A survey of mosquitoes breeding in used tires in Spain for the detection of imported potential vector species

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ABSTRACT: The used tire trade has facilitated the introduction, spread, and establishment of the Asian tiger mosquito, *Aedes albopictus*, and other mosquito species in several countries of America, Africa, Oceania, and Europe. A strategy for detecting these imported mosquito vectors was developed in Spain during 2003-2004 by EVITAR (multidisciplinary network for the study of viruses transmitted by arthropods and rodents). A survey in 45 locations found no invasive species. Eight autochthonous species of mosquitoes were detected in used tires, including *Culex pipiens, Cx. hortensis, Cx. modestus, Anopheles atroparvus, An. claviger, Culiseta longiareolata, Cs. annulata,* and *Aedes caspius*. Dominant species were *Cx. pipiens* and *Cs. longiareolata*. *Aedes caspius* was found in only once, near its natural breeding habitat. Considering the recent discovery of an established population of *Ae. albopictus* in Catalonia, the increasing commerce of used tires in Spain for recycling, storage, and recapping might greatly contribute to the rapid spread of this species across the Iberian Peninsula. ***Journal of Vector Ecology* 32 (1): 10-15. 2007.**

Keyword Index: Aedes, albopictus, *Stegomyia,* Spain, Europe, used tires.

INTRODUCTION

Discarded tires have long been recognized as breeding sites for several species of mosquitoes in rural and urban habitats (Snow and Ramdsdale 2002), and also as sources for their dispersion (Lounibos 2002). The problem of tire accumulation, disposal, and mosquito breeding has increased along with increases in population and the number of vehicles per habitant (Baumgartner 1988), increasing the worldwide dispersal of invasive mosquito species.

Several exotic mosquito vector species were detected in Europe in recent decades: *Ae. atropalpus* in Italy (Romi et al. 1997), *Ae. japonicus* in France and Belgium (Schaffner 2004) and *Ae. albopictus* in several European countries. *Ae. albopictus* in particular is an invasive species (Juliano and Lounibos 2005) with a high vectorial capacity and a high physiological, genetic, and ecological plasticity (Hawley 1988).

By the transportation of tires, the Asian tiger mosquito, *Aedes albopictus* (*Stegomyia albopicta sensu* Reinert et al. 2004), has spread worldwide from its original distribution in southeastern Asia (Hawley 1988). An impressive dispersion in just a few years was observed in the United States (Moore and Mitchell 1997), Brazil (Santos 2003), and Italy (Romi 2001). This species is capable of transmission of at least 22 arboviruses and filarial worms, with a major role as a vector of dengue in Asia (Gratz 2004). Several viruses (Cache Valley, dengue, eastern equine encephalomyelitis, LaCrosse, Potosi) and *Dirofilaria sp.* (Gratz 2004) were isolated in wild populations in America. In Europe, only *Dirofilaria repens* was isolated in natural populations from Italy (Cancrini et al. 2003). *Aedes albopictus* has also been suspected as a vector for West Nile virus (Turell et al. 2001, Holick et al. 2002, Sardelis et al. 2002).

In Europe, *Ae. albopictus* was found to be established in Albania (Adhami and Reiter 1998) and Italy (Dalla Pozza and Majori 1992). Other populations were reported in France (Schaffner and Karch 2000), Serbia and Montenegro (Petric et al. 2001), Israel, Belgium (Schaffner et al. 2004), Switzerland (Flacio et al. 2004) and Greece (Samanidou-Voyadjoglou et al. 2005). Nowadays, most of Italy is colonized by this species (Romi 2001), and the efforts in control strategies have been unsuccessful in controlling its dispersion.

In August 2004, a population of *Ae. albopictus* was detected in northeastern Spain (Sant Cugat del Vallès, Barcelona) (Aranda et al. 2006). This established population does not appear to be associated with the storage of tires or product transport, which have been identified as carriers of larval stages (Knudsen 1995, Madon et al. 2002).

The development of a system of detection of imported vectors that was focused on tire commerce and storage could facilitate the early detection of the vectors and avoid an establishment of *Ae. albopictus*. Several authors have advised on the risk of expansion of this species in southern Europe (Knudsen 1995). Considering the capacity of *Ae. albopictus* to become a serious public health threat (Romi 2001), developing mosquito surveillance systems in threatened countries is crucial (Gratz 2004).

This work describes the results of a survey to detect imported mosquito vectors in Spain. The operations were aimed at monitoring the mosquito species breeding in tires by sampling campaigns at the dump and storage places. Simultaneously, the commercial movements of used tires across the country were administratively investigated.

MATERIALS AND METHODS

A database was first organized that contained information about companies dealing with importation, storage, recapping, recycling, and burning of tires. Data sources included information obtained from infested countries, data available on the Internet, Chambers of Commerce public indexes, and direct contacts to syndicate companies and environmental agencies. Based on these data, 45 locations were chosen from 25 different provinces (Figure 1). The site selection mostly focused on areas with the highest probabilities of occurrence of non-autochthonous mosquitoes, due to the known occurrence of used tires imported from infested countries such as the U.S.A., Italy, China, Thailand, or Japan (Figure 2). Hypothesized suitable climatic areas for the establishment of *Ae. albopictus* were also considered (Eritja et al. 2005).

Larvae and adults were collected and several variables were recorded including date, regional department, locality, elevation, and type of storage (shop, national recapping storage, imported used tires storage, retreaders, scrap tire dumps, storage for incineration, and adjoining marshes), as well as the type of habitat (peridomestic or forested). Peridomestic sites had tires located a few meters from areas in which humans were readily available to mosquitoes. These sites were near homes, sport venues, industrial parks, and stores adjacent to businesses that offered tire replacement services. Forested sites were ≥100 m distant from areas of human activity (Joy et al. 2003). Individual interviews with staff members of the companies provided information about commercial fluxes, origin, and destination of the used tires.

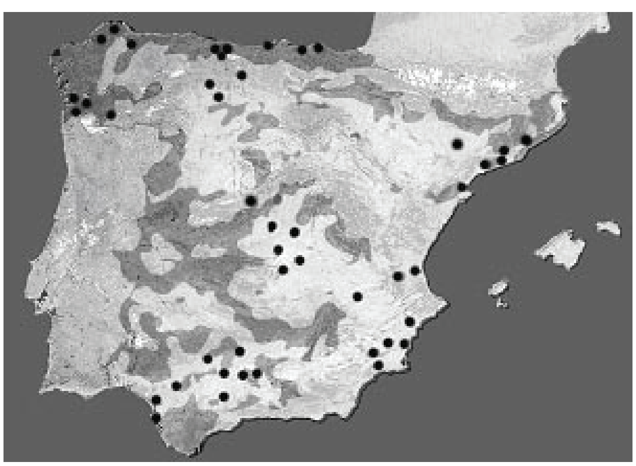

Figure 1. Collection sites of mosquitoes in used tire storages (black spots).The optimal climatic areas are drawn as gray areas (Eritja et al. 2005).

Larvae were collected using a classical dipper kept in 70% ethanol and were identified using the taxononomic keys of Schaffner et al. (2001). For each sample, we annotated the temperature of the water, origin of the tire, type of tire, fabrication date, and recapping date, if relevant. When collected, the adult specimens were captured using entomological aspirators and stored with silica gel. Specimens were permanently mounted for accurate identification to species and verification purposes. Larvae, pupae, and adults have been deposited in the collection of Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid.

The abundance was calculated as the mean number of mosquito larvae per tire in each location, and species richness by the number of species in each location. Statistical analysis of the influence of the different variables on both variables was performed using SPSS v 12.0 software. Data relating to the collections sampled in breeding sites other than tires were not included. Both described variables were transformed by the decimal logarithm for fitting a parametric distribution (Shapiro-Wilk test), followed by ANOVA.

RESULTS

No larvae of *Ae. albopictus* nor any other non-autochthonous mosquitoes were found in any of the stations sampled between October 2003 and August 2004. Among Diptera, the most common larvae found in this habitat belonged to the family Culicidae, accounting for 83% of 2,953 specimens collected in total (Figure 3). Larvae of Chironomidae were less abundant (15%). Some larvae of Syrphidae and adults of Ceratopogonidae and Micetophilidae were also found in tires. Adults of many other insect species were found drowned in the water inside tires, as these goods are quite similar to some entomological traps, such as Malaise or Pitfall (Service 1976). Larvae of Dixidae were collected only in marshes and stem water near tire stockpiles.

Eight autochthonous mosquitoes species from four genera were collected during the survey, either as larvae, as adults, or both: *Culex (Culex) pipiens* Linneaeus, 1758, *Cx. (Maillotia) hortensis* Ficalbi, 1889, *Cx. (Barraudius) modestus* Ficalbi, 1890, *Anopheles (Anopheles) atroparvus* Van Thiel, 1818, *An. (Anopheles) claviger* (Meigen, 1804), *Culiseta (Allotheobaldia) longiareolata* (Macquart, 1838), *Cs. (Culiseta) annulata* (Schrank, 1776), and *Aedes (Ochlerotatus) caspius* (Pallas, 1771).

Cx. pipiens and *Cs. longiareolata* were the most common species found in used tires (Figure 4). *Cx. hortensis* was less common and *Cs. annulata, An. claviger, Ae. caspius, Cx.*

Table 1. Abundance and ecological characteristic of the mosquitoes collected in the survey.

	Culex pipiens	*Culiseta longiareolata*	*Culex modestus*	*Culex hortensis*	*Culiseta annulata*	*Anopheles claviger*	*Anopheles atroparvus*	*Aedes caspius*
Abundance (%)	58	27	0.2	10	1	1	1	2
Vectorial potential	WN		WN?			Malaria	Malaria	WN?
Antropophilic	Yes	No	Yes	No	Yes	Yes	Yes	Yes
New report in Europe in tires	No	No	?	No	No	No	No	Under specific conditions
New regional distribution		Albacete Asturias	Murcia	A Coruña Cantabria Pontevedra Toledo	Cádiz			Alicante

Table 2. ANOVA between the abundance (logAbrel), species richness (logNsp), and type of habitat (Peridomestic and Forested).

		Sum of squares	fd	Square mean	F	Sig.
LogNsp	Between-groups	0.038	1	0.38	0.556	0.464
	Within-groups	1.1435	21	0.68		
	Total	1.473	22			
LogAbrel	Between-groups	0.262	1	0.262	1.732	0.202
	Within-groups	3.173	21	0.151		
	Total	3.435	22			

modestus, and *An. atroparvus* were found in only a few locations. Some new data were added to the known regional distribution of Culicidae in Spain (Table I).

The abundance and richness of mosquitoes in used tires was higher in the forested habitat than in the peridomestic habitat, although significant differences were not found among these variables (Figure 5, Table 2). The abundance and richness were also related to the type of storage, following a roughly similar pattern for both variables (Figure 6). Shops and national recapping stores showed a higher abundance and richness than other types of storage. The lowest richness was found in imported tire storages, and the lowest abundance was in the locations for incineration.

Administrative work disclosed that large quantities of tires (26,460,659 kg in 2003) are entering Spain from international trade sources (Anonymous 2003), as well as an equally increasing internal national trade of used tires (Figure 7).

DISCUSSION

All the species collected in tires had already been recorded in Spain (Eritja et al. 2000). *An. claviger, Cx. hortensis, Cs. longiareolata,* and *Cs. annulata* had previously been reported from tires in other European countries (Schaffner 2004). *Cx. pipiens* is commonly found in tires, as documented in France (Schaffner 2004). *Ae. caspius* was found in Jerez de la Frontera (Cádiz) in used tires that were very close to natural breeding areas. This species was already found in Greece (Schaffner 2004) under specific conditions.

For the first time in Europe, *An. atroparvus* is reported in this survey as breeding in tires, as found in Seseña (Toledo). This species was the most important malaria vector in Spain together with *An. labranchiae* (Eritja et al. 2000). The strong zoophily of *An. atroparvus* made it a malaria vector of lesser importance (Romi 1999), except under conditions of high density coupled with low standards of living (Zahar 1990). Thus, the utilization of tires by this species might not be relevant to the prevention of imported malaria.

As generalists, *Cx. pipiens* and *Cs. longiareolata* are the prevalent species in these breeding sites. *Cx. pipiens* is most common and has received some attention due to hypothesized displacement by the introduced *Ae. albopictus* in several habitats such as used tires (Juliano and Lounibos 2005). *Anopheles claviger, Ae. caspius, Cx. modestus,* and *An. atroparvus* were present in used tires in the vicinity of their natural breeding sites. Only a few adults of *Cx. modestus* were collected in a single tire storage location, so we do not consider its larval presence in used tires as common.

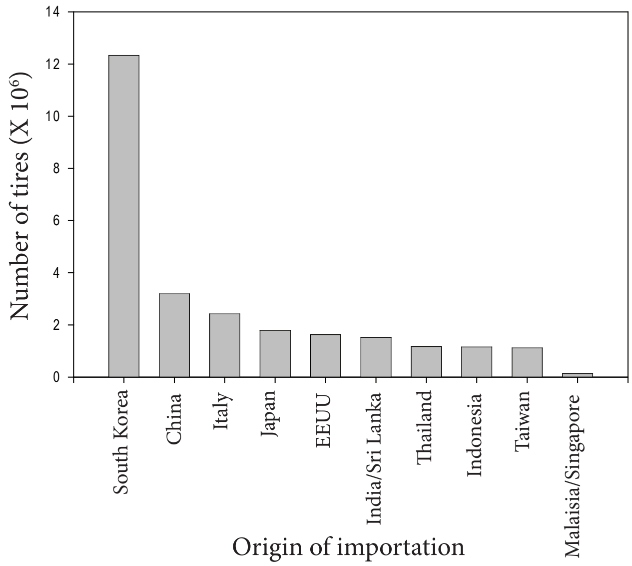

Figure 2: Importations of tires from infested countries in Spain.

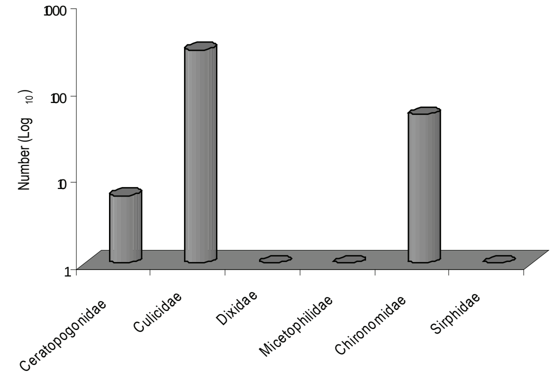

Figure 3. Individuals per family of Diptera collected.

Differences in abundance and species richness among habitats were not statistically significant. Forested habitats showed higher abundance and variety than peridomestic habitats. In a study in West Virginia, U.S.A., no general differences in population densities were reported among those habitats, and only varied between species (Joy et al. 2003). Our results corroborated this hypothesis. Abundance and richness of Culicidae are diverse between the different types of storage, neither showing significant patterns.

No evidence for the establishment of *Ae. albopictus* was detected in the tire trade in Spain. However, the recent discovery of an established population in the surroundings of Barcelona in August 2004 (Aranda et al. 2006), and the strong evidence of increasing commerce of used tires within Spain is a major concern. However, the international motorways near this introduction suggest a dispersal from European populations by adults in vehicles. In the U.S.A., Italy, and Brazil, *Ae. albopictus* has apparently been distributed through the commercial routes of the used tire trade (Reiter 1998). The situation may not be very different in this case, which would imply fast dispersion of the Asian tiger mosquito across the Iberian Peninsula.

A control strategy should be based on the grounds of used tires (source reduction) as well as keeping tire remnants dry (Hanson et al. 1996). Maintaining active mosquito sampling, local control operations where needed in the future, and product management could achieve containment of the expected spreading of *Ae. albopictus* caused by used tire transport (Novak 1995).

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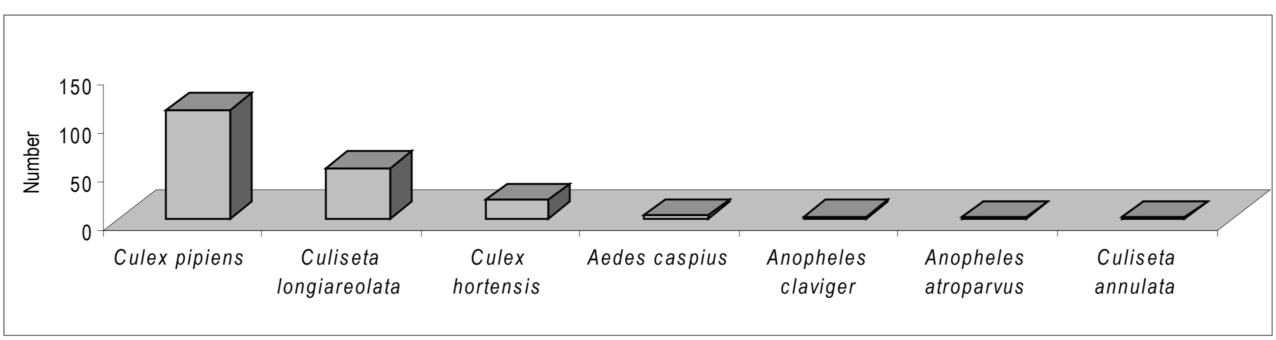

Figure 4. Abundance of the species of mosquitoes found in tires.

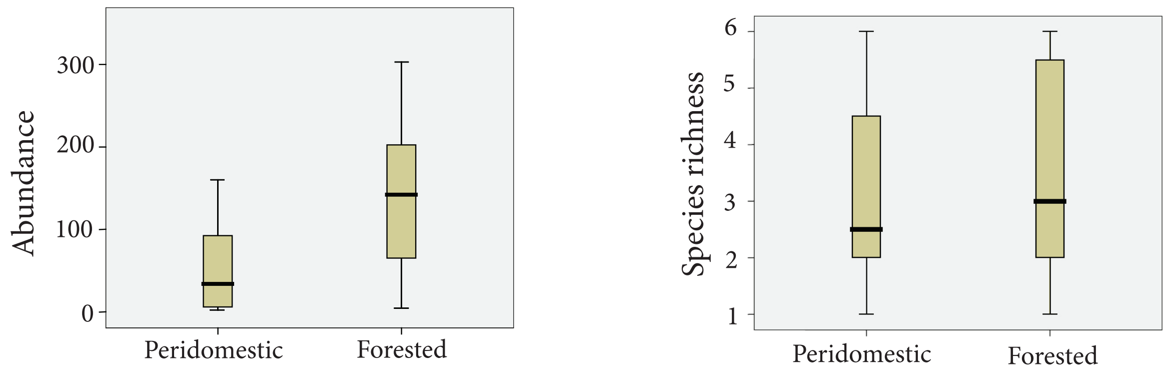

Figure 5. Box-Plot of the mean and variance of the abundance and richness among the type of habitat, the abundance, and species richness.

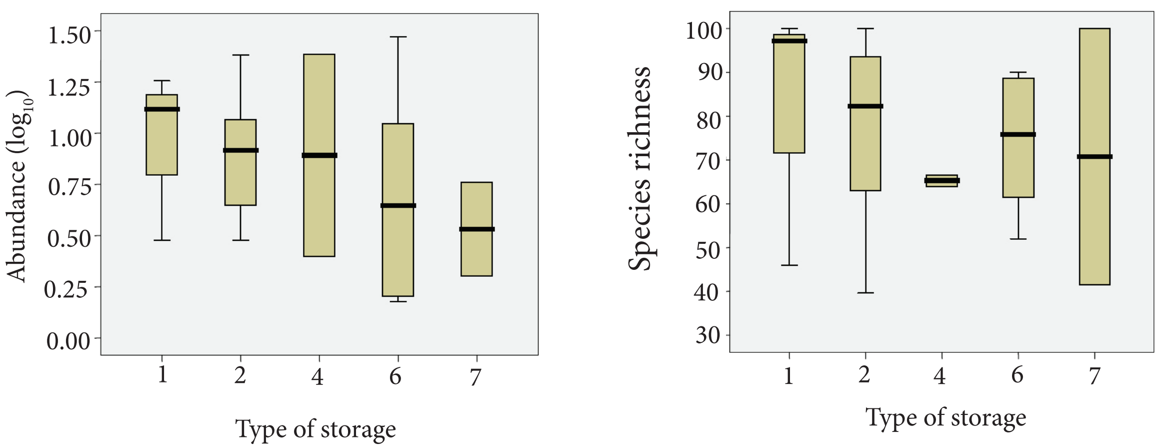

Figure 6. Influence of the type of storage (1=Shop, 2=national recapping storage, 4=imported tire storage, 6=dump, 7=storage for incineration) in the abundance and richness.

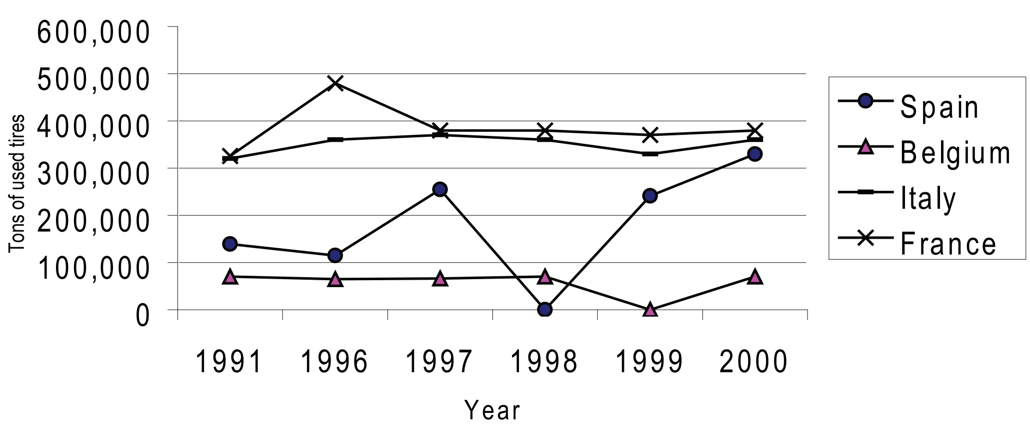

Figure 7. Evolution of national tire storages among the European countries colonized by *Aedes albopictus* (Anonymous 2003).

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