, which are mostly female-biased. The upper temperature in black traps is not the only cause of its higher captures. It is also considered that the visual attraction of *R. ferrugineus* traps could be improved by the use of a colour with a spectral reflectance as close as possible to the host structures infested by the insects. The results obtained in this work could improve the monitoring and mass trapping of *R. ferrugineus* populations.

3.5. Acknowledgements

Study of the flying ability of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) adults using a computer-monitored flight mill

Study of the flying ability of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) adults using a computer-monitored flight mill

Abstract

The Red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae), native to tropical Asian regions, has become a serious threat to palm trees all over the world. Knowledge of its flight potential is vital to improving the preventive and curative measures currently used to manage this pest. As *R. ferrugineus* is a quarantine pest, it is difficult to study its flight potential in the field. A computer-monitored flight mill was adapted to analyse the flying ability of *R. ferrugineus* through the study of different flight parameters (number of flights, total distance flown, longest single flight, flight duration, and average and maximum speed) and the influence of the weevil's sex, age, and body size on these flight parameters. Despite significant differences in the adult body size (body weight and length) of males and females, the sex of *R. ferrugineus* adults did not have an influence on their flight potential. Neither adult body size nor age was found to affect the weevil's flying abilities, although there was a significantly higher percentage of individuals flying that were 8-23 days old than 1-7 days old. Compared to the longest single flight, up to 63 % of the insects were classified as short-distance flyers (covering < 500 m) and 27.3 % and up to 9 % were classified as medium- (500-5000 m) and long-distance (> 5000 m), respectively. The results are compared with similar studies on different insect species under laboratory and field conditions.

4.1. Introduction

The Red palm weevil, *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae), is a key pest of palm trees (Arecaceae), native to tropical Asia (Wattanapongsiri 1966) and introduced worldwide (EPPO/OEPP 2008). It has a broad host range and is able to breed in a wide variety of climates (Murphy & Briscoe 1999). In the last 20 years the weevil has invaded the Middle East and the Mediterranean Basin and in 2009 reached the island of Curaçao, in the Caribbean (EPPO/OEPP 2009). The pest has killed thousands of palms in the newly invaded areas, especially *Phoenix canariensis* Hort. ex Chabaud (Arecales: Arecaceae) and *Phoenix dactylifera* L., causing serious financial losses. In the Persian Gulf, the estimated cost of pest management and eradication in a 5 % infested *P. dactylifera* plantation was around US$26 million (El-Sabea *et al.* 2009). *R. ferrugineus* is also a threat to world heritage areas such as the largest palm plantation in Europe, in Elche in the Valencia Region of Spain, a UNESCO World Heritage Site (EPPO/OEPP 2008). The weevil's expansion has been largely due to the widespread practice of shipping palm trees between different territories (Abraham *et al.* 1998). Besides human activity, the insects usually spread by flying in search of new habitats, food sources and oviposition sites (Cooter 1993).

Information on the insect's flight performance under different environmental and physiological conditions is essential for the efficient development of forecasting and control strategies (Cooter & Armes 1993). Despite the wide range of measures currently used to prevent and control *R. ferrugineus* infestations, new management strategies will have to be developed, based on a better understanding of the flying abilities and dispersion capacity of *R. ferrugineus* adults. The insect's flight range and dispersion capabilities can be analysed under field conditions using methods such as the mark-release-recapture (MRR) method. Abbas *et al.* (2006) analysed the distances covered by *R. ferrugineus* by this method, however in some regions it is difficult to study its flight potential and behaviour outdoors as it is considered a quarantine pest. Chinchilla *et al.* (1993) also analysed the migration of *R. palmarum* L. adults by MRR. In order to overcome the problems inherent in field conditions, a number of laboratory techniques have been developed to quantify insect flying abilities, including static tethering, flight mills, and flight balances and pendulums, which can be used to analyse the influence of different factors under laboratory conditions (Cooter 1993). These techniques can be used to measure an insect's flying abilities without interference from external stimuli such as pheromones or abiotic factors like wind (Sarvary *et al.* 2008). The flight mill method is

considered a model system for the laboratory analysis of insects' flight behaviour (Schumacher *et al.* 1997) and has been used successfully to study flight performance of a large number of economically important agricultural species belonging to different orders, such as *Grapholita molesta* Busck (Lepidoptera: Tortricidae) (Hughes & Dorn 2002), *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) (Wu *et al.* 2006), *Aphis glycines* Matsumura (Hemiptera: Aphididae) (Zhang *et al.* 2008), *Cylas formicarius* Fab. (Coleoptera: Brentidae) (Moriya & Hiroyoshi 1998), and *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) (Chen *et al.* 2006).

As far as we know, no laboratory study using a tethered technique had been carried out to date to determine the flight potential of *R. ferrugineus*. A basic flight mill was used by Kloft *et al.* (1986) to test whether sterilization with radioisotopes affects the flight ability of *R. ferrugineus*, but this work did not provide any data on its flight performance. Also under laboratory conditions, Weissling *et al.* (1994a) studied the sequence of events leading to flight and the influence of different climatic factors on the flying behaviour of *R. cruentatus* Fab.

The experiments conducted in this study were designed to obtain useful information on the flight abilities of *R. ferrugineus* with the aim of improving pest management strategies. An *R. ferrugineus*-adapted computer-monitored flight mill was built to analyse the influence of different biotic factors, such as sex, age, and body size on different flight parameters (number of flights, total distance flown, longest single flight, flight duration, and average and maximum flying speed).

4.2. Materials and methods

4.2.1. Experimental insects

A total of 206 *R. ferrugineus* unmated adults (115 males and 91 females) were used for the experiments, obtained from cocoons collected from infested *P. canariensis* palms in the town of Sueca, in eastern Spain (39°12′N, 00°18′W; 7 m elevation), between January and December 2012. The cocoons were held in individual sterilized 100 ml plastic containers with perforated lids and maintained in a climatic chamber at 25 ± 2 °C and 65 ± 5 % relative humidity (RH). Adult emergence was checked once a day to determine their exact age and sex, after which the newly emerged weevils were returned to the containers. A piece of apple, replaced twice a week, was provided as a food source (Llácer *et al.* 2012).

Immediately before taking part in the flight mill tests, the weevils were weighed with a precision scale (Acculab, ALC-210.4, Bradford, United States of America) and measured longitudinally with a digital calliper (Comecta Corp., Barcelona, Spain), from the beginning of the rostrum to the end of the last abdominal segment. The adults were kept in the same climatic cabinet until the tests.

4.2.2. Flight mill design

A flight mill as described in the work of Dubois *et al.* (2009), designed to test the flight potential of *Osmoderma eremita* (Scopoli) (Coleoptera: Cetoniidae), was adapted to *R. ferrugineus* with the addition of a computer-monitored system (Fig. 4.1). In order to minimize friction on the pivot caused by the lever effect of the arm, a key component of the device was a miniature ball bearing (internal Ø = 4 mm, external Ø = 8 mm, thickness = 3 mm) (Minebea Co., Japan) (Fig. 4.1.A) with a precision rate number ABEC 5 (Annular Bearing Engineering Committee scale system). The pivot (Fig. 4.1.B) was an iron rod (length = 160 mm, Ø = 15 mm) inserted into a heavy iron base (Fig. 4.1.C) (Ø = 200 mm) supported on a foam cushion to reduce any vibrations produced during the flying tests. Another finer rod (Ø = 4 mm) containing the miniature ball bearing was inserted on the pivot. The arm (Fig. 4.1.D) fastened to the ball bearing, was a 64cm carbon fibre rod (Ø = 2 mm), giving a flight path of 2.01 m per revolution. As *R. ferrugineus* is smaller than *O. eremita*, using a lighter material in the arm allowed the weevils to turn the arm without difficulty. There were two pins (Fig. 4.1.E) at the end of the arm to which the insect was attached. The weevils were tethered at the pronotum with cyanoacrylate glue (Super Glue-3®, Henkel Ibérica, Barcelona, Spain) to lengths of polyethylene foam (30 x 4 x 4 mm) fixed to the two aforementioned pins. A counterweight of adhesive paste was placed on the opposite end of the arm (Fig. 4.1.F). Two reflectors on the flight arm (Fig. 4.1.G) and a pair of infrared sensors (transmitter/receiver) (Honeywell International Inc., Mexico DF, Mexico) mounted in the frame, detected every half revolution of the flight mill arm and transferred the signal to a computer, allowing the different flight parameters to be measured easily and accurately. Five flight mills ran simultaneously in a climatic chamber, maintained at $25 \pm 2\ °C$, $65 \pm 5\ \%$ RH, and constantly lit by non-flickering 58 W fluorescent (Philips Ibérica, Madrid, Spain) and Grolux lamps (Osram Sylvania Inc, Danvers, United States of America).
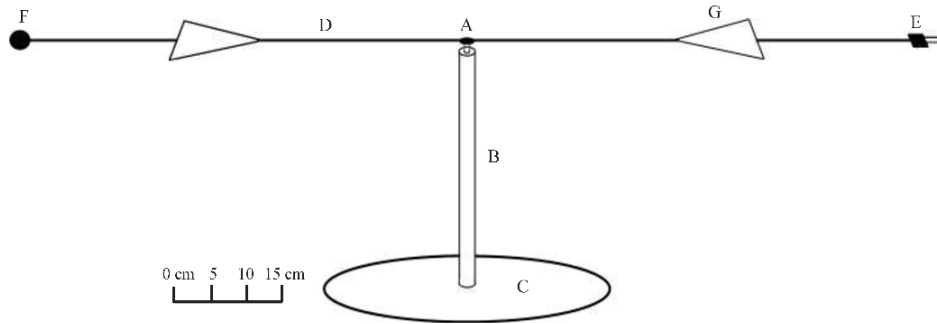
Fig. 4.1. Schematic representation of a flight mill unit: (A) Miniature ball bearing; (B) Flight mill pivot; (C) Flight mill base; (D) Carbon fibre arm; (E) Pins to attach the insect; (F) Counterweight; (G) Reflector

4.2.3. Flight parameters measured

The flight mill data were logged by a specially developed computer program which recorded each revolution and the time of its occurrence. The sequence of revolutions was interpreted in terms of single flights and breaks. A break was defined as a period of time longer than 2000 ms in which the arm did not revolve, and a single flight as the period between two breaks. In accordance with our behavioural observations (20 insects tested for 12 hours/insect), the flight time of the weevil was quantified between 400 and 2000 ms, and so the program was set to eliminate any turns with values outside this time range. The flights were monitored over a 12-h period, measuring the duration, number of turns, and number of single flights, and then computing the distance covered and speed. The flight ability of *R. ferrugineus* adults was characterized using the following flight parameters: number of flights (NOF), total distance flown (TDF), longest single flight (LSF), flight duration (FD), average flight speed (AS), and maximum flight speed (MAXS) in a 12-h trial. Only unmated males and females were tested from four age groups: from 1-3 days old, from 4-7 days old, from 8-14 days old, and from 15-23 days old. To analyse the effect of adult body size, the insects were classified by body length into three different ranges: less than 30 mm, between 31 and 34 mm, and more than 35 mm. Each flight mill was checked before each trial to ensure proper functioning.

4.2.4. Statistical analysis

Adults which did not fly were classified as "non-flyers" and were excluded from the data analysis, except for the analysis of body weight and body length. A one-way analysis of variance (ANOVA) was used to analyse the influence of sex on adult body weight and length. Regression analysis was carried out to test the relationship between body weight and length. The percentage of flyers by sex and established age ranges was compared using the *Chi-square* test. The effect of sex, age, and body length, and their interactions on each of the flight parameters were analysed using a multifactor analysis of variance (ANOVA). Means were separated using Tukey's Honest Significant Difference (HSD) test with a 95 % confidence level. As the flight parameters were not normally distributed, the data were transformed by ln (x) before the analysis. The untransformed means and their standard errors (SE) were used for graphical visualization of the data. Taking distance as the essential parameter, a regression analysis was performed to test the relationship between the total distance flown and the remaining flight parameters (number of flights, longest single flight, flight duration, average flight speed, and maximum flight speed). All the analyses were performed using Statgraphics Centurion XVI (Statgraphics 2010).

4.3. Results

4.3.1. Body size

The statistical comparison of *R. ferrugineus* adult body weight showed a significant difference between males (1.04 ± 0.02 g) and females (1.18 ± 0.03 g) (one-way ANOVA: $F = 15.99$; df = 1, 205; $P = 0.0001$). Males reached a maximum and minimum of 1.62 g and 0.53 g respectively, while females had a maximum weight of 1.68 g and a minimum of 0.40 g. The body length of the insects also showed significant differences between sexes (males: 31.17 ± 0.22 mm; females: 33.9 ± 0.27 mm) (one-way ANOVA: $F = 65.53$; df = 1, 205; $P < 0.0001$). The maximum body length values were 38 mm and 39 mm in males and females respectively, while the minimum were 24 mm in males and 26 mm in females. Adult weight showed a strong significant positive relationship with body length in males [linear regression: $R^2 = 0.7994$; $F = 455.18$; df = 1, 113; $P < 0.0001$; Body length $= 21.5017 + 9.2989$ (weight)], and females [linear regression: $R^2 = 0.7348$; $F = 250.38$; df = 1, 89; $P < 0.0001$; Body length $= 23.961 + 8.505$ (weight)]. The insect body length is a less variable parameter than body

weight, therefore the former was used instead of the latter for the subsequent analysis.

4.3.2. Effect of age and sex on the percentage of flying insects

Overall, 64.08 % of the *R. ferrugineus* adults tested were inside the established flight thresholds. The percentage of flying insects differed significantly between age groups. No significant differences were observed between the percentage of flying adults of 1-3 day old versus 4-7 day old insects (*Chi-square* test: $\chi^2 = 2.57$; df = 1; $P = 0.1090$). Nor were there any significant differences between 8-14 day old and 15-23 day old adults (*Chi-square* test: $\chi^2 = 0.10$; df = 1; $P = 0.7064$). However, the percentage of flying *R. ferrugineus* adults differed significantly between 4-7 day old and 8-14 day old insects (*Chi-square* test: $\chi^2 = 11.44$; df = 1; $P = 0.0007$), increasing substantially from 56.4 % in the former to 88.1 % in the latter case (Fig. 4.2). Based on the previous results, we tested the effect of sex on the percentage of flying insects in the 1-7 day old and 8-23 day old categories. In neither case did sex affect the percentage of flying *R. ferrugineus* (*Chi-square* test: $\chi^2 = 0.78$; df = 1; $P = 0.3767$ and $\chi^2 = 0.09$; df = 1; $P = 0.7656$, respectively).
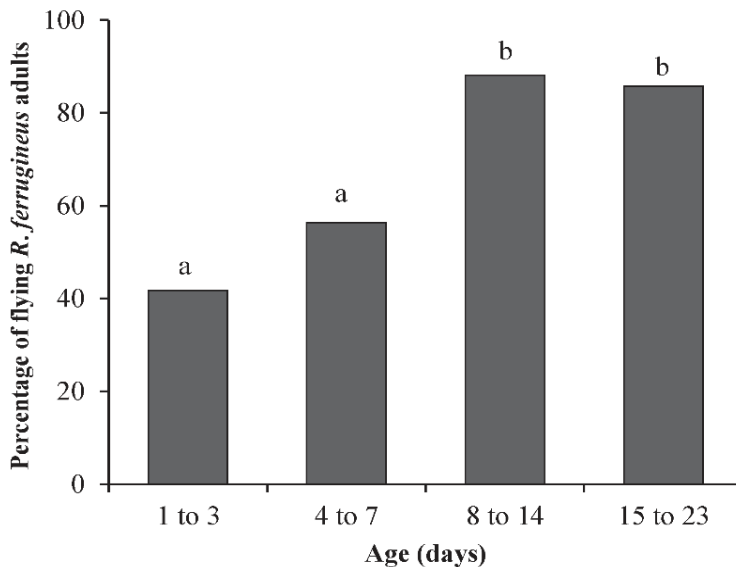
Fig. 4.2. Percentage of flying *Rhynchophorus ferrugineus* unmated adults ($n = 206$), for different age ranges (from 1-3 days old, 4-7 days old, 8-14 days old, and 15-23 days old). Different letters above the columns denote statistically significant differences at *P < 0.05* (*Chi-square* test)

4.3.3. Effect of sex, age, and body length on flight performance

The data for the different flight parameters are shown in Table 4.1. In all the established flight parameters, except in MAXS, the mean was higher in *R. ferrugineus* males than in females. It is important to highlight that the maximum values registered for the parameters examined were very high compared with the mean, especially for TDF and LSF, due to the high flight potential of some of the adults. LSF accounted for approximately 50 % of TDF by *R. ferrugineus* adults. On the other hand, AS and MAXS were less variable.

Based on the results of the percentage of flying insects, two age ranges were used for the analysis of the influence of age on the flight parameters: 1-7 days old and 8-23 days old. The data for weevil body length was classified into three categories: under 30 mm, between 30 and 35 mm, and over 35 mm. There were no significant differences in the defined flight parameters (NOF, TDF, LSF, FD, AS, and MAXS) for the three analysed factors (sex, age, and body length) (Table 4.2). Only MAXS was significantly influenced by body length, being higher in insects longer than 35 mm, but the interactions by pairs between body length, sex, and age were not significant (Table 4.2). Finally, the interaction between the three analysed factors neither influenced significantly the flight potential of weevil adults (NOF: $F = 0.9$; df = 2, 120; $P = 0.5023$; TDF: $F = 1.26$; df = 2, 120; $P = 0.2883$; LSF: $F = 1.26$; df = 2, 120; $P = 0.2880$; FD: $F = 1.50$; df = 2, 120; $P = 0.2282$; AS: $F = 0.32$; df = 2, 120; $P = 0.7283$; MAXS: $F = 0.14$; df = 2, 120; $P = 0.8676$).

We detected a significant positive relationship between TDF and NOF (linear regression: $R^2 = 0.5468$; $F = 156.75$; df = 1, 131; $P < 0.0001$; Fig. 4.3.A). Likewise, TDF and LSF showed a strongly positive relationship (linear regression: $R^2 = 0.9509$; $F = 2502.55$; df = 1, 131; $P < 0.0001$; Fig. 4.3.B). TDF and FD were strongly correlated (linear regression: $R^2 = 0.9687$; $F = 3920.32$; df = 1, 131; $P < 0.0001$; Fig. 4.3.C). Finally, the speed values (AS and MAXS) showed no correlation with TDF (AS linear regression: $R^2 = 0.2646$; $F = 46.79$; df = 1, 131; $P < 0.0001$; MAXS linear regression: $R^2 = 0.4420$; $F = 103.01$; df = 1, 131; $P < 0.0001$).

A)

y = 0.3664x − 0.1325
R² = 0.5468

ln(No. of flights)

ln(Total distance flown (m))

B)

y = 0.9947x − 0.7749
R² = 0.9509

ln(Longest single flight (m))

ln(Total distance flown (m))

C)

y = 0.7345x − 2.2408
R² = 0.9687

ln(Flight duration (min.))

ln(Total distance flown (m))
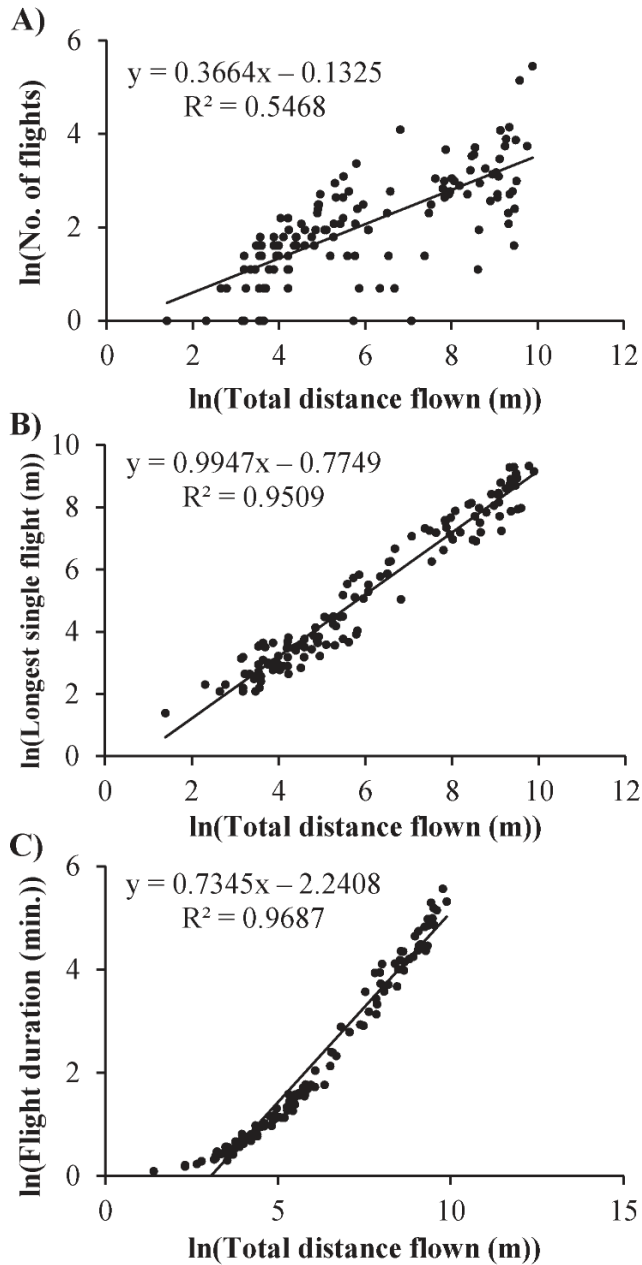
Fig. 4.3. Relationship between flight parameters for *Rhynchophorus ferrugineus* unmated adults (*n* = 132): (A) Total distance flown (TDF) and Number of flights (NOF); (B) Total distance flown (TDF) and Longest single flight (LSF); (C) Total distance flown (TDF) and Flight duration (FD)

Table 4.1. Summary of flight performance parameters (mean ± SE) of *Rhynchophorus ferrugineus* unmated adults, during 12-h tethered flight assays (25 ± 2 °C and 65 ± 5 % RH)

| Flight parameter | Total (n = 132) | | Males (n = 72) | Females (n = 60) |
	Mean ± SE*	Max*	Mean ± SE*	Mean ± SE*
No. of flights [NOF]	14.9 ± 2.3	233	18 ± 4.1	11.1 ± 1.5
Total distance flown [TDF] (m)	2615.4 ± 381	19659.6	3037 ± 549	2138.5 ± 527.6
Longest single flight [LSF] (m)	1312.5 ± 213	11244.9	1559 ± 300.6	1048.2 ± 304.4
Flight duration [FD] (min.)	30.7 ± 4.5	260.6	36.63 ± 6.52	24.08 ± 6.12
Average speed [AS] (km/h)	3.7 ± 0.07	7.05	3.73 ± 0.1	3.66 ± 0.1
Maximum speed [MAXS] (km/h)	6 ± 0.11	9.29	5.95 ± 0.16	6.07 ± 0.16

*Means and their standard errors and maximum single values measured were obtained from the untransformed data set

Table 4.2. Effect of sex, age, body length, and their interactions by pairs on flight parameters of *Rhynchophorus ferrugineus* unmated adults tested ($n = 132$), by multifactor analysis of variance (ANOVA)

Flight parameter	Sex (A)			Age (B)			Body length (C)		
	F	df	P	F	df	P	F	df	P
No. of flights [NOF]	0.00	1, 120	0.9860	0.00	1, 120	0.9482	0.54	2, 120	0.5870
Total distance flown [TDF] (m)	0.50	1, 120	0.4824	1.73	1, 120	0.1905	0.14	2, 120	0.8688
Longest single flight [LSF] (m)	1.20	1, 120	0.2749	3.11	1, 120	0.0804	0.13	2, 120	0.8741
Flight duration [FD] (min.)	0.51	1, 120	0.4745	1.81	1, 120	0.1813	0.03	2, 120	0.9678
Average speed [AS] (km/h)	3.72	1, 120	0.0562	0.21	1, 120	0.6464	2.01	2, 120	0.1384
Maximum speed [MAXS] (km/h)	2.07	1, 120	0.1526	0.13	1, 120	0.7201	4.34	2, 120	0.0151[1]

Flight parameter	Interaction A-B			Interaction A-C			Interaction B-C		
	F	df	P	F	df	P	F	df	P
No. of flights [NOF]	0.02	1, 120	0.8762	0.81	2, 120	0.4485	0.49	2, 120	0.6115
Total distance flown [TDF] (m)	0.02	1, 120	0.8811	0.99	2, 120	0.3739	1.03	2, 120	0.3589
Longest single flight [LSF] (m)	0.08	1, 120	0.7789	0.87	2, 120	0.4197	1.35	2, 120	0.2639
Flight duration [FD] (min.)	0.01	1, 120	0.9130	0.03	2, 120	0.9678	1.45	2, 120	0.2397
Average speed [AS] (km/h)	2.46	1, 120	0.1192	1.16	2, 120	0.3158	0.88	2, 120	0.4188
Maximum speed [MAXS] (km/h)	1.32	1, 120	0.2531	1.17	2, 120	0.3129	0.33	2, 120	0.7181

[1] This value shows statistical significant differences (multifactor ANOVA)

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4.3.4. Flight classification

In accordance with the individual LSF distances flown, we assigned *R. ferrugineus* adults to three arbitrary flight categories: short-distance (less than 500 m), medium-distance (between 500 and 5000 m), and long-distance flyers (more than 5000 m). Up to 63 % of the tested insects performed a LSF of less than 500 m (short-distance flyers). The percentage of medium-distance flyers was 27.3 %, whereas 9.1 % of the tested *R. ferrugineus* adults flew a distance greater than 5000 m (long-distance flyers) (Fig. 4.4).
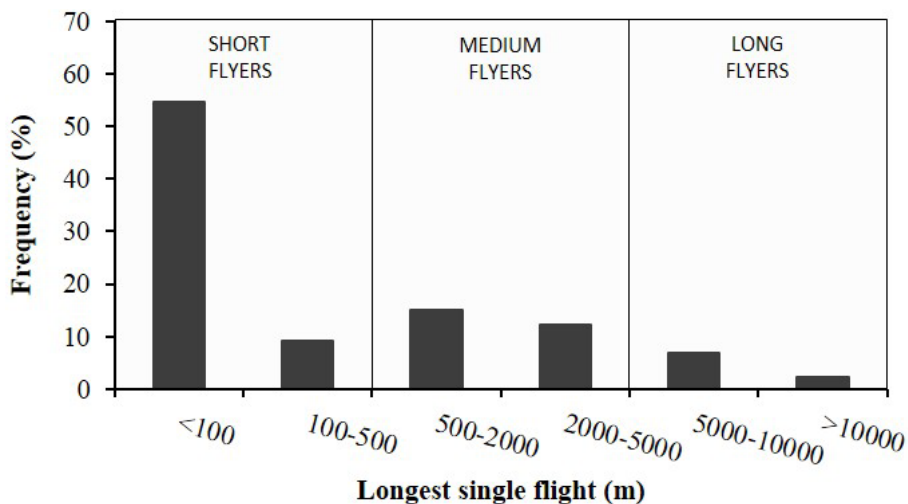
Fig. 4.4. Frequency distribution of the longest single flight (LSF) for *Rhynchophorus ferrugineus* unmated adults tested in flight mill ($n = 132$) and arbitrary flight classification categories

The first flight of the insects tested on the flight mill was the LSF in 61.4 % of cases and 71.9 % of the weevils achieved their MAXS during the LSF. In fact, the first five flights explained 77 % of the variability observed in the TDF (linear regression: $R^2 = 0.7682$; $F = 294.90$; df = 1, 89; $P < 0.0001$) (Fig. 4.5).

Fig. 4.6 shows the mean distance travelled and the average speed in relation to the elapsed time of LSF for the insects considered as long-distance flyers (LSF > 5 km). We only considered those insects which flew the longest distances without interruption, whose exhaustion was greater and therefore made a change in flying speed easier to detect. *R. ferrugineus* adults initially increased their

average speed by approximately 0.5 km/h, reaching a maximum speed at the midpoint of the flight.
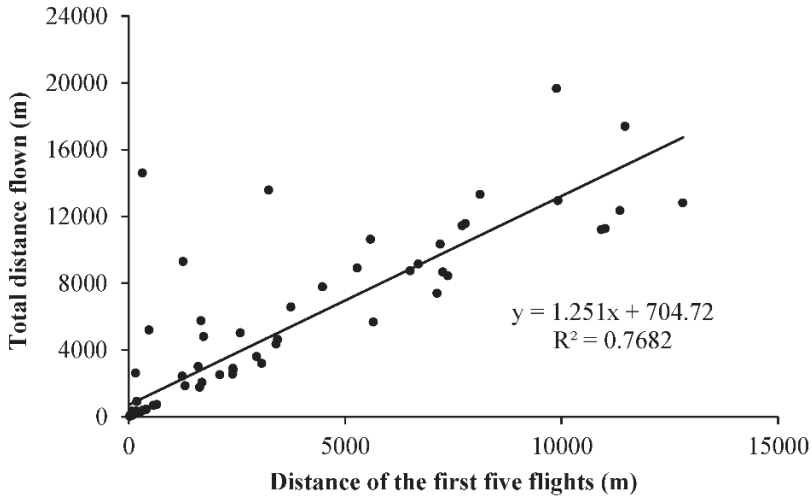
Fig. 4.5. Relationship between the total distance flown (TDF) and the distance covered during the first five flights, by *Rhynchophorus ferrugineus* unmated adults ($n = 91$)
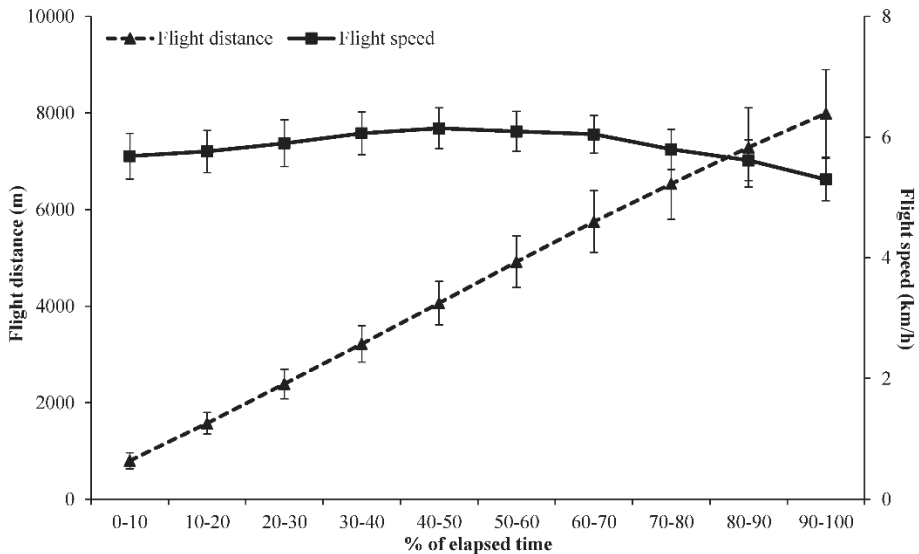
Fig. 4.6. Distance flown (m) and average speed (km/h) throughout the flight duration for the *Rhynchophorus ferrugineus* unmated adults whose longest single flights (LSF) were more than 5 km ($n = 12$). Data are presented as mean ± SE

4.4. Discussion

According to the findings *R. ferrugineus* adults have a great potential for dispersal, since, although most adults make short flights (< 500 m), 36.4 % of adults are able to perform medium- or long-distance flights (from 500 to 5000 m, and more than 5000 m, respectively). The flight potential of adults was not influenced by sex, age, or body size.

The flight mill technique is one of the best methods of analysing the flight performance of an insect under laboratory conditions (Schumacher *et al.* 1997). However, a certain percentage of the insects do not fly at all, which in the case of *R. ferrugineus,* accounted for 35.42 % of the adults tested. In studies carried out with other insect species, such as *Ips sexdentatus*, the percentage of non-flyers was similar: 31.7 ± 16.2 % of the tested insects (Jactel 1993). The percentage of non-flying insects could be a consequence of the tethered flight test method, possible morphological deficiencies that prevent them from flying, or because a portion of the individuals are not capable of flight.

The mean body size of *R. ferrugineus* (body weight and body length) differed significantly by sex. Other studies have also found sex differences in *R. ferrugineus* for these two morphological parameters (Longo 2007; Prabhu & Patil 2009). Although differences in male and female body weight and length were observed, sex did not influence the flight potential of *R. ferrugineus* unmated adults. This could possibly be due to the sexual status of the tested insects (unmated adults), since in this work mated males and females were not compared. Another possibility is that olfactory signals used by the insects for communicating with individuals of the same species would have an impact. *R. ferrugineus* adults produce a male aggregation pheromone (Hallett *et al.* 1993a) in the same way as other *Rhynchophorus* species, for example *R. palmarum* (Rochat *et al.* 1991), *R. phoenicis* (Fabricius) (Gries *et al.* 1993)*,* and *R. cruentatus* (Weissling *et al.* 1994b). The release of an aggregation pheromone causes an increase in the density of conspecifics near the pheromone source, attracting individuals of both sexes (Wyatt 2003). For this reason it is not necessary for an individual to seek the opposite sex in order to mate, and therefore both sexes have similar flight potentials. This flight behaviour can also be observed in other coleopteran species which produce male aggregation pheromones and in which sex does not influence flight potential, for example the Six-toothed pine bark beetle, *I. sexdentatus* Börner (Coleoptera: Scolytidae) (Vité *et al.* 1974; Jactel 1993), or the Plum curculio, *C. nenuphar* (Eller & Bartelt 1996; Chen *et al.* 2006). On the other hand, in species which do produce sex

pheromones, such as *O. eremita*, males produce the pheromone (Larsson *et al.* 2003) and females have greater flight potential (Dubois *et al.* 2010). In the Sweetpotato weevil, *C. formicarius,* females produce the sex pheromone (Heath *et al.* 1986) and males have greater flight ability (Moriya & Hiroyoshi 1998).

Rhynchophorus ferrugineus body size did not influence its flight ability, except for adults (males and females) with a body length greater than 35 mm, which had a significantly higher maximum flight speed. This may be because they are equipped with better musculature to reach maximum speeds at peak times. Some studies also found an absence of correlation between the flight potential and the parameters used to determine the body size of *Ips typographus* and *I. sexdentatus* (Botterweg 1982; Jactel 1993, respectively).

The age of *R. ferrugineus* adults affected the percentage of flying insects but had no influence on their flight potential. The current results therefore suggest that *R. ferrugineus* adults of all ages may contribute to a greater or lesser extent to the dispersal of the pest. The percentage of flying weevils in adults from 1-7 days old was significantly lower than in 8-23 days old. *R. ferrugineus* adults remain inside the cocoon an average of 8 days after emergence from the pupal case (Menon & Pandalai 1960). Probably the high percentage of non-flying 1-7 day old insects could be a consequence of incomplete development of the muscles needed for flight. In agreement with our findings, Tanaka & Yamanaka (2009) pointed out that the percentage of *Ophraella communa* LeSage (Coleoptera: Chrysomelidae) flyers increased with age, from days 1 to 5 and thereafter remained at a high level. The life average span of *R. ferrugineus* adults is 1.5-3 months (Esteban-Durán *et al.* 1998), but we cannot say what would have happened if we had tested weevils more than 23 days old.

The flight capability data obtained in the study indicate that *R. ferrugineus* tends to fly short distances. Most of the tested adults were classified as short-distance flyers (63.6 %), covering less than 500 m. A high number of consecutive short flights may play an important role in the dispersal of *R. ferrugineus,* as demonstrated by the strong positive correlation between the total distance flown and the number of flights. Oehlschlager *et al.* (1992) pointed out in their MRR study with *R. palmarum* that the highest percentage of recaptures occurred at 500 m from the release point, the shortest of the distances tested. In other studies carried out with migratory insects such as the Beet armyworm, *Spodoptera exigua* Hübner, more than 60 % of the moths tested flew more than 10 km and 5 h during a tethered flight of 12-hours (Xinfu *et al* 1999). Kennedy (1985) defines "migratory flight" as an active process: persistent, straight, and undistracted

movement. We believe that according to this definition, the behaviour of *R. ferrugineus* adults during its dispersion does not correspond to a migratory flight, but rather to a "trivial flight" (Southwood 1962). In the study carried out by Abbas *et al.* (2006) under field conditions using MRR, some *R. ferrugineus* adults were recaptured at 7 km from the release point. In our laboratory study, unmated weevil adults flew a mean total distance of around 2.5 km and were able to cover distances of about 20 km. In addition, the fact that up to 9 % of the adults were able to fly more than 5000 m corroborates that some *R. ferrugineus* have the ability to fly considerable distances, which would heighten the pest's dispersal despite not exhibiting migratory behaviour.

Another indicator of the high flight potential of *R. ferrugineus* is its flying speed, with a mean around 4 km/h, similar to studies on other coleopteran species, *e.g. I. sexdentatus* and *O. eremita*, whose average speed was between 4 and 5 km/h (Jactel & Gaillard 1991; Dubois *et al.* 2010). Interestingly, the speed of *R. ferrugineus* was fairly constant, even when it travelled more than 5 km non-stop, which meant that the distance travelled also increased progressively, contrary to the findings of Lu *et al.* (2007), in which the *Lygus lucorum* Meyer-Dür (Heteroptera: Miridae) flying speed decreased gradually, so that the increase in the covered distance was also reduced.

In the Mediterranean Basin *P. canariensis* and *P. dactylifera*, the main hosts of *R. ferrugineus,* are the two major palm species used as ornamental plants (Ferry & Gomez 2002). Palm tree distribution densities vary from residential areas, nurseries, avenues, and promenades, with dense uniform distributions, to natural areas where palm trees grow wild and scattered, with light distributions. The ability of *R. ferrugineus* to adapt to different environmental conditions, the trade and transport of infested plant material, the abundance of palm trees in most southern European countries in which the pest is present, and the high dispersal potential of the pest itself, as confirmed by the results of the present study, could explain its rapid and widespread dispersion in the last twenty years.

The flight ability of *R. ferrugineus* was not found to be influenced by sex, age, or body size in the conditions tested. Although weevil adults do not have a potential for long-range migratory flights, they are capable of covering long distances in a series of short flights, which contributes significantly to their potential for spreading. While the data obtained on their flying ability under laboratory conditions should not be interpreted as an exact reflection of the performance of an insect in its natural environment, we believe this information could be useful for improving strategies currently in place for the management of

this pest, such as olfactory trapping. It will also allow us to better define critical areas around pest outbreaks, to intensify inspections, and improve the phytosanitary treatment of palm trees.

4.5. Acknowledgements

Flight behaviour and dispersal of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) adults using mark-release-recapture method

Flight behaviour and dispersal of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) adults using mark-release-recapture method

Abstract

Rhynchophorus ferrugineus Oliv. (Coleoptera: Dryophthoridae) has spread worldwide from its origins in Southeast Asia and Melanesia, and it still causes serious damage to palm trees. Knowing the flight patterns of this insect can help us to better understand its rapid spread and to improve the strategies used against it. To analyse the flight performance of *R. ferrugineus*, laboratory experiments were conducted, but these data must be complemented with field tests that allow us to know the real flight behaviour and dispersal patterns of this insect. Two mark-release-recapture experiments were conducted in areas with infestation of *R. ferrugineus*. In the first, the effects of different biotic and abiotic factors in the take-off and flight mobility of weevil adults were analysed. The second experiment aimed to determine the maximum flight distance travelled by adults in field. The take-off rate of *R. ferrugineus* adult males was significantly greater than females, and was positively influenced by temperature and solar radiation. Female weevil recaptures were significantly higher, especially as temperature and solar radiation increased. Dispersal distances of weevil adults increased when temperatures rose, and while most of the insects flew short distances (< 500 m), some were able to cover up to 7 km. The dispersal of the *R. ferrugineus* adults occurred the first week after their release, and when relative humidity increased, their dispersal time was reduced. Similar field experiments and a flight performance study of *R. ferrugineus* using a flight mill were analysed and compared with data obtained in the present study.

Keywords: Red palm weevil, mark-release-recapture, flight behaviour, dispersal, take-off, flight potential.

5.1. Introduction

The Red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae), is still the primary pest of palm trees (Arecaceae) worldwide (EPPO/OEPP 2008). This weevil is native to Southeast Asia and Melanesia, where it is the key pest of Coconut palms, *Cocos nucifera* L. (Arecales: Arecaceae) (Viado & Bigornia 1949). Although *R. ferrugineus* has a broad host range, it favours certain *Phoenix* species, such as *P. canariensis* Hort. ex Chabaud and *P. dactylifera* L. (Arecales: Arecaceae) (Murphy & Briscoe 1999; Faleiro 2006a). This pest invaded the Middle East in the mid-1980s, where it has taken over 50 % of the date palm-growing countries, causing major economic losses (Faleiro 2006a). *R. ferrugineus* has spread around the Mediterranean basin in the last two decades, also reaching Australia (EPPO/OEPP 2008) and the American continent (EPPO/OEPP 2009). At present, its dispersal continues to new countries, reaching Yemen in 2013 (Assggaf 2013) and Russia in 2014 (Karpun *et al.* 2014).

The dispersal of insects can be carried out in different ways, through their own movement or transported by humans. The former involves performing vital roles such as feeding, mating, oviposition, or migration (Goldsworthy & Wheeler 1989). In the case of *R. ferrugineus,* factors such as the movement of palm trees between territories (Abraham *et al*. 1998; Rugman-Jones *et al*. 2013), and the high flight potential of this weevil (Ávalos *et al*. 2014), have facilitated its dispersal. There are several techniques used to evaluate, in laboratory and field conditions, the flight performance and behaviour of insects and to determine the influence of biotic and abiotic factors. In laboratory, the flight mill technique allows the aforementioned aspects to be studied. However, flight data obtained from tethered insects should not be interpreted as an exact reflection of their flight performance in field (Cooter 1993), and it is essential to complement these data with field trials. Several methods and devices can be used outdoors to track the insect's movement: radio telemetry, harmonic radars, vertical-looking entomological radars, and radio frequency identification, among others (Chapman *et al.* 2011; Kissling *et al*. 2013). Other less complex and less expensive methods can also be used to study the insect's flight range and its dispersal capabilities under field conditions. One of these is the mark-release-recapture (MRR) method, which has been used successfully to study the flight behaviour and dispersal of numerous agricultural pests, including *Ceratitis capitata* Wied. (Diptera: Tephritidae) (Gavriel *et al*. 2012), *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Margaritopoulos *et al*. 2012), *Homalodisca coagulata*

Say (Hemiptera: Cicadellidae) (Coviella *et al*. 2006), and *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) (Szendrei *et al*. 2009).

The prevention and control of the infestations produced by *R. ferrugineus* require an integrated pest management program based on olfactory trapping with pheromones and kairomones, application of chemical or biological insecticides, early detection of infestations, destruction of infested plant material, and mechanical sanitation (Hallett *et al*. 1999; Abbas *et al*. 2001; Faleiro 2006a; EPPO/OEPP 2008; La Mantia *et al*. 2008*)*. However, these approaches to management are not very efficient, besides they have a high economic and environmental cost, and thus they should be improved. Knowing and understanding the flight capability and behaviour of the *R. ferrugineus* adults is one way to improve the current management programs used against this pest. Several authors have analysed some of the insect's behavioural aspects, including Ávalos *et al.* (2014), who studied the flying ability of *R. ferrugineus* adults under laboratory conditions, comparing different flight parameters and the influence of the weevil's sex, age, and body size. On the other hand, Abbas *et al.* (2006) evaluated under field conditions the distances covered by the weevil adults in *P. dactylifera* plantations using the MRR method. Finally, Oehlschlager *et al*. (1992) studied the migration of another *Rhynchophorus* species*, R. palmarum* L. (Coleoptera: Curculionidae), and estimated its populations in Oil palm plantations, *Elaeis guineensis* Jacq. (Arecales: Arecaceae). Nonetheless, despite the data gathered in these studies, there is still a lack of information regarding the flight behaviour and dispersal of *R. ferrugineus* adults under field conditions.

The present study aimed to analyse the flight behaviour and dispersal of *R. ferrugineus* adults, and the influence of several biotic and abiotic factors in it, so as to obtain information that allows for the improvement of the different control strategies used against this weevil. To this end, two MRR experiments were conducted in different areas with infestation of the pest. The influence of the *R. ferrugineus* sex, temperature, relative humidity, and solar radiation, was analysed in two measured phases of their flight, take-off and flight mobility. Moreover, their dispersal distances and times were also calculated. Results were compared with previous MRR experiments and with the study of the flight performance of this insect under laboratory conditions.

5.2. Materials and methods

5.2.1. Experimental insects

The *R. ferrugineus* unmated adults used in the experiments were obtained from cocoons collected from infested *P. canariensis* palm trees in the town of Algemesí, in eastern Spain (39°11′N, 00°26′W; 17 m elevation), between March 2009 and January 2012. The cocoons were held in individual sterilized 100 ml plastic containers with perforated lids, and kept in a climatic chamber at 25 ± 2 °C and 65 ± 5 % relative humidity. Adult emergence was checked once a day to determine the age and sex, after which the newly emerged weevils were returned to the containers. A piece of apple was provided twice a week as a food source (Llácer *et al*. 2012). Weevil adults used in the MRR experiments were less than 30 days old, and were kept in the climatic chamber until the trials.

Because *R. ferrugineus* is considered as a quarantine pest in some regions of the world, including Spain, and to avoid infestation of palm trees by mating and oviposition of adult weevils, insects used in the MRR experiments were subjected to a process of manual sealing. The method consisted in sealing with cyanoacrylate glue (Super Glue-3®, Henkel Ibérica, Barcelona, Spain) the insect's pygidium, where the aperture of the reproductive system is located. Eleven couples of *R. ferrugineus* adults (males and females, less than seven days old) were manually sealed in order to know their lifespan, to ensure the sealing remained in place, and to confirm that oviposition was absent. Each couple of insects was placed in a separated box with a food source and were kept in a climatic chamber at 25 ± 2 °C and 65 ± 5 % relative humidity. Moreover, in order to determine if the sealing system influenced the flying ability of *R. ferrugineus* adults, the flight performance of 231 (118 males and 113 females) sealed and 206 (115 males and 91 females) non-sealed adults, between 1 and 23 days old, were compared using the flight mill technique and the experimental procedure, exactly as described by Ávalos *et al.* (2014). In the aforementioned work, the flight potential of a *R. ferrugineus* adult was defined as the number, distance, duration, and speed of its flights. In the present study, the flight performance parameters compared were the number of flights, total distance flown, longest single flight, and flight duration.

5.2.2. Marking technique

Released *R. ferrugineus* adults were marked by painting their pronotum with a white dot using correction fluid (Tipp-Ex®, Societé BIC, Île-de-France,

France), and covering it with a thin layer of transparent cyanoacrylate glue (Super Glue-3®, Henkel Ibérica, Barcelona, Spain). In order to avoid confusions between successive releases in recaptured insects, the mark was alternated in each release, using one or two white dots. Twenty adults were marked and placed during 30 days in a box containing pieces of palm tree stem and fibres, and kept in a climatic chamber at 25 ± 2 °C and 65 ± 5 % relative humidity, proving that the marking system used was distinctive, durable in all the tested insects, and did not affect the behaviour or survival of adults.

5.2.3. MRR experiment 1

The first MRR experiment was conducted in the maritime area of the town of Castellón, in eastern Spain (39°59′N, 00°01′W; 10 m elevation), an area with a high infestation level of *R. ferrugineus*. The release point, as well as 37 white bucket traps, scattered according to the possibilities so as to fit a uniform distribution around the spot at distances between 220 and 1390 m (362 ha), were placed in the study area (Fig. 5.1). Bucket traps, with 10 litres capacity, were baited with a *R. ferrugineus* male aggregation pheromone (Ferrolure+, composed of 4-methyl-5-nonanol and 4-methyl-5-nonanone [9:1], and containing 700 mg of 95 % pure active ingredients), kairomone (composed of ethyl acetate, and containing 40 ml of 95 % pure active ingredient) (Econex Ltd., Murcia, Spain), a piece of infested *P. canariensis* petiole (sized 8 x 5 cm), and water. The recommended lifespan of the pheromone and kairomone dispensers was three months (Econex 2013a; Econex 2013b), being replaced after this time, together with the palm tree petioles. To conduct the releases in a wide range of climatic conditions, four temperature ranges were established, taking into account the average monthly temperatures from the previous five years in the study area (Weather station in the experiment area: 39°90′N, 00°01′W; 16 m elevation): below 15 °C, between 15 and 20 °C, between 20 and 25 °C, and over 25 °C. Three releases between 66 and 180 adults of both sexes, per temperature range, were done from June 2009 to January 2012 (Table 5.1). Traps were inspected 1, 3, 7, 15, and 21 days after each release day, recording the number and sex of recaptured marked weevils. In addition, the effects of temperature (°C) and relative humidity (%) on the flight mobility of weevil adults were analysed.

Taking advantage of each *R. ferrugineus* release, the take-off of 60 of the released weevils (30 males and 30 females) was analysed (except for the first release), by direct observation (Table 5.2), during 30 min, at four times of day: 7:00 a.m., 10:00 a.m., 1:00 p.m., and 4:00 p.m. (15 adults per time tested), in

order to determine if the weevil sex, temperature, relative humidity, or solar radiation had some effect on their take-off.

The climatic data, used to analyse their effect on the take-off and flight mobility of the *R. ferrugineus* adults, were obtained at the moment of the weevil release, and every ten minutes during the 21 days following each release, from the aforementioned weather station (Table 5.1).

Fig. 5.1. Trapping grid of MRR experiment 1 with 37 *Rhynchophorus ferrugineus* bucket traps (circles) placed at different distances from the release point (square)

5.2.4. MRR experiment 2

In order to measure the maximum flight distance travelled by *R. ferrugineus* adults in field, a second MRR experiment was carried out from June to October 2011 around the Albufera Natural Park, in eastern Spain (39°24′N, 00°31′W; 7 m elevation). This area was chosen because there was a low density of palm trees and due to its orography, a large extension of completely flat rice fields with no geographical barriers, which facilitates weevil flight. The release point was surrounded by a ring of eight bucket traps, placed equidistant between

them (same model and bait as in MRR experiment 1). Initially traps were placed at 4 km from the release point. Between 62 and 100 marked *R. ferrugineus* adults of both sexes were released each time, depending on the availability of cocoons in field (Table 5.3). Traps were inspected 1, 3, 7, 15, and 21 days after each release day. If at least one weevil adult was recaptured, the distance between the traps and the release point was increased and a new release was carried out. The maximum flight distance was considered the previous one to the release in which no weevil adult was recaptured after four releases for the same tested distance.

5.2.5. Statistical analysis

A multifactor analysis of variance (ANOVA) was performed to determine the effect of pygidium sealing, sex, and age of weevil adults, and their interactions, on the parameters established to measure their flight performance. Means were separated using Tukey's Honest Significant Difference (HSD) test with a 5% significance level. Due to the periodicity of the countings after each release, the flight performance parameters were analysed grouping the ages in three ranges: from 1 to 6, from 7 to 14, and from 15 to 23 days old. Insects that did not fly were excluded from the data analysis. Given the positive asymmetry characteristic of this type of data, these were transformed by ln (x) before the analysis. A two-sample *t*-test was used to compare the effect of sex on the longevity of the sealed adults. A binary logistic regression model was used to determine the significance of the weevil sex, temperature, relative humidity, and solar radiation on the insect take-off. Due to the distribution of the recaptures, the mean values of the climatic factors during the seven days after each release were used for the subsequent analyses. In order to detect any effect of weevil sex, temperature, and relative humidity on insect recaptures a binary logistic regression model was carried out. To study the influence of weevil sex, temperature, and relative humidity on the dispersal distances a multiple regression model was used. A *Chi-square* test was used to compare the differences in recaptures between three tested trap distance ranges from the release point: less than 500 m (9 traps), between 500-1000 m (19 traps), and between 1000-1390 m (9 traps) from the release point. A regression model with interval-censored data, assuming a lognormal distribution for time variable, was used to examine the influence of weevil sex, temperature, and relative humidity on the insect's recapture time. Finally, to detect the differences in recaptures between the different surveyed time ranges after the release, a *Chi-square* test was used. All the analyses were performed using Statgraphics Centurion XVI (Statgraphics 2010) and Minitab 14 (Minitab 2004).

Table 5.1. Summary of the *Rhynchophorus ferrugineus* adult releases: Release dates, mean (± SD) values of climatic conditions, number of released adults, and percentage of recaptured weevils

Release No. and Date	Climatic conditions*		No. of released adults			% of recaptured adults		
	Temp. (°C)	Rel. hum. (%)	Males	Females	Total	Males	Females	Total
1 - 01/06/2009	22.2 ± 1.94	66.7 ± 18.65	49	26	75	8.0	17.3	25.3
2 - 29/07/2009	26.6 ± 1.17	73.2 ± 10.10	17	49	66	0.0	4.5	4.5
3 - 10/11/2009	16.9 ± 2.66	68.8 ± 15.14	101	79	180	8.3	6.1	14.4
4 - 04/01/2010	07.6 ± 3.14	65.9 ± 23.01	60	60	120	0.0	0.0	0.0
5 - 07/07/2010	25.7 ± 1.35	75.9 ± 06.61	43	43	86	2.3	2.3	4.6
6 - 07/09/2010	23.7 ± 2.57	60.4 ± 16.04	50	50	100	3.0	2.0	5.0
7 - 26/10/2010	16.6 ± 2.95	55.5 ± 17.15	59	89	148	8.1	15.5	23.6
8 - 30/11/2010	11.8 ± 4.52	57.6 ± 14.83	55	55	110	0.0	0.9	0.9
9 - 17/05/2011	19.5 ± 1.98	69.6 ± 10.15	51	60	111	5.4	13.5	18.9
10 - 26/07/2011	28.5 ± 2.76	77.4 ± 07.93	50	52	102	5.9	6.8	12.7
11 - 08/11/2011	21.6 ± 1.97	79.4 ± 07.44	59	59	118	14.4	17.8	32.2
12 - 10/01/2012	14.2 ± 2.48	78.5 ± 08.83	49	49	98	1.0	0.0	1.0
Total			643	671	1314	5.2	7.5	12.6

*Climatic conditions for each release period corresponds to the mean values of the seven days after each release.

Table 5.2. Summary of take-off observation of the *Rhynchophorus ferrugineus* adults in MRR experiment 1: Release dates and percentage of adults taking-off (30 males and 30 females tested in each release).

Release No. and Date	% of adults taking-off		
	Males	Females	Total
*1 - 01/06/2009	-	-	-
2 - 29/07/2009	23.3	33.3	28.3
3 - 10/11/2009	13.3	6.7	10.0
4 - 04/01/2010	0.0	0.0	0.0
5 - 07/07/2010	53.3	40.0	46.7
6 - 07/09/2010	43.3	40.0	41.7
7 - 26/10/2010	33.3	30.0	31.7
8 - 30/11/2010	6.7	0.0	3.3
9 - 17/05/2011	26.7	43.3	35.0
10 - 26/07/2011	73.3	50.0	61.7
11 - 08/11/2011	46.7	60.0	53.3
12 - 10/01/2012	26.7	13.3	20.0
Total	31.5	28.8	30.2

*Take-off not observed for this release

Table 5.3. Summary of the *Rhynchophorus ferrugineus* adult releases in MRR experiment 2: Trap distance from release point, release dates, number of released adults, and number of recaptured weevils and their sex

Trap distance to release point	Release No. and Date	No. of released adults			Recaptured adults and sex	
		Males	Females	Total		
4 km	1 - 31/05/2011	31	31	62	Yes	1 female
6 km	2 - 15/06/2011	35	35	70	No	-
	3 - 22/06/2011	34	34	68	No	-
	4 - 31/06/2011	34	34	68	Yes	1 male
8 km	5 - 06/09/2011	38	38	76	No	-
	6 - 13/09/2011	36	36	72	No	-
	7 - 21/09/2011	40	40	80	No	-
	8 - 30/09/2011	50	50	100	No	-
7 km*	9 - 12/10/2011	45	45	90	Yes	1 female
Total		343	343	686		

*Maximum flight distance at which a *Rhynchophorus ferrugineus* adult was recaptured

5.3. Results

5.3.1. Features of experimental insects

A comparative analysis of the influence of the pygidium sealing method on the flight potential of the *R. ferrugineus* adults was performed using a flight mill test. A total of 138 (82 males and 56 females) sealed and 132 (72 males and 60 females) non-sealed weevils flew in the flight mill. The mean ($\pm$ SE) number of flights was 14.3 ± 1.99 flights for the sealed insects, and 14.9 ± 2.30 flights for non-sealed ones. Likewise, the mean ($\pm$ SE) total distance flown for both kind of insects was 2344.6 ± 337 and 2615.4 ± 381 m, respectively. In their longest single flight, the sealed insects covered 1240.2 ± 194 m, and the non-sealed ones 1312.5 ± 213 m. Finally, the mean ($\pm$ SE) flight durations for sealed and non-sealed insects were 27.6 ± 3.9 and 30.7 ± 4.5 min, respectively. According to these values, statistical analysis showed no differences in the flight potential of sealed and non-sealed *R. ferrugineus* adults (Table 5.4).

Additionally, the effect of the age range and sex on the flight performance of the experimental adults was analysed. The *R. ferrugineus* age range did not influence the flight potential of the sealed or non-sealed insects. On the other hand, the sex of both weevils, sealed and non-sealed, significantly affected their flight performance, being greater in males, except for the number of flights. The interactions by pairs between the pygidium sealing method, age range, and weevil sex showed no significant influence on the flight performance parameters studied (Table 5.4). Finally, the triple interaction between the analysed factors neither influenced significantly the flight performance of weevil adults (Number of flights: $F = 1.30$; df = 2, 258; $P = 0.2735$; Total distance flown: $F = 0.63$; df = 2, 258; $P = 0.5355$; Longest single flight: $F = 0.28$; df = 2, 258; $P = 0.7539$; Flight duration: $F = 0.41$; df = 2, 258; $P = 0.6663$).

Regarding the lifespan of the *R. ferrugineus* adults as a consequence of the pygidium sealing method, their mean ($\pm$ SE) longevity was reduced to 16.7 ± 1.9 days (19.55 ± 2.8 days in males and 13.8 ± 2.5 days in females), with no significant differences between sexes (*t*-test: $t = 1.54$; df = 20; $P = 0.1381$). In addition, the seal remained intact until the end of their lives and no egg was laid.

Overall, the flight potential of experimental insects was not influenced by the pygidium sealing method, being their longevity the only analysed factor that was affected due to sealing. To avoid the influence of sealing on weevil longevity, the evaluation period in subsequent MRR experiments was adjusted to the experimental insects' lifespan.

Table 5.4. Effect of sealing, sex, age range, and their interactions by pairs on flight parameters of *Rhynchophorus ferrugineus* adults tested ($n = 270$), by multifactor analysis of variance (ANOVA)

Flight parameter	Sealing (A)			Sex (B)			Age range (C)		
	F	df	P	F	df	P	F	df	P
Number of flights	2.31	1, 258	0.1295	0.97	1, 258	0.3253	0.06	2, 258	0.9463
Total distance flown (m)	1.22	1, 258	0.2709	5.79	1, 258	0.0168[1]	0.42	2, 258	0.6599
Longest single flight (m)	0.46	1, 258	0.4964	6.69	1, 258	0.0102[1]	0.44	2, 258	0.6449
Flight duration (min.)	0.19	1, 258	0.6651	4.46	1, 258	0.0356[1]	0.47	2, 258	0.6284

Flight parameter	Interaction A-B			Interaction A-C			Interaction B-C		
	F	df	P	F	df	P	F	df	P
Number of flights	1.14	1, 258	0.2870	0.93	2, 258	0.3956	0.38	2, 258	0.6848
Total distance flown (m)	2.10	1, 258	0.1483	0.13	2, 258	0.8790	0.02	2, 258	0.9788
Longest single flight (m)	0.88	1, 258	0.3481	0.04	2, 258	0.9582	0.09	2, 258	0.9169
Flight duration (min.)	0.53	1, 258	0.4683	0.03	2, 258	0.9660	0.05	2, 258	0.9559

[1]This values show statistical significant differences (multifactor ANOVA)

5.3.2. Weevil take-off

Considering the total number of insects released in field, the take-off of 660 *R. ferrugineus* adults was tested. The total percentage of weevil adults which took-off was 30.2 %, being 33.1 and 28.7 % in males and females, respectively, reaching a maximum value of 61.7 % in one of the releases (Table 5.2).

The influence of different biotic and abiotic factors on the take-off of the *R. ferrugineus* adults was analysed. Concerning the biotic factors, the take-off probability was significantly higher in males compared to females. Regarding the abiotic factors analysed, temperatures during the experiment reached minimum and maximum values of 8.8 and 32.3 °C, respectively. Solar radiation varied between 0 and 883.5 W/m^2, and relative humidity between 21 and 95 %. The statistical analysis showed that according to the generated binary logistic regression model for take-off with forward selection (Table 5.5), with a solar radiation of 400 W/m2, a relative humidity of 65 %, and a temperature of 10 °C, the probability of take-off for males was 0.2189. With the same relative humidity and solar radiation, but at 17 °C, an intermediate temperature, this probability increased to 0.8308. Under the aforementioned conditions, and with a higher temperature (25 °C), this probability rose to 0.9372, the maximum according to the model prediction. Therefore, a rise in temperature and solar radiation significantly increased the probability of take-off in the *R. ferrugineus* adults. This increase was less pronounced for high values of these climatic parameters, even producing a reduction in its take-off. On the other hand, relative humidity did not influence the weevil take-off.

Table 5.5. Summary of the stepwise binary logistic regression model (forward selection) for the take-off of *Rhynchophorus ferrugineus* adults in MRR experiment 1

Parameter	Estimated	Standard error	*P*-value
Constant	-12.4218	1.75908	-
Temperature	0.8949	0.161133	0.0000
Temperature^2	-0.01811	0.003653	0.0000
Solar radiation	0.01046	0.001368	0.0000
Solar radiation^2	-0.00001	0.000001	0.0000
Sex = F	-0.38391	0.199937	0.0540

Hosmer-Lemeshow Goodness-of-fit test: $\chi^2 = 5.009$; df = 3; $P = 0.1711$

5.3.3. Weevil flight mobility

A total of 1314 insects were released, and the mean percentage of recaptured weevils was 12.6 %, reaching a maximum value of 32.2 % in one of the releases. Concerning the total number of recaptured adults per sex, of the 643 males and 671 females released, 10.6 and 14.6 %, respectively, were recaptured with a ratio ($\pm$ SE) of 1.4 ± 0.2 recaptured females per male (Table 5.1).

Biotic and abiotic factors influencing flight mobility

The analysis of the influence of biotic factors on the recapture probability of *R. ferrugineus* showed that, according to the generated model, the weevil sex had a significant influence, being the recaptures female-biased. Regarding abiotic factors, and according to the predictions of the generated binary logistic regression model with forward selection (Table 5.6), the probability of being recaptured increased significantly with a rise in temperature, reaching a maximum at 21 °C (30 %). This probability increase was less pronounced with high temperature values, and diminished with low temperatures, being above 10% for females, for values between 15 and 27 °C. On the other hand, relative humidity did not significantly influence the weevil recaptures.

Table 5.6. Summary of the stepwise binary logistic regression model (forward selection) for the recapture of *Rhynchophorus ferrugineus* adults in MRR experiment 1

Parameter	Estimated	Standard error	*P*-value
Constant	-13.985	2.046	-
Temperature	1.214	0.203	0.0000
Temperature^2	-0.029	0.005	0.0000
Sex = Female	0.396	0.172	0.0208

Hosmer-Lemeshow Goodness-of-fit test: $\chi^2 = 4.126$; df = 3; $P = 0.2481$

Dispersal distances and times

The analysis of dispersal distances of *R. ferrugineus* adults indicated that the highest percentage of recaptured insects (77.1 %) was recovered in traps placed under 500 m from the release point (Fig. 5.2), and significant differences were observed for the three range distances established (less than 500 m, between 500-1000 m, and between 1000-1390 m from the release point) (*Chi-square* test:

$\chi^2 = 147.52$; df = 2; $P = 0.0000$). On the other hand, in MRR experiment 2, 7 km was the maximum flight distance at which a marked *R. ferrugineus* adult was trapped, and although weevils were recaptured at closer distances (4 and 6 km), none were recaptured when traps were placed at 8 km from the release point (Table 5.3).
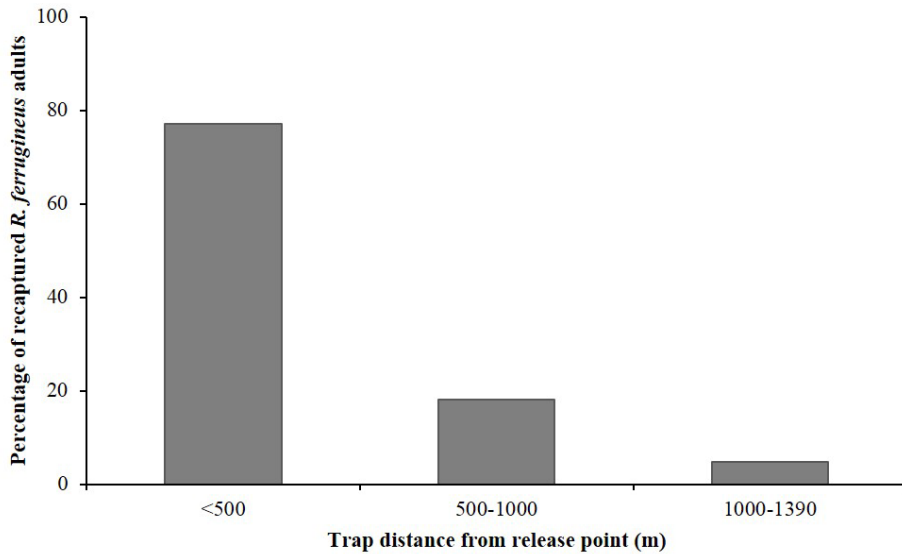
Fig. 5.2. Percentage of marked *Rhynchophorus ferrugineus* adults, recaptured in three established distance intervals in MRR experiment 1

For the analysed distances in MRR experiment 1, more than 90 % of the recaptured *R. ferrugineus* adults were recovered in the first seven days after their release (Fig. 5.3), showing significant differences between for the surveyed times (*Chi-square* test: $\chi^2 = 84.78$; df = 4; $P = 0.0000$). Likewise, it is important to note that in MRR experiment 2, the marked adult trapped at 7 km from the release point was recaptured in less than five hours after its release.

The influence of weevil sex on the dispersal distances and times of the *R. ferrugineus* adults was analysed and no significant effect was found (Tables 5.7 and 5.8). Regarding the influence of climatic factors, the dispersal distances were significantly increased by rising temperatures (Table 5.7). On the other hand, the dispersal time of the weevils was significantly influenced only by relative humidity, thus being reduced when humidity was higher (Table 5.8).
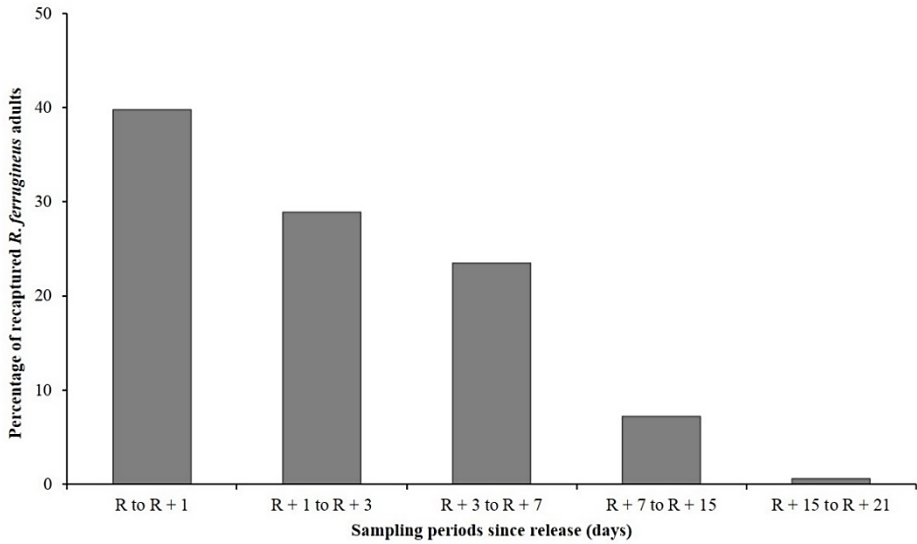
Fig. 5.3. Percentage of marked *Rhynchophorus ferrugineus* adults, recaptured in each sampling period after the release day (R) in MRR experiment 1

Table 5.7. Summary of the stepwise multiple regression model (forward selection) for dispersal distances of *Rhynchophorus ferrugineus* adults in MRR experiment 1

Parameter	Estimated	Standard error	*P*-value
Constant	121.347	108.246	0.2639
Temperature	14.937	5.244	0.0050

Coefficient of determination $R^2 = 4.7$ %

Table 5.8. Summary of the regression model with interval-censored data (lognormal distribution) for dispersal times of *Rhynchophorus ferrugineus* adults in MRR experiment 1

Parameter	Estimated	Standard error	*P*-value
Constant	3.906	0.788	0.0000
Temperature	-0.035	0.035	0.3120
Relative humidity	-0.042	0.016	0.0070
Sex = Male	0.187	0.189	0.3230
Scale	1.094	0.086	-

5.4. Discussion

Considering the influence of the *R. ferrugineus* sex and different abiotic factors in the two adult flight phases analysed, take-off and flight mobility, the results of MRR experiments 1 and 2 will be discussed.

5.4.1. Influence of sex on take-off and flight mobility

The take-off probability of the *R. ferrugineus* adults was greater in males; however, in the following studied phase, flight mobility, more females were recaptured. Therefore, the insect sex seems to have different effects depending on the phase of flight and as a consequence of differences in its dispersal behaviour. Take-off was significantly greater in *R. ferrugineus* males, in accordance with the analysis of their flight performance under laboratory conditions, which shows that males have a greater flight potential. Ávalos *et al.* (2014) observed a tendency towards a greater flight potential in males but no statistically significant differences were found, probably because the sample size was not large enough. Therefore, the greater probability of take-off in males may be a consequence of their greater flight potential. On the other hand, recaptures of *R. ferrugineus* females were significantly greater. As observed in the present study, Abraham *et al.* (1999), Faleiro & Rangnekar (2000), and Al-Saoud (2011), among others, found that sex ratio captures in pheromone traps range from 1.94 to 2.7 females per male. Moreover, tests performed under controlled conditions using olfactometers also showed that females were more attracted to pheromones than males (Giblin-Davis *et al*. 2013). This could be because of their requirement for mating places, new food sources and oviposition sites (Soroker *et al*. 2005). On the other hand, Ávalos & Soto (2015) observed that when traps were not baited with olfactory lures, the sex ratio of captures was not always female-biased. Therefore, the observed differences between sexes in both analysed flight phases could be an indication of the different dispersal behaviour depending on the weevil sex. A possible hypothesis is that *R. ferrugineus* males, which have a higher flight potential and take-off rate, are responsible for the first step of colonizing a new host. When a *R. ferrugineus* male arrives to a new palm tree, he releases the aggregation pheromone that attracts both weevil sexes, but mainly females, increasing the likelihood of a successful colonization.

5.4.2. Weevil take-off

Insect dispersal begins with take-off and needs some external stimuli and specific climatic conditions to induce flight (Johnson 1969). In the present study

the influence of abiotic factors was analysed, and take-off of *R. ferrugineus* adults was significantly influenced by temperature, increasing as the ambient temperature increased, being optimal the intermediate temperatures (between 17 and 25 °C). In other *Rhynchophorus* species, such as *R. cruentatus* Fab., temperatures above 15 °C also increase take-off probability (Weissling *et al*. 1994a). Solar radiation, highly correlated with temperature, also influenced take-off of the *R. ferrugineus* adults, which increased as solar radiation increased. With regard to this aspect, Johnson (1969) pointed out that some coleopteran species have an asynchronous fibrillar type of flight muscles, preparing for flight by heating their thorax before unfolding their wings. Therefore, and despite that *R. ferrugineus* being a tropical insect, intense solar radiation seems to have decisive implications in their take-off. The present results, and the expansion of this weevil to a large number of countries, with very different climates, confirm their great adaptability to a wide range of climatic conditions, compared with climatic conditions in their origin areas.

5.4.3. Weevil flight mobility

The dispersal of *R. ferrugineus* was analysed and quantified through their release and recapture in traps placed in an area with a high density of infested palm trees. As occurred in the MRR studies performed by Abbas *et al*. (2006) and Oehlschlager *et al*. (1992), with *R. ferrugineus* and *R. palmarum*, respectively, in the present study very low recaptures of *R. ferrugineus* adults were obtained (12.6 %). The high number of palm trees in the experimental area, which offer an optimal niche for the insect, and therefore represent its perfect attractant, could be one of the reasons for the low percentage of recaptures. Moreover, Kalshoven (1981) and Abbas *et al*. (2006) reported that a high infestation level in the study area is correlated with a higher emission rate of pheromones and kairomones, which attract more weevil adults than compounds used to bait the traps, this explaining the low percentage of recaptures observed. In other MRR experiments with insect borer species such as *Anoplophora glabripennis* Motschulsky and *Monochamus galloprovincialis* Oliv. (Coleoptera: Cerambycidae), low rates of recaptures (1.13 and 6.76 %, respectively) were also reported (Smith *et al.* 2001; Hernández *et al.* 2011).

Abiotic factors influencing flight mobility

Ecological factors are directly associated with *R. ferrugineus* mobility, mating season, and efficacy in searching for food sources; therefore, they play an important role in determining its activity (Faleiro 2006a; Haris *et al*. 2014). In the

present study, the recaptures of the *R. ferrugineus* adults were significantly influenced by temperature, being very low with values under 15 °C and above 27 °C, and reaching a maximum at 21 °C. Regarding temperatures, Ávalos *et al.* (2011) reported that in the Valencia Region, *R. ferrugineus* adults' activity is higher during autumn, with values around 20 °C, and decreases during winter months with cold temperatures. Moreover, Faleiro (2006a) pointed out that in general, moderated temperature values increase the flight of tropical weevils. On the other hand, temperatures around 15 °C may be considered a threshold, below which the *R. ferrugineus* adult's flight is limited. With regard to this aspect, El-Garhy (1996) analysed the monthly number of captured *R. ferrugineus* adults in pheromone traps, reporting that insect captures were very low during months with an average daily temperature range below 12-14 °C.

Dispersal distances

The dispersal distance of the *R. ferrugineus* adults tends to be short, since most of the weevils were recaptured at less than 500 m from the release point (77.1 %), and a small percentage (4.8 %) were collected in the farthest traps (between 1000-1390 m). Besides, as shown in MRR experiment 2, despite this tendency to fly short distances, *R. ferrugineus* was able to travel up to 7 km from the release point. In previous studies performed by our research group under laboratory conditions, without friction that hinders the insect flight, this tendency of weevils to fly short distances and their ability to cover up to 20 km was observed (Ávalos *et al.* 2014). However, as the findings from present study suggest, the percentage of weevils that can travel these long distances seems to be very low. Abbas *et al.* (2006) managed to capture *R. ferrugineus* adults which migrated up to 7 km. Likewise, the study by Oehlschlager *et al.* (1992) provided information about the distances travelled by other *Rhynchophorus* species, such as *R. palmarum*, concluding that most adults, as well as *R. ferrugineus* ones, were recaptured in traps placed at 500 m from the release point. On the other hand, the dispersal distance of *R. ferrugineus* adults was significantly influenced by temperature, as occurred with take-off and recaptures, increasing when temperatures rose.

Dispersal times

Regarding dispersal times for the distances analysed in MRR experiment 1, the *R. ferrugineus* adults were quickly dispersed after their release, decreasing their recaptures to very low values from the seventh day onwards. A possible reason for this decrease in recaptures is the differences in the flight potential of experimental insects depending on their age. Nevertheless, the flight performance

study performed herein shows that the flight potential of *R. ferrugineus*, regardless of pygidium sealing, was not influenced by age. Therefore, this quick dispersal may be a consequence of the dispersal behaviour of the insect, which seeks and finds new hosts immediately after leaving a palm tree. Other MRR studies conducted by Abbas *et al*. (2006) and Oehlschlager *et al*. (1992) indicated that most of the released weevil adults are recaptured between the second and fifth day after their release. On the other hand, the dispersal time of the *R. ferrugineus* adults was significantly reduced with high levels of atmospheric humidity, requiring less time to cover the same distance when relative humidity increased. In accordance with the generated model, for relative humidity values between 80 and 100 %, the probability of increasing the dispersal time was reduced between 40 and 60 %, respectively. Leefmans (1920) pointed out that *R. ferrugineus* adults seek moist harbourages until they find a new host, and Aldryhim & Al-Bukiri (2003) suggested that irrigation management and soil moisture are key factors in the dispersion of this weevil. Therefore, dry conditions do not favour the dispersal of *R. ferrugineus* adults. Regarding other weevil species such as *R. cruentatus*, Weissling & Giblin-Davis (1993) indicated that this insect presents high cuticular permeability and is susceptible to significant water loss in dry environments. Besides, in laboratory test the insect chooses high over low relative humidity. In this sense, and as occurs with temperatures, Faleiro (2006a) indicated in his work that high values of relative humidity enhance the flight of tropical weevils. Other species, such as *Conophthorus coniperda* Schwarz (Coleoptera: Scolytidae) cease to fly when exposed to dry air (Henson 1962), while the flight of *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) is intermittent in dry air, and more continuous in humid air (Waloff 1953).

5.5. Conclusions

In conclusion, the results obtained show the influence of different biotic and abiotic factors on the two analysed phases of the *R. ferrugineus* flight, take-off and flight mobility, besides their flight behaviour and dispersal. The weevil adult flight begins with take-off, which was greater in males, increasing as temperature and solar radiation rose. The next analysed flight phase, flight mobility, was greater in females, and increases as temperature rose. The differences in flight behaviour and dispersal between *R. ferrugineus* males and females in the two analysed flight phases could be a consequence of their dispersal flight behaviour. The dispersal distances and times of *R. ferrugineus* adults are influenced by temperature and relative humidity, respectively. These adults tend to fly short distances (< 500 m), but are able to fly up to 7 km.

Moreover, their flight dispersal mainly occurs during the first week after release. This information complements the studies of flight performance conducted under laboratory conditions, allowing us to identify the dispersal behaviour of this pest with greater accuracy. By enhancing our knowledge about *R. ferrugineus* behaviour it is possible to better implement trapping systems or better define critical areas around *R. ferrugineus* outbreaks that will consequently allow for better intensive surveillance and better application of phytosanitary treatment strategies for palm trees.

5.6. Acknowledgements

General discussion

General discussion

The Red palm weevil, *Rhynchophorus ferrugineus*, has caused a significant decline in palm tree populations, both in natural palm groves and in palm trees used in landscaping. In areas infested by the weevil the survival of this plant is threatened, and therefore all sectors that are directly or indirectly involved in their growth are affected economically. The low efficacy of preventive and curative methods used against the pest is one of the main causes of its severity. Moreover, the lack of information about the behaviour of *R. ferrugineus*, especially about its mobility, contributes to an inefficient design of management programs. New knowledge about the behavioural aspects of the weevil allows for the improvement of the management techniques used against the pest, and to explain the potential causes of its rapid spread.

The ability of insects to distinguish between different colours and shapes allows them to be oriented or to detect a suitable host (Mazokhin-Porshnyakov 1969; Prokopy & Owens 1983), and therefore, indirectly influence their mobility and dispersal. Their visual preferences can be analysed using pheromone/food-based trapping systems which in addition to contributing to pest monitoring and control, have greatly enhanced our knowledge about the dispersal and behaviour of a large number of economically important pests (Hardee & Mitchell 1997; Howse *et al.* 1998; El-Sayed *et al.* 2006). The results obtained in the present doctoral thesis clearly demonstrate the preference of *R. ferrugineus* adults for black. The comparison of the spectral reflectance of studied colours shows that the wavelength spectrum of the most attractive ones and of analysed palm tree tissues were very similar. This preference of weevil adults towards these colours could be a consequence of their similarity with the colour of host tissues that allows it to detect a suitable host, or because of the cryptic habits of the insect. In addition, management programs based on a pheromone/food-based trapping system reduce the higher reproductive potential of *R. ferrugineus* populations. This reproductive strategy, determined by the continuous egg-laying of females throughout the year (Faleiro 2006a), adds more difficulties to its control. Although weevil sex ratio in natural populations is one female per male, adult captures in traps baited with olfactory attractants are female-biased, because of their higher attraction towards compounds used to bait traps. Therefore, this technique can help us to reduce indirectly the pest damage to palm trees.

The analysis of different aspects regarding the mobility of *R. ferrugineus* adults, such as their flying ability and flight dispersal, and how these are influenced by different biotic and abiotic factors, highlights the high flight

potential of this insect and how its dispersal occurs. Biotic factors such as the sex of weevil adults have a significant influence on their flight. *R. ferrugineus* males present a significantly higher flight potential, which may be the cause of their higher probability for take-off. Therefore, the question should focus on the possible causes of flight differences between sexes in the different analysed flight phases (take-off and flight mobility). The aforementioned differences may be a consequence of the sexual condition of the analysed adults, unmated males and females. Another hypothesis is that males have a greater flight potential because they may be the ones that first colonize new palm trees, producing then an aggregation pheromone which attracts adults of both sexes, but mainly weevil females (Hallett *et al.* 1993a; Wyatt 2003; Ávalos & Soto 2015). Females show a higher attraction than males to pheromones because of their requirement for mating places, new food sources and oviposition sites (Soroker *et al.* 2005). In this way, the founding of a new colony is more likely to be successful. Additionally, other biotic factors such as the adult body size and age do not influence their flight potential significantly. Nevertheless, the percentage of flying adults increases with age, as occurs in other insect species (Tanaka & Yamanaka 2009), probably because young insects need to finish developing the muscles required for flight.

Likewise, ecological factors are directly associated with the *R. ferrugineus* mobility, mating season, and efficacy in searching for food sources (Haris *et al.* 2014), and thus play an essential role in determining their activity (Faleiro 2006a). As the results obtained in the present thesis show, abiotic factors such as temperature, solar radiation, and relative humidity, are of great importance in the dispersal of this insect, both in their flight initiation and in their subsequent flight. An increase in the temperature and solar radiation produces an increase in the weevil flight initiation and in the distances covered. Further, an increase in relative humidity reduces its dispersion time. Faleiro (2006a) pointed out that flight of tropical weevils is expected to be higher with greater relative humidity values and moderate temperatures. Therefore, it is essential to know which biological and weather conditions influence the mobility of *R. ferrugineus* adults, to predict their dispersion using forecasting models, permitting the adaptation of the different strategies used in their management. In addition, the present results, together with the large number of countries with different climates where the pest is present, confirm the great adaptability of this tropical insect to different climatic conditions.

Further evidence for the high flight potential of dispersal of *R. ferrugineus* is the great distances covered by the insect and the quick dispersal times of adults.

R. ferrugineus are quite capable colonizing new areas and establishing new colonies, despite their tendency to fly short distances (less than 500 m). The weevil adults are able to cover 7 km under field conditions and 20 km according to studies carried out in the laboratory. Although as field studies show, it is very improbable that long flight distances obtained under laboratory conditions occur in the field. The European Commission (2011) establishes "demarcated areas", including outbreaks and a buffer zone of 10 km around it, where eradication measures must be applied. Considering the aforementioned information and the results obtained about *R. ferrugineus* flight distances, proposed management measures by European Commission seem appropriate regarding the areas that must be intensively monitored around an outbreak. Regarding dispersion times, results demonstrate that weevil adult dispersion occurs rapidly, during the first week after release. The same tendency has been observed in studies conducted by Abbas *et al.* (2006) and Oehlschlager *et al.* (1992) for dispersal times of *R. ferrugineus* and *R. palmarum*, respectively. This may indicate that *R. ferrugineus* adults tend to seek and find new hosts immediately after leaving a palm. The exact reasons for leaving are unknown, probably due to a lack of food resources in the host, or because their behaviour implies that a portion of the population must leave the colony to establish new ones.

Finally, the causes of their rapid spread in the last two decades, mainly in southern European countries, include the high dispersal potential of *R. ferrugineus* adults through flight, together with the great adaptability of the insect to new hosts and climatic conditions, the high abundance of their main hosts, and the substantial movement of plant material. Overall, the analysis of the flight and dispersal parameters will permit a better implementation of trapping grids, both to monitor and reduce populations of the weevil. Moreover, the detection of new infested areas, and the size definition of regions around *R. ferrugineus* outbreaks could be established and adjusted, permitting better intensive surveillance, and therefore better applications of phytosanitary treatment strategies.

Conclusions

Conclusions

Analysis of chromatic preference of *Rhynchophorus ferrugineus* adults

i. *Rhynchophorus ferrugineus* adults showed chromatic preference, being the black coloured traps, both with and without olfactory attractants, the ones that captured a higher number of adults when compared with red and white traps.

ii. The great similarity between the spectral reflectance of the black colour, and the fibres of *Phoenix canariensis,* may be one of the main reasons for the greatest preference of the *R. ferrugineus* adults to the black traps.

iii. Another possible reason for the greatest preference of the *R. ferrugineus* adults to black traps could be the greater heating of this colour. Despite its greater heating when compared with white traps, the difference of temperatures between both coloured traps occurred during a short time period and never exceed 2.9 °C.

iv. *R. ferrugineus* field populations showed a sex ratio of one female per male.

v. Traps baited with olfactory attractants captured more *R. ferrugineus* females regardless of their colour. This did not occur when the pheromonal and kairomonal attractants were not used; therefore, the olfactory attractants may be the responsible for their female-biased captures.

Study of the flying ability and flight behaviour and dispersal of *Rhynchophorus ferrugineus* adults

i. The flight potential and take-off of male weevils was significantly greater than for females. Despite this, recaptures in traps were female-biased, due to the higher attraction of females to olfactive attractants used to bait traps.

ii. Flight potential of *R. ferrugineus* adults was not significantly influenced by age or body size, although the percentage of flying weevils was higher in adults aged 8-23 days old than in adults aged 1-7 days old.

iii. The flight initiation of the *R. ferrugineus* adults was significantly influenced by temperature and solar radiation, increasing when these parameters rose.

iv. The recaptures of *R. ferrugineus* adults were significantly influenced by temperature, increasing when this climatic factor increased.

v. Most *R. ferrugineus* adults tended to fly short distances (below 500 m). However, a small percentage was able to travel long distances, up to 7 and 20 km, under field and laboratory conditions, respectively.

vi. Dispersion distances of the weevil adults were significantly higher when temperatures increased.

vii. The dispersal of the *R. ferrugineus* adults occurred mainly during the first week after their release. The dispersal time was significantly reduced when relative humidity increased.

Overall, the behavioural aspects analysed in the present doctoral thesis are of great relevance to improve the management of *R. ferrugineus* worldwide, and thus to protect and preserve the wealth that palm trees represents in our world. The study of the vision of the weevil adults through its chromatic preference, can allow for the addition of a new visual stimulus in pheromone/food-based trapping systems to attract and capture more insects, and hence to improve one of the main techniques to analyse their flight behaviour and mobility. Likewise, the analysis of different aspects of the flight of the *R. ferrugineus* adults, such as their flight potential and their flight behaviour, can allow for a better understanding of the weevil dispersal. This knowledge about weevil adult mobility will allow for a better implementation of trapping systems or a better definition of critical areas around *R. ferrugineus* outbreaks. Consequently, a better intensive surveillance and a better application of a phytosanitary treatments strategy of palm trees can be developed.

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