Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2010 8(3), 0-0
ISSN: 1695-971-X

Short communication. The detection of Israeli Acute Paralysis virus (IAPV), fipronil and imidacloprid in professional apiaries are not related with massive honey bee colony loss in Spain

E. Garrido-Bailón[1], R. Martín-Hernández[1], J. Bernal[2], J. L. Bernal[2], A. Martínez-Salvador[3], L. Barrios[4], A. Meana[5] and M. Higes[1]*

[1] *Bee Pathology Laboratory. Cenro Apícola Regional. JCCM. 19180 Marchamalo. Spain*
[2] *IU CINQUIMA. Analytical Chemistry Group. Facultad de Ciencias. Universidad de Valladolid. 47011 Valladolid. Spain*
[3] *Department of Epidemiology. Animal Health and Livest Co. (Tragsega). 28006 Madrid. Spain*
[4] *Statistics Department. CTI. Consejo Superior de Investigaciones Científicas. 28006 Madrid. Spain*
[5] *Animal Health Department. Facultad de Veterinaria. Universidad Complutense de Madrid. 28040 Madrid. Spain*

Abstract

Soil crusting is a serious problem in numerous irrigated soils of Aragon. It reduces water infiltration rate, seedling emThis study is presented in order to determine the presence and impact of some factors related with honeybee colony losses such as Israeli Acute Paralysis virus (IAPV) and certain pesticides. Samples (house worker bees and stored pollen from brood chamber) were selected from one hundred apiaries, half of them with a clear underpopulation, in accordance with region and time of the year. Total prevalence of IAPV either in spring or in autumn was 18% (CI95% = 9.9-26.0; $p < 0.0001$), no relationship between IAPV and depopulated colonies was established. Fipronil was only detected in two samples of stored pollen from asymptomatic colonies and imidacloprid was not detected in any sample. Like IAPV, neither fipronil nor imidacloprid appeared to be directly related with the generalized problem of colony death and honey bee losses in professional Spanish apiaries.

Additional key words: *Apis mellifera*, honeybee colony loss, neonicotinoids, pesticides, viruses.

Resumen

Comunicación corta. La detección de IAPV, fipronil e imidacloprida en colmenares profesionales no está relacionada con la masiva pérdida de colonias de abejas en España

Se llevó a cabo un estudio para estudiar la presencia y el impacto de algunos factores relacionados con las pérdidas de colonias de abejas, como el virus IAPV (virus israelita de parálisis aguda) y ciertos pesticidas. Se incluyeron en el estudio muestras (abejas adultas y polen almacenado de la cámara de cría) procedentes de 100 colmenares profesionales, la mitad de ellas con claros síntomas de despoblamiento de acuerdo con la región y el momento del año. La prevalencia total del virus IAPV fue del 18% (CI95% = 9,9-26,0; $p < 0,0001$), tanto en otoño como en primavera y su presencia no estaba directamente relacionada con la manifestación del síntoma de despoblamiento en las colonias de abejas. El fipronil se detectó solo en dos muestras de polen almacenado en la cámara de cría de las colmenas, en ambos casos en colonias asintomáticas. No se detectó imidacloprida en ninguna muestra. Nuestros resultados sugieren que IAPV, fipronil e imidacloprida no están relacionados con las muertes masivas de colonias de abejas detectadas en colmenares profesionales en España.

Palabras clave adicionales: *Apis mellifera,* fipronil, IAPV, imidacloprida, pérdida de colonias de abejas.

In recent years, high losses of worker honey bees have been reported worldwide. Accordingly, unexpected honey bee colony losses have been reported by beekeepers in Spanish apiaries since autumn/winter 2004, with mortality rates of up to 85% of the colonies in certain areas of the country. Indeed, from 2006, this

* Corresponding author: mhiges@jccm.es
Received: 08-12-09; Accepted: 28-04-10.

Abbreviations used: CCD (colony collapse disorder), IAPV (Israeli Acute Paralysis Virus).

problem has become more widespread across the entire Spanish territory. Some hypotheses regarding the origin of this phenomenon have been linked to the deleterious effect of certain pesticides (Chauzat *et al*., 2006), the possible introduction of a novel infectious agent, such as *Nosema ceranae* (Martín-Hernández *et al*., 2007; Higes *et al*., 2008; revised by Williams, 2009) or of the Israeli Acute Paralysis Virus (IAPV: Cox-Foster *et al.*, 2007), as well as other aetiologies (*e.g.,* environmental and climatic changes, radiation, the synergistic action of different agents). However, these latest hypotheses have not always been supported by well founded scientific data.

In this study, a total of 100 samples were included in the study from 100 different professional apiaries from 33 different Spanish provinces. The case definition for the disease was: the unusual presence of few adult bees from the hives with no or little builds up of dead bees in or in front of the colony compared with highly productive colonies in the same region and time of the year.

Each sample was composed of adult house worker honey bees ($n > 200$) and stored pollen (> 100 g) from brood chamber combs. The samples were collected by the veterinary services of beekeeping and included a report about the sanitary status of the colonies in the moment of sampling. Samples were obtained in the spring ($n = 50$) and autumn ($n = 50$) of 2006 (Figs. 1a and 1b) and while 55 of them came from diseased colonies ($n = 28$ spring samples, $n = 27$ autumn samples), the other 45 were from asymptomatic colonies ($n = 22$ spring samples, $n = 23$ autumn samples).

IAPV analyses were carried out as described previously (Maori *et al*., 2007) in the virus laboratory of Hebrew University of Jerusalem (Israel). The presence of fipronil and imidacloprid in stored pollen were carried out as described previously (Jiménez *et al.,* 2007; Higes *et al*., 2008).

To define the correlation between the presence of each one of these three agents and colony depopulation, three successive and independent contrasting hypotheses were proposed based on the Chi-squared asymptotic test and calculating the exact probabilities. The statistical analyses were all performed using SPSS 15.0.1.

Total prevalence of IAPV in the period of sampling was 18% (CI95% = 9,9-26,0; $p < 0.0001$), and 9 apiaries from 8 different Spanish provinces were IAPV positive in spring. Similar results were obtained in autumn 2006, when 8 apiaries were found as IAPV positive (Figs. 1a and 1b). No significant correlation was established between IAPV detection and depopulation symptoms (Chi-square, $p > 0.05$, Table 1). Fipronil was only detected in 2 samples of stored pollen from asymptomatic colonies in two provinces during spring (Fig. 1a), and it was not related with colony depopulation (Chi-square, $p > 0.05$). Imidacloprid was not detected in any of the stored pollen samples.

IAPV was first described in Israel (Maori *et al*., 2007) and it was associated with bee mortality, even though only some hives in an apiary were affected. It was suggested that genetic diversity among honey bees could explain the different susceptibility/resistance

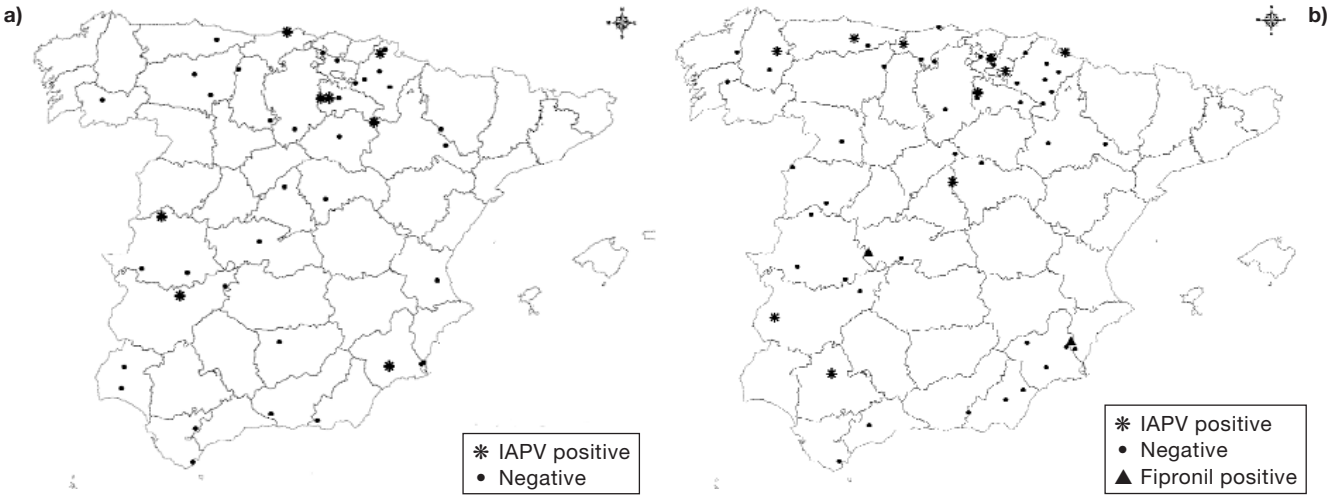

Figure 1. Collection sites of the Spanish apiaries monitores in a) spring 2006 (N = 50) and b) autumn 2006 (N = 50). • Samples negative to both Fipronil and IAPV. ▲ Samples positive to Fipronil. ✱ Samples positive to IAPV. None sample was positive to both of studied factors.

Table 1. Frequencies of detection of studied factors (IAPV and Fipronil) in colonies showing depopulation symptoms or asymptomatic

	Depopulation symptoms			Asymptomatic		
	Spring	Autumn	Chi-square	Spring	Autumn	Chi-square
IAPV	6/31	3/25	$p > 0.05$	3/19	6/25	$p > 0.05$
Fipronil	0/31	0/25	$p > 0.05$	2/19	0/25	$p > 0.05$

between hives in an apiary. Thus, it was proposed that IAPV is related to the problem of bee colony losses in the USA (Cox-Foster *et al*., 2007) and that it is a significant marker for Colony Collapse Disorder (CCD). However, further works found IAPV to be present in U.S. bees since prior to CCD, and that U.S. isolates of IAPV are not substantially different from those found in Israel (Chen and Evans, 2007). Indeed, no association was established between this virus and the severe winter losses in France, since the prevalence of IAPV in this country is 14% (Blanchard *et al*., 2008), indicating that other pathogens/factors may be responsible for the colony loss. In our research work we found no relationship between IAPV and depopulated colonies, our results are consistent with these latter conclusions. Indeed, the prevalence of IAPV (18%) is very low compared with other pathogens that are more prevalent in Spain such as *Nosema ceranae* (Higes *et al*., 2006; Martín-Hernández *et al*., 2007) and *Varroa destructor* (Bee Pathology laboratory, Centro Apícola Regional, unpublished data) do appear to affect the health status of our apiaries. Thus, IAPV does not seem to be a good marker for depopulation and colony loss, although the exact pathogenic role of this virus in Spanish honey bee colonies remains to be defined.

It has been proposed that bees come into contact with pesticides through the pollen and/or nectar they consume. Accordingly, stored pollen could be a chronic source of toxic to the bees (Faucon *et al*., 2005), which would mainly exert their influence in autumn/winter since stored pollen is practically the only pollen that the bees consume in this season (Seeley, 1985). The toxicity of sub-lethal doses of fipronil in honey bees has been demonstrated in laboratory studies but until now, not in field conditions (El Hassani *et al*., 2005). In Spain, its use has been authorized for sunflower (*Helianthus annuus*) and other crops since 2004. As a consequence, its application to sunflower seeds is very recent and it is limited to very specific geographic sites in central and southern Spain. Indeed, in 2007 fipronil residues were only detected in two samples but they were not directly related with unhealthy colonies. Imidacloprid is a neonicotinoid that is toxic to honey bees, and authorized in Spain to treat corn (*Zea mays*) crops, fruit trees and vegetable crops but their prevalence in stored pollen samples from Spain is very low as described previously and not related with colony losses from professional apiaries (Higes *et al*., 2009b). Actually the bee losses observed by beekeepers in France or Belgium are not attributed to imidacloprid and other causes such as pathogens or other unidentified synergistic factors have been considered in the origin (Schmuck, 2004; Faucon *et al.*, 2005; Nguyen *et al*., 2009).

Thus, like IAPV, neither of these two insecticides we tested appears to be directly related with the generalized problem of colony death and honey bee losses in professional Spanish apiaries.). Honey bee losses are likely to be provoked by different causes but the recent pandemic produces weakness, depopulation or death clinical features similar to those caused by the emergent pathogen *N. ceranae*, known to be a short-term bee pathogen (Higes *et al.,* 2007; Paxton *et al*., 2007; Chen *et al*., 2009a,b) and a long-term colony pathogen (Higes *et al*., 2008, 2009a; Korpela, 2009). Along similar lines, well-known pathogens like *V. destructor*, one of the main pathogens of *A. mellifera,* can cause a similar illness and act as a re-emerging agent if not suitably controlled. In our country, the combination of both pathogens and the highly pathogenic viruses associated with them could increase the risk of death in infected colonies, without pesticides exerting a significant effect (Higes *et al*., 2009b).

Acknowledgements

The authors wish to thank the Spanish beekeeper Associations for supplying the samples and Dr. Sela's lab from Hebrew University of Jerusalem for IAPV analysis. This study was supported by the Junta de Comunidades de Castilla-La Mancha (Consejería de Agricultura) and INIA (RTA2005-152 and RTA 2008-

00020-C02-01). R. Martín-Hernández was co-financed by the Junta de Comunidades de Castilla-La Mancha (JCCM) and INIA-European Social Found.

References

BLANCHARD P., SCHURR F., CELLE O., COUGOULE N., DRAJNUDEL P., THIÉRY T., FAUCON J.P., RIBIÈRE M., 2008. First detection of Israeli acute paralysis virus (IAPV) in France, a dicistrovirus affecting honeybees (*Apis mellifera*). J Invertebr Pathol 99, 348-350. doi: 10.1016/j.jip.2008.07.006.

CHAUZAT M.P., FAUCON J.P., MARTEL A.C., LACHAIEZE J., COUGOULE N., AUBERT M., 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. J Econ Entomol 99, 253-262. doi: 0022-0493/06/0253?0262$04.00/0.

CHEN Y., EVANS J.D., 2007. Historical presence of Israeli Acute Paralysis Virus in the United States. A Bee J, 147.

CHEN Y.P., EVANS J.D., MURPHY C., GUTELL R., ZUKER M., GUNDENSEN-RINDAL D., PETTIS J.S., 2009a. Morphological, molecular and phylogenetic characterization of *Nosema ceranae*, a Microsporidian parasite isolated fro the European honey bee *Apis mellifera*. J Eukaryot Microbiol 56, 142-147. doi: 10.1111/j.1550-7408.2008.00374.x.

CHEN Y.P., EVANS J.D., ZHOU L., BONCRISTIANI H., KIMURA K., XIAO T., LITKOWSKI A.M., PETTIS J.S., 2009b. Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. J Invertebr Pathol 101, 204-209. doi: 10.1016/j.jip.2009.05.012.

COX-FOSTER D.L., CONLAN S., HOLMES E., PALACIOS G., EVANS J.D., MORAN N.A., *et al*., 2007. A metagenomic survey of microbes in honey bee Colony Collapse Disorder. Science 318, 283-287. doi: 10.1126/science.1146498.

EL HASSANI A.K., DACHER M., GAUTHIER M., ARMENGAUD C., 2005. Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*), Pharmacol Biochem Behav, 82, 30-39,

FAUCON J.P., AURIÈRES C., DRAJNUDEL P., MATHIEU L., RIBIÈRE M., MARTEL A.C., ZEGGANE S., CHAUZAT M.P., AUBERT M., 2005. Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. Pest Manag Sci 61, 111-125. doi: 10.1002/ps.957.

HIGES M., MARTÍN-HERNÁNDEZ R., MEANA A., 2006. *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. J Invertebr Pathol 92, 93-95. doi: 10.1016/j.jip.2006.02.005.

HIGES M., GARCÍA-PALENCIA P., MARTÍN-HERNÁNDEZ R., MEANA A., 2007. Experimental infection of *Apis mellifera* honeybees with the Microsporidia *Nosema ceranae*. J Invertebr Pathol 94, 211-217. doi: doi:10.1016/j.jip.2006.11.001

HIGES M., MARTÍN-HERNÁNDEZ R., BOTÍAS C., GARRIDO-BAILÓN E., GONZÁLEZ-PORTO A.V., BARRIOS L., DEL NOZAL M., BERNAL J.L., JIMÉNEZ J.J., GARCÍA-PALENCIA P., MEANA A., 2008. How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environ Microbiol 10, 2659-2669. doi: 10.1111/j.1462-2920.2008.01687.x.

HIGES M., MARTÍN-HERNÁNDEZ R., GARRIDO-BAILÓN E., GONZÁLEZ-PORTO A.V., GARCÍA-PALENCIA P., MEANA A., DEL NOZAL M.J., MAYO R., BERNAL J.L., 2009a. Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. Environ Microbiol Reports 1, 110-113. doi: 10.1111/j.1758-2229.2009.00014.x.

HIGES M., MARTÍN-HERNÁNDEZ R., MARTÍNEZ-SALVADOR A., GARRIDO-BAILÓN E., GONZÁLEZ-PORTO A.V., MEANA A., BERNAL J.L., NOZALM.J., BERNAL J., 2009b. A preliminary study of the epidemiological factors related to honey bee colony loss in Spain. Environ Microbiol Reports, (accepted). doi: 10.1111/j.1758-2229.2009.00099.x.

KORPELA S., 2009. *Nosema* situation in Finland. Proc 5th COLOSS Conference. Montpellier, France, Sept. 14-15, p. 48.

JIMÉNEZ J.J., BERNAL J.L., DEL NOZAL M.J., MARTÍN M.T., MAYO R., 2007. Comparative study of sample preparation procedures to determine fipronil in pollen by gas chromatography with mass spectrometric and electron capture detection. J Chromatogr A 1146, 8-16. doi: 10.1016/j.chroma.2007.01.117.

MARTÍN-HERNÁNDEZ R., MEANA A., PRIETO L., MARTÍNEZ-SALVADOR A., GARRIDO-BAILÓN E., HIGES M., 2007. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Appl Environ Microbiol 73, 6331-6338. doi: 10.1128/AEM.00270-07.

MAORI E., TANNE E., SELA I., 2007. Reciprocal sequence exchange between non-retro viruses and hosts leading to the appearance of new host phenotypes. Virology 362, 342-349. doi: 10.1016/j.virol.2006.11.038.

NGUYEN B.K., SAEGERMAN C., PIRAD C., MIGNON J., WIDART J., THIRIONET B., VERHEGGEN F.J., BERKVENS D., PAUW E., HAUBRUGE E., 2009. Does imidacloprid seed-treated maize have an impact on honey bee mortality? J Econ Entomol 102, 616-623. doi: 10.1603/029.102.0220.

PAXTON R., KLEE J., KORPELA S., FRIES I., 2007. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie 38, 558-565. doi: 10.1051/apido:2007037.

SEELEY T.D., 1985. Honeybee ecology: a study of adaptation in social life. Princeton Univ Press, Princeton, NJ, USA.

SCHMUCK R., 2004. Effects of a chronic dietary exposure of the honeybee *Apis mellifera* (Hymenoptera: Apidae) to imidacloprid. Arch Environ Contam Toxicol 47, 471-478. doi: 10.1007/s00244-004-3057-6.

WILLIAMS B.A.P., 2009. Unique physiology of host-parasite interactions in microsporidia infections. Cell Microbiol 11, 1551-1560. doi: 10.1111/j.1462-5822.2009.01362.x.