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Review article

Review of the methods to determine the hazard and toxicity of pesticides to bumblebees

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Abstract – Methods to determine the impact of pesticides on bumblebees are described. They are classified into laboratory tests to determine the acute toxicity and the hazard to bumblebees, (semi) field tests, and brood tests. The reproducibility and the significance of the data for practical purpose are discussed. Standardized laboratory toxicity tests supply reproducible data. In hazard tests, both in the laboratory and semi field tests, the exposure is not proportionate to the number of adult insects and the brood. Field tests provide realistic data on the hazard of a pesticide to bumblebee colonies but when the results are interpreted it must be taken in account that the test plot is only a portion of the total foraging area of a bumblebee colony. In a brood nest, due to the disorderly structure, only major effects can be recognized. Laboratory rearing of bumblebee brood should be developed to produce a standardized brood test that supplies reproducible data.

Bombus terrestris / pesticides / toxicity / LD_{50} tests / laboratory test / semi field tests / brood test / sublethal effect

1. INTRODUCTION

Over the last decade, bumblebees (*Bombus* sp.) have played an increasingly important role in horticulture as pollinators. In the year 2000, about 40 000 bumblebee colonies were used for pollination in horticulture in The Netherlands alone. Information about the toxicity of pesticides for bumblebees is essential for successful pollination management. Several tests to determine the impact of pesticides on bumblebees have been developed recently. These tests are classified as laboratory tests, (semi) field tests, brood tests and tests to determine the sublethal effects. The methods of each test are discussed.

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2. LABORATORY TESTS

Laboratory tests are conducted to determine the acute toxicity of a pesticide to bumblebees, expressed as a lethal dose (LD_{50}). They are also conducted to determine the hazard of a pesticide when used in the recommended concentration in the field. The lethal dose (LD_{50}) is the dose at which 50% of the test bumblebees die within a defined period.

2.1. Acute oral LD_{50} test

In 1993 Gretenkord, Schaefer and van der Steen formed a working group of the International Commission for Plant-Bee Relationships (ICPBR) to develop methods to determine the acute oral and contact LD_{50} for bumblebees. The tests described by Gretenkord (1997) and van der Steen et al. (1996) are similar in outline and are described hereafter as one test. This test is derived from the OECD test (1992). Bumblebee (*Bombus terrestris* L.) workers are fed individually with a range of concentrations of a pesticide dissolved in a 50% sugar solution. Each individual bumblebee is fed 10 µl of the test solution. Prior to offering the test solution, the bumblebees are starved for 2–3 hours. Per concentration, 30 bumblebees must have consumed the test solution within 2 hours, the maximum intake time. The LD_{50} is calculated with the mortality data, obtained 24, 48 or 72 hours after intake. The LD_{50} is expressed in µg a.i. or formulation/bumblebee.

2.2. Acute contact LD_{50} test

The acute contact LD_{50} tests described by Tasei (1987, 1993, 1994), van der Steen (1994 and 1996) and Gretenkord (1997) are fundamentally similar. In the tests, bumblebee workers (*B. terrestris*) are narcotised with CO_2 and 1 µl of test solution (pesticide dissolved in acetone) is administered on the thorax. The observation time is 72 hours. A range of concentrations is tested and at least 30 bumblebees workers are treated per concentration. The LD_{50} is expressed in µg a.i. or formulation/bumblebee. Gretenkord and van der Steen recommend that the test solution be administered on the ventral part of the thorax between the 2nd and 3th pair of legs respectively, to avoid grooming attempts using their legs on the dorsal part of the thorax by recovering individuals.

2.3. Laboratory hazard test

Schaefer (1993) describes laboratory hazard tests via oral, contact and respiratory routes. These tests, developed for the honeybee, are derived from Stute (1991). For the oral route, groups of 10 *B. terrestris* bumblebees (males plus workers) are fed 2 mL of a 1% test substance in honey solution. If this concentration shows effects, 0.5% and 0.1% concentrations are tested. The intake time is 24 hours, based on the average amount a bumblebee consumes of a 150 µl 30% sucrose solution within 24 hours. The precise amount consumed is determined by repipetting the test solution left after 24 hours. The starvation period, prior to feeding is 2 hours.

For the two contact tests and the respiratory test, the test substance is administered in a concentration that is twice the recommended dose for field application. For the first contact route, groups of 10 bumblebees are housed for 72 hours in cages covered inside with 156 cm^2 paper sprinkled with 20 ml test solution in water and afterwards air-dried. In the second contact test, the bees are sprinkled directly with 1 mL test substance in water. In the laboratory hazard test via the respiratory route, bumblebees are exposed for 72 hours to the test substance in water by placing a test cage that has holes in the bottom over a petri dish containing the test solution.

Sechser (1996) describes a sequential test scheme that includes a feeding test, three

contact tests, and a cage test. The test substance is applied in the recommended dose for field application. In all tests, the test groups consist of 5 bumblebee workers (*B. terrestris*). In the feeding test, the test groups are housed in plexiglass cylinders and are fed 2 mL test solution per day for 5 days (pesticide in the recommended dose in fructose solution). In the first contact test, the bottom of the cylinder is sprayed with pesticide solution in the recommended concentration and later dried. Next bumblebee workers are housed in the cylinder. In the second contact test 1 µl test solution is dripped on the thorax of the bumblebee, and in the third contact test the bumblebees are dipped into the test solution for 5 seconds. In the feeding test and the contact tests, the observation period is 5 days.

2.4. Comments on the laboratory tests

As mentioned above, two types of laboratory tests are used. In the toxicity test, the impact of a pesticide, irrespective of the recommended dose for field application, is determined. The advantage of this test is that the impacts of pesticides, expressed in the LD_{50}'s, are comparable to each other. To translate the LD_{50} into the impact of the pesticide in the field, data obtained from field tests and practice and the LD_{50} must be linked to each other. This is done for honeybees (*Apis mellifera* L.) by calculation of the hazard ratio, as described in 'Organisation Européenne et méditerranéenne Pour la Protection de Plantes (OEPP)/European and Mediterranean Plant Protection Organization (EPPO), 1992, "Decision making scheme for the environmental risk assessment of plant protection products" Chapter 10, Honeybees'. As bumblebee tests are relatively new, data from field tests and practice are scarce so far.

In contrast to the toxicity test, the hazard test determines the hazard of a pesticide to bumble bees at the recommended concentration for field purposes. However, this pseudo-realistic approach is questionable. Continuous exposure, both oral and contact, during 24 hours or longer results in extreme overexposure and does not give a realistic picture of the impact of the pesticide. The main disadvantage is that the data obtained from these tests can not be compared to each other.

Individual feeding and group feeding: Bumblebees do not share food by trophallaxis. There is great variation in the amount of test solution that a bumblebee consumes at one time. It is obvious that in group feeding, the test solution will not be equally shared among the bees. Together with the phenomenon of torpor, to be discussed later, it is clear that group feeding is unsuitable for bumblebee testing.

Torpor: During laboratory tests, bumblebees frequently fall into a state of torpor. This has consequences for the feeding tests. Gretenkord (1997) and van der Steen (1996) disrupt this behaviour by switching the light on and off and by moving the test cups.

Unlike honeybees, individually caged bumblebees do have difficulties in finding the test solution. They seem unable to detect the location of the test solution and they must stumble across it to find it. The test solution has to be offered outside the cage or via a micropipette to prevent pollution of the test solution, Gretenkord (1997) and van der Steen (1996) mark the opening in the cage through which the individual bumblebee has to take in the test solution with sugar solution.

Weight influence upon sensitivity: There is a significant link between the size of the bumblebee and its susceptibility to a pesticide. The bigger the bumblebee, the less susceptible it is to a pesticide (Drescher, 1991; van der Steen, 1994; Philipsen, unpublished data). That is why overly small and overly big bumblebees must be excluded from a test group (van der Steen, 1996; Gretenkord, 1997). There is a significant link between weight and size of the thorax, so weight is a good criterion for selection.

Although the bumblebees can consume up to 25% of their weight (Schaefer, 1993), it has been observed that the effect of differences in crop content is limited when the bees are taken directly from the colony (Philipsen, unpublished data).

Males/workers: Since bumblebee males also visit flowers and participate in pollination activity, it would be interesting to determine the impact of a pesticide on these bumblebees. But, as long as there are no data available concerning the impact on males, males and workers should not be mixed and the standard tests should be carried out on females only.

Duration of the test: In acute oral toxicity tests, it has been observed that the effect of organophosphates increases over time (Drescher, 1991; Philipsen, unpublished data). Because of this phenomenon, the observation time must be at least 72 hours.

Bumblebee species: With respect to methomyl, here is no significant difference in the sensitivity of *B. terrestris* and that of *Bombus lapidarius* L. (Drescher, 1991).

3. (SEMI) FIELD TESTS

3.1. Protocols

Gretenkord (1996, 1997) described a tent test with standardized colonies. This protocol is an improved version of his tent test of 1993 in which he worked with colonies of up to 80 workers. The improvements will be discussed in "comments on (semi) field tests". In the protocol of 1997, a healthy queenright bumblebee colony (*B. terrestris*) with at least 100 workers is placed in a cool box in the ground, outside the tent, in order to protect it from overheating. By means of a tube, this box is connected to a gauze tent of $3 \times 4 \times 2$ meters placed over *Phacelia tanacetifolia*. When a constant foraging activity of about 10 workers is reached, the connection tube is closed during the day, the colony is standardized, and after that,

the crop is sprayed. The standardized colony consists of a queen, about 10 foragers, 5 nurse bees, 4–6 egg clumps, a clump with 1–2 day old larvae, a clump with 3–4 day old larvae, a clump with 5–6 day old larvae and a clump with 10–15 pupae. The test colony remains in the test cage for 2–3 weeks and afterwards for 2 weeks in the laboratory to check the emerged bees for malformations.

Tasei (1987, 1993) conducted experiments in glasshouse compartments. Two colonies (*B. terrestris*) were placed in glasshouse compartments of 3×2 meter with a crop area of 2 m^2. Dead bees were collected every 24 hours. Each treatment was repeated after 7 days. Sechser (1996) placed a free flying colony in a tent of 5–9 m^2 that was sprayed with the pesticide in the recommended concentration. The colony was fed fructose solution with a pesticide at the recommended rate. After 6 weeks all stages were evaluated.

3.2. Comments on (semi) field tests

The main problem with tent tests is that the crop area and the size of a colony, that is used for pollination activity, are not proportionate. There is not enough pollen and nectar available in the cages for a colony of normal size. Addition of pollen and sugar syrup dilutes the possible effects caused by the pollen and nectar from the cage. This problem is insuperable and it makes the results of the tests by definition hard to interpret. To make sure that the pollen and nectar from the test plot are used for colony development, Gretenkord (1996, 1997) reduces the colony artificially. This facilitates the observations but the interpretation problem remains because the colony structure is not normal and it is not clear whether the crop area and the standardized colony size are in proportion or not.

4. FIELD TEST

4.1. Protocol

Schaefer (1995) describes a field test in which six bumblebee colonies are placed in a 2400 m^2. phacelia field. In the field, fallow lanes of 1.5 m width are laid out and covered with plastic to collect dead bees. Collection activity is monitored with observations on 5×1 m^2 for 1 minute. Observations are carried out daily for 4 days. In the observations, the adults are counted, the colonies are checked for dead larvae and photographs of the broodnest are taken daily.

4.2. Comment on field tests

In spite of the limitation that the test field is always just a portion of the total foraging area of a bumblebee colony, a field test gives the most realistic information of the impact of a pesticide on a bumblebee colony. Dead bee traps, plastic covered fallow lanes and daily observation of the brood give sufficient information to recognize a major impact. Collecting information on the impact on the brood is very hard because the location of the brood stages changes continuously. Photographing the brood nest daily is a useful tool in the interpretation of major effects on the brood. Data on the impact of a pesticide on honeybees cannot be transferred to bumblebees, among other things because of the different surface to volume ratio. In general, the greater the surface to volume ratio of individual bees the more susceptible bees become to field residues (Johansen, 1983).

5. BROODTEST

5.1. Protocols

Three brood tests with *B. terrestris* colonies have been described by van den Eijnde (unpublished data), de Wael (1995)

and Gretenkord (1996, 1997). Van den Eijnde tested the impact of Nomolt by feeding a colony 25 ml of a 50% sucrose solution with the test substance. This amount of food represents the average quantity a colony with about 50–80 workers and all stages of brood consumes in 24 hours. After the test solution has been consumed, the colony is offered a 50% sucrose solution ad libitum. By noting the presence of egg clumps, open cells with larvae, and cells with pupae 3 times a week during 3 weeks, the effect on the brood is checked. De Wael (1995) offers the pesticide dissolved in 50% sucrose solution for 24 hours to 6 colonies with 30–50 workers of *B. terrestris.* After that, 50% sucrose solution and pollen are available ad libitum. The observations consist of a daily count, the removal of dead adults and larvae and photographing the brood nest daily from a fixed position, starting a week prior to administering the test solution. Gretenkord (1996) chooses a totally different approach: laboratory rearing of the brood and treatment of the brood under laboratory conditions. Eggs are removed from colonies and kept in the incubator at 32 °C and 55–60% RH until hatching. Subsequently, the number of larvae per cup is standardized to 10 by removing or adding larvae. Then the larval cups are placed separately in small rearing boxes with 3 worker bumblebees at 28 °C and 50 ± 5% RH. The test group is offered sucrose solution and pollen dough. The exposure of the larvae to the test substance is carried out with larvae 1, 4 or 6 days old, each for 24 hours. On the 7th day, the first larvae begin to pupate. After pupation of all the larvae, the workers are removed and the pupae are kept until the adults emerge. To determine the amount of food consumed by the larvae, the amount consumed by a test group of larvae and a test group of 3 workers without larvae are compared. With these data the average consumption of each larva can be estimated.

5.2. Comments on brood tests

The impact of a pesticide on the brood is of major importance. A broodnest that contains all stages of development, stimulates pollination activity while a decrease of the broodnest, due to brood mortality inhibits pollination activity. The effect of a pesticide that has a lethal effect on the brood will show up several days after application of the pesticide. Due to the fact that the broodnest of bumblebees has a disorderly structure, it is impossible to determine the exact impact of a pesticide on the different broodstages. The number of dead larvae and pupae is an unreliable parameter because cannibalism occurs. Photographs of the brood surface taken daily can show major effects. However, in this way, minor effects in a normal developing broodnest cannot be detected. Rearing and treating the brood in the laboratory can demonstrate these effects. Gretenkord has begun developing a method of testing the impact on the brood in detail. Efforts must be undertaken to improve laboratory rearing for testing toxicity on the brood. A laboratory brood test carried out with a range of concentrations to determine a lethal dose will supply reproducible and comparable information. Brood tests pose a special challenge in connection with newly developed Insect Growth Regulators (IGR's).

6. SUBLETHAL DOSES

Tasei (1993, 1994) conducted tests to investigate the sublethal effect of pesticides by checking the food consumption and longevity of adults with feeding tests involving a pesticide in a sublethal dose. Feeding contaminated pollen to hibernated queens tested the effect on the initiation phase of a colony. The parameters tested were the initiation of oviposition, the emergence of the first brood and the number of workers in the first brood. These tests give useful additional information about the long-term effects.

7. CONCLUSION

Laboratory toxicity tests that have been developed so far to establish the acute oral and contact LD_{50}, produce reliable and reproducible results on the toxicity of test substances for adult bumblebees.

Hazard tests, developed so far only provide information about the impact of the overexposure to a pesticide. This applies to both laboratory and cage tests. In cage tests, the plant surface area is by definition not proportionate to a healthy normally developed bumblebee colony.

Due to the structure of a bumblebee's broodnest, it is impossible to obtain detailed information about the impact of a pesticide on the brood under practical circumstances. Only field tests provide reliable information on the actual hazard of a pesticide to bumblebee colonies.

Efforts to determine the impact of pesticides on adult bumblebees should focus on the improvement of standardized toxicity tests and on field tests. Because of the importance of brood to colony development and pollination performance, a standardized laboratory rearing method for bumblebee brood should be developed in order to determine the toxicity of pesticides on brood.

Résumé – Mise au point sur les méthodes pour déterminer le risque et la toxicité des pesticides pour les bourdons (*Bombus* sp.). Depuis que l'élevage des bourdons sous abris s'est développé, ces insectes jouent un rôle important dans la pollinisation des cultures horticoles. Il est essentiel de disposer de données concernant l'impact des pesticides sur les bourdons pour gérer avec succès la pollinisation. Nous décrivons ici les méthodes mises au point pour déterminer cet impact. Les méthodes ont été classées en quatre groupes : (i) tests de laboratoire pour déterminer la toxicité aiguë, (ii) tests de laboratoire pour déterminer le risque

encouru par les bourdons, (iii) tests en conditions de (semi) plein champ et (iv) test sur le couvain. La reproductibilité et l'importance des données obtenues pour la pratique sont discutées.

Les tests de laboratoire pour déterminer la toxicité aiguë orale et de contact (tests de DL_{50}) peuvent être facilement standardisés et ils fournissent des données reproductibles. Les tests de laboratoire mis au point pour déterminer le risque dû à un pesticide donné vis-à-vis des bourdons sont basés sur une approche pseudo-réaliste du problème. Il y a très souvent une surexposition au pesticide pour une certaine concentration. Les données obtenues ne peuvent pas être comparées entre elles. Dans les tests en semi plein champ, le problème majeur est que l'aire de butinage n'est pas proportionnée à la taille de la colonie de bourdons. Il faut donc adapter la taille de la colonie à la quantité de nourriture qui peut être prélevée, mais cette intervention rend les résultats difficiles à interpréter. Un test en champ fournit des données réalistes sur le risque que représente un pesticide pour une colonie de bourdons mais, lors de l'interprétation des résultats, il faut toujours tenir compte du fait que la parcelle testée n'est qu'une partie de l'aire de butinage globale d'une colonie de bourdons. La présence de couvain est importante pour l'activité pollinisatrice. Le nid à couvain des bourdons est désordonné. Dans un nid à couvain normal, seuls des effets importants peuvent être reconnus. Il faut donc mettre au point l'élevage en laboratoire du couvain de bourdon afin de développer un test standardisé qui fournisse des données reproductibles.

***Bombus terrestris* /pesticide / toxicité / test LD_{50} / test de laboratoire / effet sublétal / test semi plein champ / test sur couvain**

Zusammenfassung – Methoden zur Bestimmung der Giftigkeit von Pestiziden für Hummeln und deren Gefährdung: Eine Übersicht. Seit der Einführung von Methoden zur Hummelzucht in geschlossenen Räumen haben diese Insekten zunehmende Bedeutung als Bestäuber im Gartenbau eingenommen. Kenntnisse zum Einfluss von Pestiziden auf Hummeln sind daher für eine erfolgreiche Bestäubungspraxis von groβer Bedeutung. Hier werden Methoden beschrieben, die entwickelt wurden um diesen Einfluss zu bestimmen. Diese Methoden können in verschiedene Bereiche eingeteilt werden: Labortests zur Bestimmung der akuten Toxizität und Gefährdung der Hummeln, Freiland- und Halbfreilanduntersuchungen, und Bruttests. Die Wiederholbarkeit der Ergebnisse und deren Bedeutung in praktischer Hinsicht wird diskutiert.

Labortests zur Bestimmung der akuten oralen und Kontaktgiftigkeit (LD_{50} Tests) können sehr leicht standardisiert werden, diese Tests liefern zumeist reproduzierbare Ergebnisse. Die zur Bestimmung einer Gefährdung der Hummeln durch bestimmte Pestizide entwickelten Labortests stellen allerdings nur eine pseudorealistische Annäherung an das Problem dar. Oftmals werden die Tiere einem Pestizid in einer bestimmten Konzentration überexponiert, die Ergebnisse solcher Tests sind untereinander oft nicht vergleichbar. In Halbfreilanduntersuchungen ist ein hauptsächliches Problem, dass das Sammelareal und die Gröβe der Hummelkolonie nicht in angemessenem Verhältnis stehen. Daher muss die Koloniegröβe an die sammelbare Futtermenge angepasst werden, dieser Eingriff macht die Ergebnisse allerdings schwer interpretierbar. Feldtests liefern zwar realistische Angaben zur Gefährdung von Hummelnestern durch Pestizide, bei der Interpretation der Ergebnisse muss aber berücksichtigt werden, dass das Testareal nur einen Teilbereich des gesamten Sammelareals der Kolonien darstellt. Die Anwesenheit von Brut ist wichtig für die Bestäubungsaktivität. Brutnester von Hummelnestern sind allerdings von unregelmäβiger Anordnung, daher können normalerweise erst gröβere Schadeinflüsse

wahrgenommen werden. Es wäre daher erstrebenswert, durch Aufzucht von Hummelbrut im Labor einen Standard-Bruttest zu entwickeln, der zur Reproduzierbarkeit der Ergebnisse beiträgt.

***Bombus terrestris* / Pestizide / Giftigkeit / LD_{50}-Tests / Laboruntersucheungen / Halbfreilanduntersuchungen / Bruttests / Sublethale Effekte**

REFERENCES

Drescher W., Geusen-Pfister H. (1991) Comparative testing of the oral toxicity of acephate, Dimethoate and methomyl to honeybees, bumblebees and Syrphidae, Acta Hortic. 288, 133–138.

Gretenkord C., Drescher W. (1993) Development of a cage test method for the evaluation of pesticide hazard to the bumble bee *Bombus terrestris* L., Proc. 5th Int. Symp. on the hazard of pesticides to bees (ICPBR), October 25–28 Wageningen, The Netherlands, pp. 151–154.

Gretenkord C., Drescher W. (1993) Die Auswirkungen von vier Pflanzenschutzmitteln (Decis, Metasystox, Pirimor, Rubitox) auf die Dunkle Erdhummel (*Bombus terrestris* L.): Bestimmung der oralen LD_{50} und erste Erfahrungen mit Zeltversuchen, Apidologie 24, 519–521.

Gretenkord C., Drescher W. (1996) Laboratory and cage test methods for the evaluation of the effects of insect growth regulators (Insegar, Dimilin) on the brood of *B. terrestris*, Proc. 6th Int. Symp. on the hazard of pesticides to bees (ICPBR), September 17–19, Braunschweig, Germany, appendix 29.

Gretenkord C. (1997) Laborzucht der Dunklen Erdhummel *Bombus terrestris* und toxikologische Untersuchungen unter Labor- und Halbfreilandversuchen, Dissertation Rheinischen Friedrich-Wilhelms-Universität Bonn, Germany.

Johansen C.A., Mayer D.F., Eves J.D., Kious C.W. (1983) Pesticides and bees, Environ. Entomol. 12, 1513–1518.

OEPP/EPPO (1992) Decision making scheme for the environmental risk assessment of plant protection products, Honeybees, EPPO/OEPP Bull. 10, 151–159.

OEPP/EPPO (1992) Guideline on test methods for evaluating the side effects of plant protection products No. 170, Honeybees, OEPP/EPPO Bull. 22, 203–216.

Schaefer H.D. (1993) Entwicklung einer Labormethode zur Pflanzenschutzmittel-Prüfung an Erdhummeln (*Bombus terrestris* L.) und die Konzeption einer kontinuierlichen Laborzucht, Diplomarbeit Universität Bielefeld, pp. 22–66.

Schaefer H., Mühlen W. (1996) Erste Erfahrungen zur Prüfung der Auswirkungen des Pflanzenschuzmittels Alsystin an der Erdhummel *Bombus terrestris* L. im Freiland, Apidologie 27, 273–275.

Sechser B., Reber B. (1996) Using a sequential testing scheme under laboratory and field conditions with the bumble bees *Bombus terrestris* to evaluate the safety of different groups of insecticides, Proc. Conf. Ecotoxicology Pesticides and Beneficial Organisms, Oct. 14–16 1996, Cardiff, Wales, UK.

Steen J.J.M. van der (1994) Method development for the determination of the contact LD_{50} of pesticides for bumble bees (*Bombus terrestris* L.), Apidologie 25, 463–465.

Steen J.J.M., Gretenkord C., Schaefer H. (1996) Methods to determine the acute oral LD_{50} and acute contact LD_{50} of pesticides for bumble bees (*Bombus terrestris* L.), Proc. 6th Int. Symp. on the hazard of pesticides to bees (ICPBR), September 17–19, Braunschweig, Germany, appendix 28.

Stute K. (1991) Richtlinien für die Prüfung von Pflanzenschutzmittel im Zulassungsverfahren Auswirkungen von Pflanzenschutzmitteln auf die Honigbiene. Teil VI. Biologische Bundesanstalt für Land- und Forstwirtschaft der Bundesrepublik Deutschland, Saphir Verlag, Ribbersbüttel, Deutschland.

Taséi J.N., Carré S., Grondeau C., Hureau J.M. (1987) Effets d'application insecticides à l'égards d'apoïdes pollinisateurs autres que l'abeille domestique (*Megachile rotundata* F. et *Bombus terrestris* L.), Conf. Int. sur les ravageurs en agriculture, Paris, ACTA, pp. 127–136.

Taséi J.N., Sabik H., Pirastru L., Langiu E., Blanche J.M., Fournier J., Taglioni J.P. (1993) A sequential study on the effects of deltamethrin (Decis CE) on bumble bees, Proc. 5th Int. Symp. on the hazard of pesticides to bees (ICPBR), October 25–28, Wageningen, The Netherlands, pp. 155–160.

Taséi J.N., Sabik H., Pirastru L., Langiu E., Blanche J.M., Fournier J., Taglioni J.P. (1994) Effects of sublethal doses of deltamethrin (Decis) on *Bombus terrestris*, J. Apic. Res. 33, 129–135.

Wael L. de, Greef M. de, Laere O. van (1995) Toxicity of pyriprofen and fenoxycarb to bumblebee brood using a new method for testing growth regulators, J. Apic. Res. 34, 3–8.