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An extended laboratory test to evaluate the effects of pesticides on bumblebees. Preliminary results

Francesca INCERTI[1], Laura BORTOLOTTI[2], Claudio PORRINI[3], Anna MICCIARELLI SBRENNA[1], Giovanni SBRENNA[1]

[1] *Dipartimento di Biologia, Università di Ferrara, Italy*
[2] *Istituto Nazionale di Apicoltura, Bologna, Italy*
[3] *Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Italy*

Abstract

In recent years, several researches on the acute laboratory toxicity of pesticides to bumblebees have been carried. However, only a few researches on the toxicity of pesticides to bumblebees in semi-field and field-tests have bee conducted, and thus little information on this subject is available. The Italian Ministry of Agricultural Resources and Forestry financed the "AMA" Project (Ape-Miele-Ambiente) with the aim to intensify researches on toxicological impacts of pesticides on Apoidea. Within this project, we tried to develop an extended laboratory bioassay to evaluate the effects of insecticides and acaricides on bumblebees.

In order to record the behaviour and mortality of bumblebee workers after exposure to plants sprayed with pesticides, special cages offering the bees ample flying space, were built. Prior being transferred into the cages, potted flowering cucumber (*Cucumis sativus*) plants were sprayed with different pesticides at the field rate. Three different classes of pesticides were tested: phosphorganics (chlorpyriphos-methyl, dichlorvos, dimethoate, heptenophos and quinalphos), azotorganics (ethiofencarb and imidacloprid) and pyrethroids (acrinathrin and bifenthrin). In a first test, in each cage, thirty bumblebees were put into contact with the plants 1 hour after the treatment. For the next three days, every 24 hours, abnormal behaviour and bumblebee mortality were recorded. In a second test, the introduction of the bumblebees into the cages was delayed up to 24 hours after the treatment; behaviour and mortality were recorded over the next two days. The cages were housed in a greenhouse and temperature was recorded throughout the testing period.

Phosphorganics had the highest level of toxicity, especially dimethoate and quinalphos, whereas the lowest hazard was recorded for pyrethroids, for heptenophos and for ethiofencarb. Imidacloprid did not lead to high mortality rates, but interrupted the insects' activity for several hours. A consistent hazard decrease after delayed introduction was registered only for phosphorganics.

The method seems to provide reproducible and useful results for the evaluation of pesticide toxicity. It may be used to reinforce results obtained from common laboratory toxicity tests (contact and oral), and may help to evidence repellence effects of pesticides. However, in order to standardise this testing method, several aspects require improvement, and climatic conditions, such as temperature and photoperiod, should not vary during the testing period.

Key words: *Bombus terrestris*, pesticides, test methodology, extended laboratory test, acute contact toxicity.

Introduction

Bombus terrestris (L.) (Hymenoptera Apidae) is an important pollinator for both cultivated crops and wild plants. The misuse of pesticide can threaten its existence and decrease its beneficial effects on agriculture. Although *B. terrestris* is widely used as pollinator of glasshouse crops, such as tomatoes, paprika and courgettes, studies about the effects of pesticides on bumblebees are scarce (van der Steen, 2001a; Tasei, 2002). Conclusions are often inferred from already existing data on honeybees, but several morphological and biological differences between the two species exist, and therefore studies on the toxicological effects of pesticides specifically on bumblebees are necessary (Thompson, 2001; Tasei, 2002).

The Italian Ministry of Agricultural Resources and Forestry financed the "AMA" Project (Ape-Miele-Ambiente) with the aim to intensify both laboratory and semi-field researches on toxicological impacts of pesticides on Apoidea. The toxicity of several pesticides to *B. terrestris* in the laboratory was determined (Bortolotti *et al*., 2001; Marletto *et al*., 2003). Laboratory tests with the thirty most widely used pesticides in agriculture were conducted; the methods proposed by ICP-BR were used (van der Steen *et al*., 1996).

Few semi-field researches on bumblebees exist (review in van der Steen, 2001b). The hitherto developed cage tests (Gretenkord and Drescher, 1993, 1996) and greenhouse tests (Tasei *et al*., 2001; Tornier, 2001) require the use of queenright bumblebee colonies, not easy to achieve by relying only on commercial colonies. The aim of the Extended Laboratory Test (ELT) is to set up a simple and reproducible method to investigate the toxicity of pesticides to adult bumblebees in a more realistic condition than the laboratory.

Materials and methods

Adult bumblebees were collected from commercial queenright colonies consisting of about 100 workers. Colonies were kept in a climate room at 27°C in complete darkness. Medium size workers were collected operating under red light, and newly emerged workers were discharged. For each test, the same number of

workers was collected from two different colonies.

In order to record the behaviour and mortality of bumblebee workers after exposure to plants sprayed with pesticides, special cages offering the bees ample flying space, were built. The bottom of the box (plywood) measured 0.5 x 0.5 meters, while the top and the walls (plastic) were 1 meter high, with openings to allow the introduction of insects and plants.

We chose cucumber plants (*Cucumis sativus*), because they have large leaf surface, grow fast, and because they potentially attract bumblebees with both pollen and nectar. Seeding occurred around 40 days before the test. Plants were placed in a climate chamber at 24°C for germination. The soil (standard conditioning loam) of each pot was isolated with plastic film to prevent insects from finding a refuge. Prior to the beginning of the test, the plants were provided with a drop irrigation system, connecting the upper part of the cage with the pots. Once in bloom (approximately 12 flowers per plant), each plant was sprayed with 25 ml of a given pesticide solution at field dose. Other plants were sprayed with water, thus acting as control.

In each cage, containing two treated plants, thirty bumblebee workers were introduced. To prevent lack of nectar in case of insufficient production by the plants, each cages was also provided with 30 grams of commercial sucrose solution. All the operations following the spray occurred in a greenhouse.

Two different tests were performed: in the first test, bumblebees were introduced in the cage one hour after the treatment (ELT), while in the second test the introduction of the bumblebees was delayed up to 24 hours after the treatment (Delayed ELT = DELT). In both tests, temperature, abnormal behaviour and bumblebee mortality were recorded until the third day after the treatment, i.e. 24, 48 and 72 hours after the introduction of the insects in ELT, and 24 and 48 hours after the introduction of the insects in DELT.

Three different classes of pesticides were tested in ELT: phosphorganics, azotorganics and pyrethroids (table 1); only dimethoate and imidacloprid were tested in DELT. One cage containing plants sprayed with water acted as control.

Results

Behaviour of bumblebees in the cages

The bumblebee workers adapted well to the cage conditions. They were attracted by the flowers, and *C. sativus* seems to be a suitable plant for ELT and DELT, since open flowers were available throughout the testing period (three days). Anyway, the number of flowers was not sufficient for the foraging bumblebees. In fact, the bees consumed large amounts of the supplied sucrose solution (not measured value).

During the last hours of the test, flight activity was almost absent and the bumblebees tended to concentrate in the corners of the cage, aggregated in groups. In many cases they were observed building wax cups on the bottom of the cage.

Toxicity of pesticides

In the ELT, phosphorganics had the highest level of toxicity, especially dimethoate, chlorpyriphos-methyl and quinalphos (85% after 72 hours), whereas a lower hazard was recorded for the pyrethroids, for heptenophos and for ethiofencarb (figure 1, 2 and 3).

With phosphorganics, mortality increased gradually during the three days following the spray; the same occurred with the pyretroids, but at lower rates. With azotorganics two different behaviours were observed: with ethiofencarb mortality did not exceed 15% even three days after the introduction of the bumblebees, whereas with imidacloprid mortality was around 30% already after 24 hours, and did not increase during the subsequent two days. Even though with imidacloprid mortality rates were lower than those recorded with phosphorganics, the product interrupted the bees' activity for several hours, causing a temporary "knock down effect" (in many cases bumblebees seemed to be dead at the first mortality check, but they recovered in the following days).

Mortality in the control was always below 10%, as required by official guidelines and common protocols (OEPP/EPPO, 1992, 1993; van der Steen *et al.*, 1996).

Table 1. Tested pesticides and relative field dose.

Active ingredient	Trade name	Field dose	Percentage of a.i.
acrinathrin	Rufast®	50 ml/hl	15.69
bifenthrin	Brigata®	150 ml/hl	11
chlorpyriphos-methyl	Reldan 22®	200 ml/hl	22.1
dichlorvos	Dedevap®	200 ml/hl	50
dimethoate	Dacol L 40®	200 ml/hl	38
ethiofencarb	Croneton®	150 ml/hl	46
heptenophos	Hostaquick®	100 ml/hl	50
imidacloprid	Confidor 200 SL®	50 ml/hl	17.8
quinalphos	Ekalux®	150 ml/hl	25

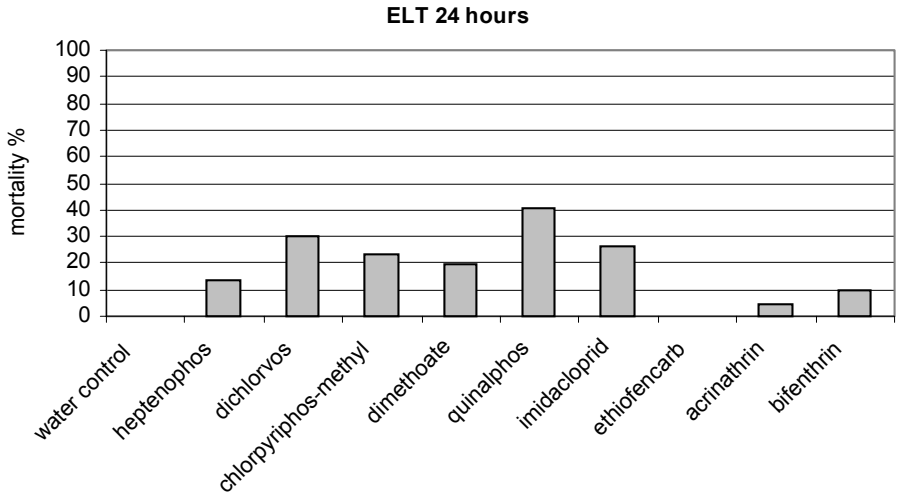

Figure 1. Mortality in ELT (Extended Laboratory Test) 24 hours after the introduction of the bumblebees.

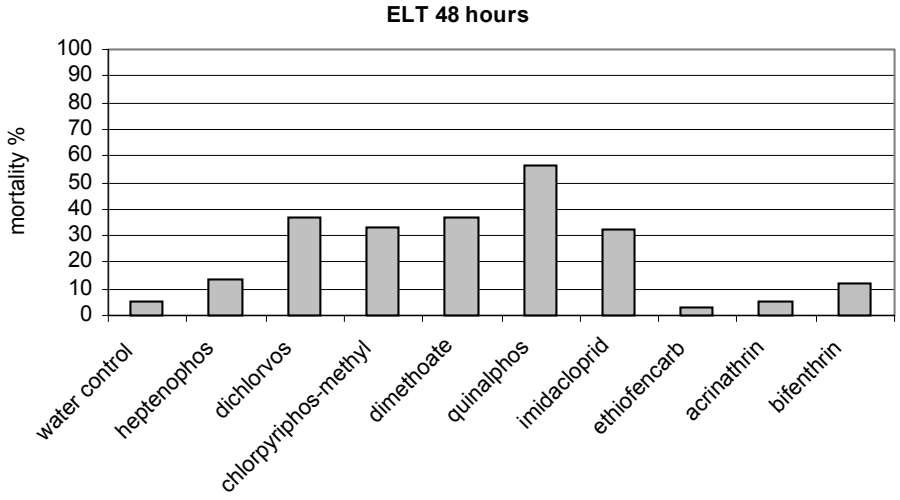

Figure 2. Mortality in ELT (Extended Laboratory Test) 48 hours after the introduction of the bumblebees.

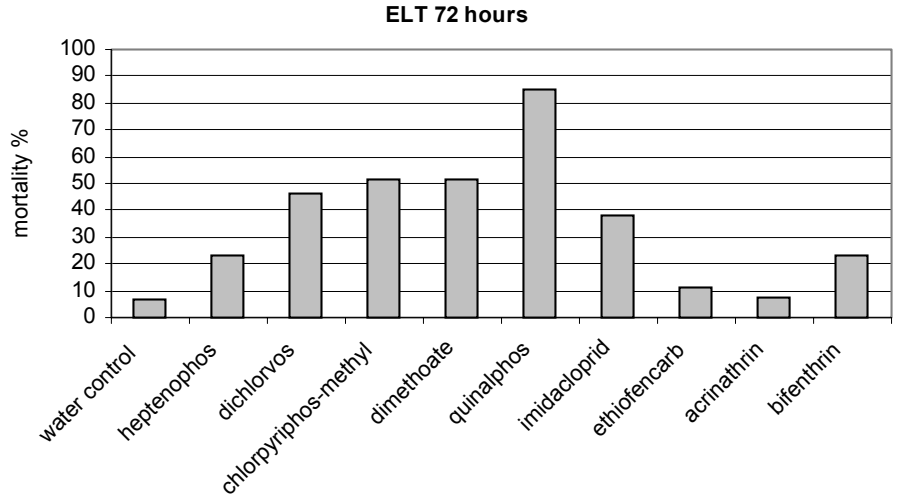

Figure 3. Mortality in ELT (Extended Laboratory Test) 72 hours after the introduction of the bumblebees.

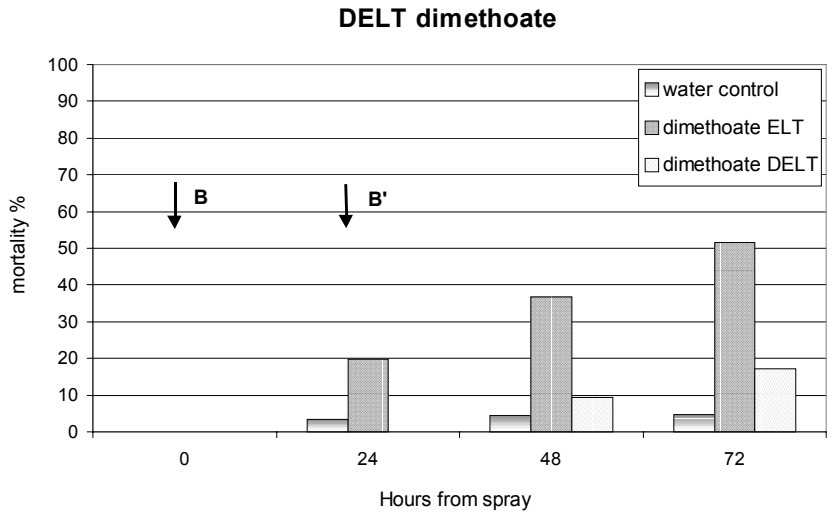

Figure 4. Mortality in DELT (Delayed Extended Laboratory Test) with dimethoate. B = introduction of bumblebees in ELT; B' = introduction of bumblebees in DELT.

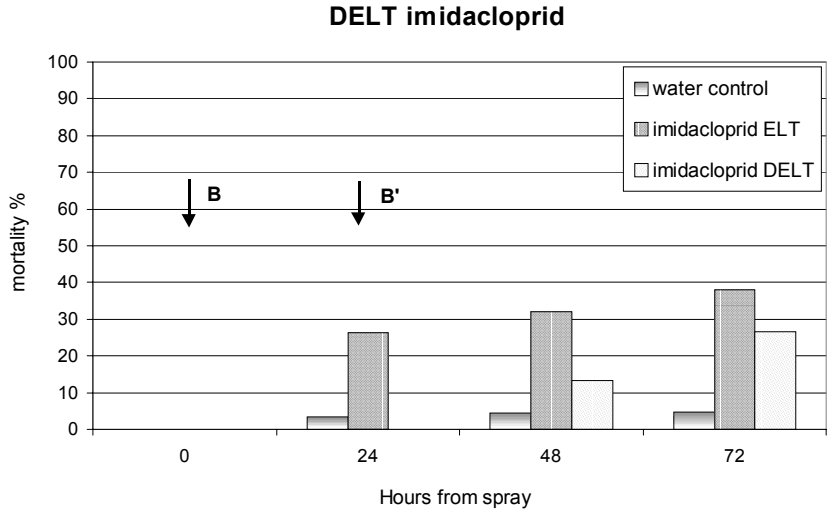

Figure 5. Mortality in DELT (Delayed Extended Laboratory Test) with imidacloprid. B = introduction of bumblebees in ELT; B' = introduction of bumblebees in DELT.

The results obtained with the delayed methodology (DELT) in comparison with those obtained in ELT for the two active ingredients, dimethoate and imidacloprid, are shown in figures 4 and 5. With dimethoate, the delayed bumblebee introduction determined a decrease in toxicity on the third day: in DELT bumblebees mortality was 35% lower than in ELT (figure 4). With imidacloprid, the decrease in bumblebees mortality 72 hours after the treatment is not as high as with dimethoate (figure 5).

Based on our results of ELT and those of the Acute Contact Tests (ACT) carried out during the AMA project (Bortolotti *et al*., 2001), we classified the active ingredients into hazard classes, according to the classification used for beneficial insects (Hassan, 1995). The classification is listed in table 2.

With four active ingredients, the hazard class was higher in the ACT than in the ELT (the two pyretroids, and dichlorvos and quinalphos among the phosphorganics).

The two phosphorganics, chlorpyriphos-methyl and heptenophos, and the azotorganics show similar toxicity in both ELT and ACT.

Only the hazard class of dimethoate seems to be higher in the ELT than in the ACT, and further studies are warranted for confirmation.

Table 2. Classification of toxicological risk.

Active Ingredient	ELT	ACT
acrinathrin	+	++
bifenthrin	+	++
chlorpyriphos-methyl	++	++
dichlorvos	++	++++
dimethoate	+++	++
ethiofencarb	+	+
heptenophos	+	+
imidacloprid	++	++
quinalphos	+++	++++

ACT hazard classes		ELT hazard classes	
< 30 % = low	(+)	< 25 % = low	(+)
30-80% = weak	(++)	25-50% = weak	(++)
80-99% = moderate	(+++)	51-75% = moderate	(+++)
> 99 % = high	(++++)	>75% = high	(++++)

Discussion

The aim of this study was the establishment of on extended laboratory test that could be routinely applied to establish the hazard of pesticides to bumblebees.

The method seems to provide reproducible and useful results for the evaluation of pesticide toxicity, which can be combined with the results from acute laboratory toxicity tests (contact and oral). Being a first approach to a semi-field test, ELT could be included in the Decision Making Scheme for the environmental risk assessment of plant protection products (OEPP/EPPO, 1993). However, in order to standardise this testing method, several aspects should be improved:

- Test plants. Even though seeds were sawn contemporaneously and seedlings were kept in a climate chamber, the plants did not always have a sufficient number of flowers at the same time, thus hindering test planning. Furthermore, as reported above, the number of flowers was not sufficient, and the bumblebees could not rely only on the pollen and nectar of the flowers as food supply. A sucrose solution was thus provided as an alternative food source. We recommend the use of plants with more open flowers, or the use of more plants per cage. It is obviously important to avoid the use of plant protection products on the test plants. If necessary, natural enemies should be used. The few infestations by aphids that occurred during our tests, were controlled with *Harmonia axyridis* (Pallas).
- Test insects. The selection of the bumblebee workers should be standardised: criteria, such as weight and thorax width of the bees, should be considered (van der Steen, 2001b).

Both the workers building wax cups in the cages and the lack of activity on the third day suggest that the bees need a nesting side. The cage could be connected to a small colony; in this way, more accurate observations on the insects' behaviour could be made, and the effects on brood and colony development could be investigated.

A video record support during the first hours of the test could provide further useful information, and would help to evidence possible repellent effects of the pesticides.

- Test conditions. Difficulties in controlling the climatic conditions in the greenhouse containing the cages emerged. Seasonal variations determined high fluctuations in temperature and photoperiod among replicates. Housing the cages in a climate room during the test could help to standardise test conditions.

Notwithstanding the ELT and DELT must be improved, both tests evidenced that pesticides affect the foraging activity and survival of bumblebees. Pesticides may therefore also curtail the pollination activity of the colony. Adequate bumblebee hive management practices, such as a delayed introduction of colonies after chemical treatments in the greenhouse, are essential. However, a 24 hour-delay may not be sufficient to actually contain the risks of pesticide exposure.

In conclusion, also on bumblebees, for each pesticide, mortality should be established, and repellence or sublethal effects should be investigated.

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References

BORTOLOTTI L., GRAZIOSO E., PORRINI C., SBRENNA G., 2001.- Effect of pesticides on the bumblebee, *Bombus terrestris* L. in the laboratory.- In: *Proceedings of the 7th International Symposium "Hazards of pesticides to bees"*, September 7-9, 1999, Avignon, France (BELZUNCES L. P., PÉLISSIER C., LEWIS G. B., Eds). *Les Colloques de l'INRA*, 98: 217-225.

GRETENKORD C., DRESCHER W., 1993.- Development of a cage test method for the evaluation of pesticide hazards to the bumble bee *Bombus terrestris* L..- In: *Proceedings of the 5th International Symposium on the Hazards of Pesticides to Bees*, October 26-28, 1993, Wageningen, The Netherlands (HARRISON E.G., Ed.). Appendix 20.

GRETENKORD C., DRESCHER W., 1996.- Laboratory and cage test method for the evaluation of the effects of insect growth regulators (Insegar®, Dimilin®) on the brood of *Bombus terrestris* L..- In: *Proceedings of the 6th International Symposium on Hazards of pesticides to bees*, September 17-19 1996, Braunschweig, Germany (LEWIS G. B., Ed.). Appendix n. 29.

HASSAN S. A., 1995.- Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS Working Group "Pesticides and Beneficial Organisms".- *EPPO Bulletin*, 15: 214-255.

MARLETTO F., PATETTA A., MANINO A., 2003.- Laboratory assessment of pesticide toxicity to bumblebees.- In: *Proceedings of the 8th International Symposium "Hazards of pesticides to bees"*, September 4-6, 2002, Bologna, Italy (PORRINI C., BORTOLOTTI L., Eds). *Bulletin of Insectology*, 56 (1): 155-158.

OEPP/EPPO, 1992.- Guideline for the efficacy evaluation of plant protection products - Side-effects on honeybees.- Directive PP 1/170 (2). *Bull. OEPP/EPPO*, 22: 203-216.

OEPP/EPPO, 1993.- Decision making scheme for the environmental risk assessment of plant protection products. Chapter 10 Honeybees.- *Bulletin OEPP/EPPO*, 23: 151-165.

STEEN J. J. M. VAN DER, 2001a.- Review of the methods to determine the hazard and toxicity of pesticides to bumblebees. *Apidologie*, 32: 399-406.

STEEN J. J. M. VAN DER, 2001b.- The effect of the size of the bumblebee (*Bombus terrestris* L.) on the susceptibility to the pesticide dimethoate 40%.- In: *Proceedings of the 7th International Symposium "Hazards of pesticides to bees"*, September 7-9, 1999, Avignon, France (BELZUNCES L. P., PÉLISSIER C., LEWIS G. B., Eds). *Les Colloques de l'INRA*, 98: 213-216.

STEEN J. J. M. VAN DER, GRETENKORD C., SCHAEFER H., 1996.- Method to determine the acute oral LD_{50} of pesticides for bumblebees (*Bombus terrestris* L.).- In: *Proceedings of the 6th International Symposium on Hazards of pesticides to bees*, September 17-19, 1996, Braunschweig, Germany (LEWIS G. B., Ed.). Appendix n. 27.

TASEI J. N., RIPAULT G., RIVAULT E., 2001.- Effect of Gaucho® seed coating on bumblebees visiting sunflowers.- In: *Proceedings of the 7th International Symposium "Hazards of pesticides to bees"*, September 7-9, 1999, Avignon, France (BELZUNCES L. P., PÉLISSIER C., LEWIS G. B., Eds). *Les Colloques de l'INRA*, 98: 207-212.

TASEI J. N., 2002.- Impact of agrochemicals on non-*Apis* bees.- In: *Honey bees: Estimating the Environmental Impact of Chemicals*. (DEVILLERS J., PHAN-DELÈGUE M. H., Eds), Taylor & Francis, London and New York, 101-131.

THOMSON H. M., 2001.- Assessing the exposure and toxicity of pesticides to bumblebees.- *Apidologie*, 32: 305-321.

TORNIER I., 2001.- Side effects of an insect growth regulator on bumble-bees and honey-bees.- In: *Proceedings of the 7th International Symposium "Hazards of pesticides to bees"*, September 7-9, 1999, Avignon, France (BELZUNCES L. P., PÉLISSIER C., LEWIS G. B., Eds). *Les Colloques de l'INRA*, 98: 299.

Corresponding author: Giovanni SBRENNA, Dipartimento di Biologia, Università di Ferrara, via Borsari 46, 44100 Ferrara, Italy. E-mail: sbr@unife.it