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Introduction of indices for the evaluation of tent tests and field tests with honeybees

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Abstract

According to the EPPO-guideline 170 different evaluations are requested in tent tests and field tests with honeybees. Many data are generated as counted or measured values, e.g. for mortality, foraging activity and brood development. Although they can be presented in tables or in graphs, these absolute figures are open for any interpretation.

It is proposed to calculate averages of the respective parameters for data before application and for data after application for all bee colonies in one test unit. This concentrates the data and allows the comparison of the state after application with the state before application. A simple mathematical division converts the absolute data into relative data.

The use of the proposed indices, representing relative data instead of absolute ones, could be a helpful tool for the interpretation of the obtained data in tent tests and field tests with honeybees.

Key words: tunnel test, field test, threshold, mortality, foraging activity, brood assessment.

Introduction

Principles of the laboratory test

Each plant protection product has to be tested for its bee safety. Respective testing procedures exist since almost 50 years. Originally they differed from country to country, but meanwhile the EPPO-guideline no. 170 is an accepted framework.

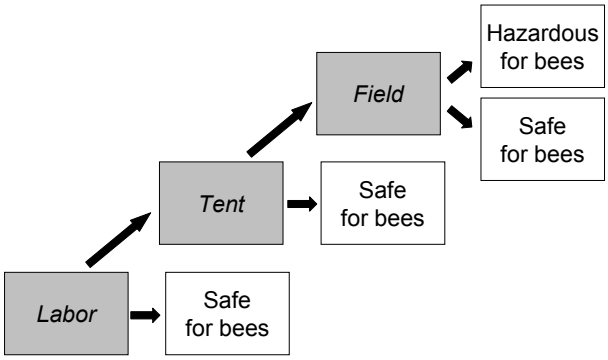

Figure 1. Sequential testing scheme for bee safety.

The sequential testing scheme (Lab – Tent – Field, figure 1) is generally adopted. The testing starts in the laboratory, where two LD 50-values are determined. The two LD 50-values (oral and topical) represent primarily the intrinsic toxicity of the test substance. A first information of the bee safety of a substance can be deducted from these laboratory tests. It is a very comfortable figure for the comparison of different compounds. A kind of a threshold exists. A LD 50 above 200 µg per bee is considered as non-toxic for bees. In other words: If the bees tolerate a high amount, the test substance is considered as not toxic for bees.

$$HQ = \frac{\text{rate in g/ha}}{\text{LD 50 in µg / bee}}$$

HQ < 50 harmless HQ > 50 continue testing

$$\text{Example (pyrethroid)}\quad HQ = \frac{\text{15 g a.i. / ha}}{\text{0,16 µg / bee}} = 93{,}75$$

Figure 2. Hazard Quotient.

Although the laboratory test with the determination of the LD 50 is an essential tool for the knowledge about the bee safety it has some disadvantages:

- only adult worker bees are tested and the influence on the juvenile stages (brood) is disregarded;
- only individual bees are tested and the influence on the whole bee colony is eliminated;
- the test substance is applied directly to bees and not like in the practice onto flowering plants;
- it is not possible to test granules or seeddressings correctly in the laboratory;

- it is a compulsory testing and the bees have no choice to search for alternative food;
- a range of rates is tested and the recommended field rate of the test substance is not the focus.

The latter aspect is recognised and a hazard quotient is introduced. The field rate (given in g a.i./ha) is divided by the LD 50 (figure 2). If this figure is below 50, no critical effects are expected. If the field rate is high and the LD 50 is low, a hazard quotient above 50 signals that harmful effects on bees are very likely. The given example uses a pyrethroid with a high oral toxicity (=low LD50 value) in the laboratory test. Due to its low rate per hectare the hazard quotient appears not too serious. It is not possible to obtain more information from a laboratory test.

Principles of the tent test

The advantages of a tent or field test are:

- the substance is usually applied on flowering plants;
- the bees live in a real, but small bee colony containing a queen;
- sublethal effects on the behaviour or the degree of pollination can be observed;
- besides mortality new parameters can be observed like: foraging activity, nectar and pollen accumulation in the combs, development of the brood, weight of the hive as an indicator for the honey yield;
- weather factors are included;
- it is possible to test granules and seeddressings.

Nevertheless, the exposition in a tent test is yet increased, because the bees are forced to forage exclusively on treated plants. They cannot escape or avoid the treated area for foraging like in a field test. On the other hand that means that a tent test is very suitable to show possible effects. It overdemonstrates the reaction of the bees under the enforced conditions. The normal exposition in a field trial may reflect better the reality.

The aim of bee testing is the prevention of a damage. As a damage is normally considered:

- a high mortality or even the extinction of whole colonies;
- a depression of the brood leading to a delayed development of whole colonies;
- a reduction of the nectar accumulation expressed as a reduced honey yield.

A reduced foraging activity, normally called repellence, is not a real damage to the bee colony. It is a symptom indicating that the bees have recognised the substance. We consider it as a protection from intoxication. If none of the other measurable damages is manifest, e.g. no mortality, the repellence itself is not a harm on the bee colony. - That a repellent effect is fatal for crops depending from insect pollination is a true but different aspect and it has nothing to do with a damage to the bees.

Materials and methods

Time schedule of a tent test

The bee colony is introduced into the tent at the beginning of the flowering period and before the test substance is applied (except for granules and seeddressings). This serves the purpose of adaptation of the colonies and implies that the above mentioned parameters are evaluated before the test substance is applied.

It is well known that bee colonies differ in their vitality and vary in their reaction under tent conditions. Therefore it is common practice to limit or balance this variation by using sister queens. After the application of the test substance the evaluation of the same parameters as before application continues. A reaction of a bee colony is usually recognised by a difference in the level of the parameters after and before application.

The obtained data can be compiled into two sets:

- evaluation before application,
- evaluation after application.

The data can be concentrated as an average for each set. The guideline EPPO 170 does not give any assistance, what to do with the elaborated data and how to interpret them. At present nobody knows exactly whether 20 or 50 or 100 dead bees per day are acceptable or what level of mortality is considered as a damage. There is no official threshold like it is given for testing beneficials by the IOBC.

There are two possible ways to make the evaluation more transparent (figure 3):

a) the evaluations are expressed in percent relative to the control;

b) by comparison of the evaluations obtained from the same colony after application with the evaluations before application.

The approach a) is like the Abbott formula based on the untreated control as a calculation basis. It can be used when it is certain that the bee colonies in untreated and in treated behave and develop identical. That is not always the case. It might be more important to demonstrate a change in the evaluated parameters for the same bee colony. If this is linked with the test substance, the approach b) might offer the better way to disclose an effect.

$$\text{a) in \% relative to untreated} = \frac{\text{test substance}}{\text{untreated}} \times 100\ \%$$

$$\text{b) as an index} = \frac{\text{evaluations after application}}{\text{evaluations before application}}$$

(use average of evaluations)

Figure 3. Expression of efficacy.

Proposal of an Index

For the interpretation of the data obtained in a bee test we propose to use an index like b) comparing the data after application with the data before application for the same bee colony. All following examples stay for tent tests as well as for field tests irrespective of the size of the beehives used in the test system.

Results and discussion

Mortality

Dead bees are collected in front of the beehives once per day usually at the same time to maintain a 24-hour interval. In a tent test with flowering *Phacelia* we found the daily mortality values reported in table 1. There is a certain variation and an increase of dead bees after the application. The graph visualises better, that the product causes an increased mortality (figure 5). The question remains unanswered by the graph, whether this is a damage to the bee colony or not.

The following simple calculation may assist in such an assessment. The average of dead bees per day after application is formed and divided by the average of dead bees per day before application (table 1). The quotient represents a move away from absolute figures to relative figures. This is a better basis for the interpretation of a result, because the absolute figures can cover a wide range and they have different levels from test to test. The index converts absolute figures of all ranges into easy to interpret figures, which are applicable to every bee test.

If only natural mortality occurs, the number of dead bees per day does not change very much and the index (quotient) is closed to 1. We see this for the untreated control (table 1). If the test substance induces an increased mortality, than the quotient exceeds 1. A quotient of 2 indicates that the mortality is twofold of the natural mortality. Does this offer a tool for the introduction of a threshold? We feel that if the index is around 2 or even higher, one must ask for the reason. Are other reasons besides the application of the test substance involved, must the test be repeated or is there a clear link to the application of the test substance?

When the index also applied to the control and both indices are compared by a simple mathematical division we come to a clearing quotient I_M for mortality (figure 4). It illustrates the deviation of the test substance from the untreated control. Please pay attention that we do not compare absolute figures, but relative figures, which is fairer.

$$Q_M = \frac{\text{average number of dead bees per day after application (5 days)}}{\text{average number of dead bees per day before application}}$$

Q is only valid for the test substance and compares the reaction of the bees after application with their reaction before application

$$I_M = \frac{Q_M}{K_M}$$ (K_M like Q_M, but for control)

I is a clearing quotient and compares the test product with the untreated control

Figure 4. Index for mortality

We should discuss the following misuse: when we evaluate mortality, then we look for acute effects. It might be of less interest how many bees die 10 days after the application. If the increased mortality is restricted to the day 1 and 2 after application and this is included into an average mortality of 10 days after application, then the figure might be too low. To avoid that the mortality is underrepresented the average should be limited to cover only 5 days after application. This is justified because longer lasting effects should be visible during the evaluation of the strength of colony. This does not mean to stop the evaluation of mortality 5 days after application. We just want to introduce a time frame for the calculation of the index.

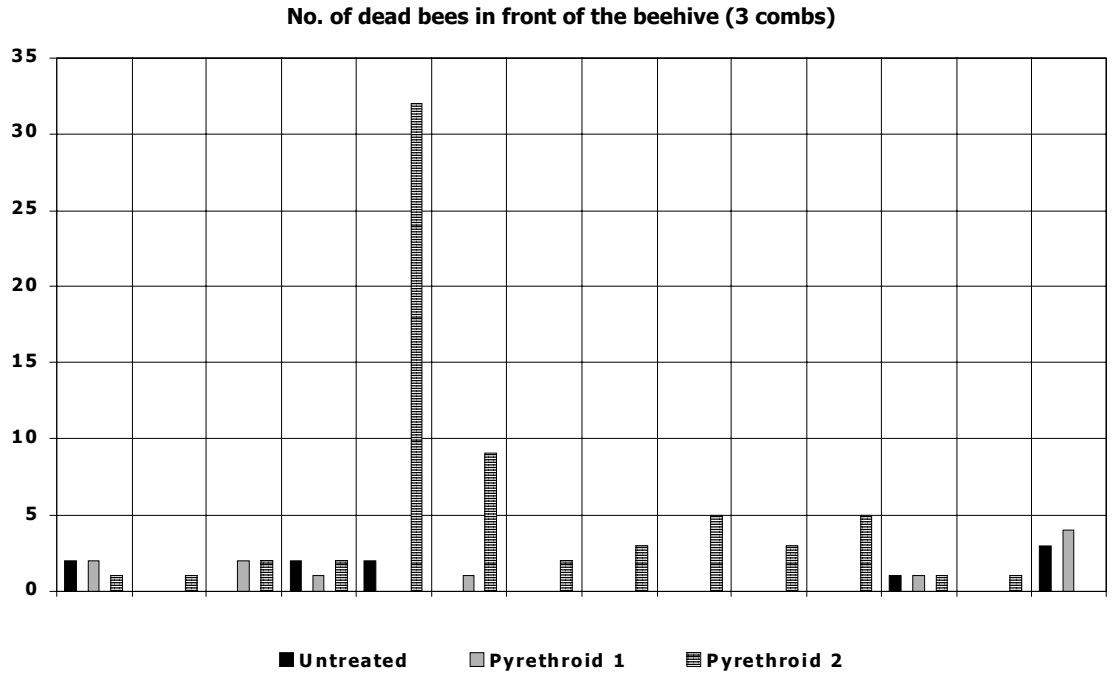

Figure 5. Mortality in a tent test sprayed 18.06.1997 on 50 m^2 rape

Table 1. Mortality in a tent test, sprayed 18.06.1997 on 50 m^2 rape.

	No. of dead bees in front of the beehive (3 combs)		
Day	Untreated	Pyrethroid 1	Pyrethroid 2
-3	2	2	1
-2	0	0	1
-1	0	2	2
-0	2	1	2
+0	2	0	32
+1	0	1	9
+2	0	0	2
+3	0	0	3
+4	0	0	5
+5	0	0	3
+6	0	0	5
+7	1	1	1
+8	0	0	1
+9	3	4	0
Total of 5 days after appl.	2	1	54
After appl. per day	0.33	0.17	9
Total before appl.	4	5	6
Before appl. per day	1	1.25	1.5
Index after:before	0.33	0.14	6.0
Clearing Index Substance/Untreated		0.42	18.18

Table 2. Foraging activity in a tent test, sprayed 18.06.1997 on 50 m^2 rape.

	No. of bees on 2 x 1 m^2 flowering plants		
Day	Untreated	Pyrethroid 1	Pyrethroid 2
-2	17	21	16
-1	22	15	25
-1	18	23	27
-1	22	22	24
-0	15	14	5
-0	13	16	17
5 min.	9	9	0
20 min.	11	2	0
30 min.	13	1	0
50 min.	18	10	2
2 h	21	15	0
4 h	28	19	0
5 h	17	16	0
+1	29	25	1
+1	42	29	2
+1	48	36	2
+2	19	16	9
+4	22	20	11
+6	12	6	0
+6	9	6	0
+7	7	5	3
+7	11	8	3
Total of 3 days after appl.	255	178	16
After appl. per day (11 eval)	23.2	16.2	1.5
Total before appl.	107	111	114
Before appl. per day (6 eval)	17.8	18.5	19.0
Index after/before	1.3	0.88	0.08
Clearing Index Substance/Untreated		0.68	0.06

Foraging activity

As foraging activity the number of bees is considered landing on flowers for the collection of nectar or pollen within 1 minute on 1 m^2 of flowering plants. Bees crossing the area without landing on a flower are disregarded. In a strict sense, we are mainly interested in measuring the activity, which is the visitation of flowers.

We assess the activity several times before spraying and calculate an average from all assessments of the days before application (table 2). The assessments of several days after application are used for the calculation of the average of the activity after application. We divide the average after application by the average before application (table 2). We should limit the basis for this calculation to the same number of days before and after application, e.g. to three days before and to three days after application. The number of assessments can differ, which is included and balanced by the average.

If this index is closed to 1, then the sprayed product does not influence the foraging activity. If the index is 0,5 or even less, an avoidance of the treated crop can be stated. It is obvious, here is again the chance given for the introduction of a threshold. As the index of 0,5 stays for a reduction of 50% we propose this as a threshold for a distinct reduction of the foraging activity. However, we recall that the repellent effect is transient and is not regarded per se as a damage to the bees.

The index offers a better interpretation of the obtained data than a graph would give (figure 7).

Let us discuss the following case. The foraging activity drops after application of the test substance and in the untreated control as well (perhaps due to weather conditions). What are we doing, when the index for the untreated control deviates too much from 1? Under such circumstances it is necessary to compare the index of the test substance with the index of the untreated control by a simple division (figure 6). This is again a clearing quotient I_F. It indicates by which factor the test substance differs from the control. We propose as a tolerance limit the range between 0,5 and 2, which we consider as the normal reactivity of the bees. If the clearing quotient calculates beyond this range, this is a hint for an effect.

$$Q_F = \frac{\text{average number of bees per square and per evaluation after appl. (3 days)}}{\text{average number of bees per square and per evaluation before application}}$$

Q is only valid for the test substance and compares the reaction of the bees after application with their reaction before application

$$I_F = \frac{Q_F}{K_F}$$ (K_F like Q_F, but for control)

I is a clearing quotient and compares the test product with the untreated control

Figure 6. Index for foraging activity = flower visitation.

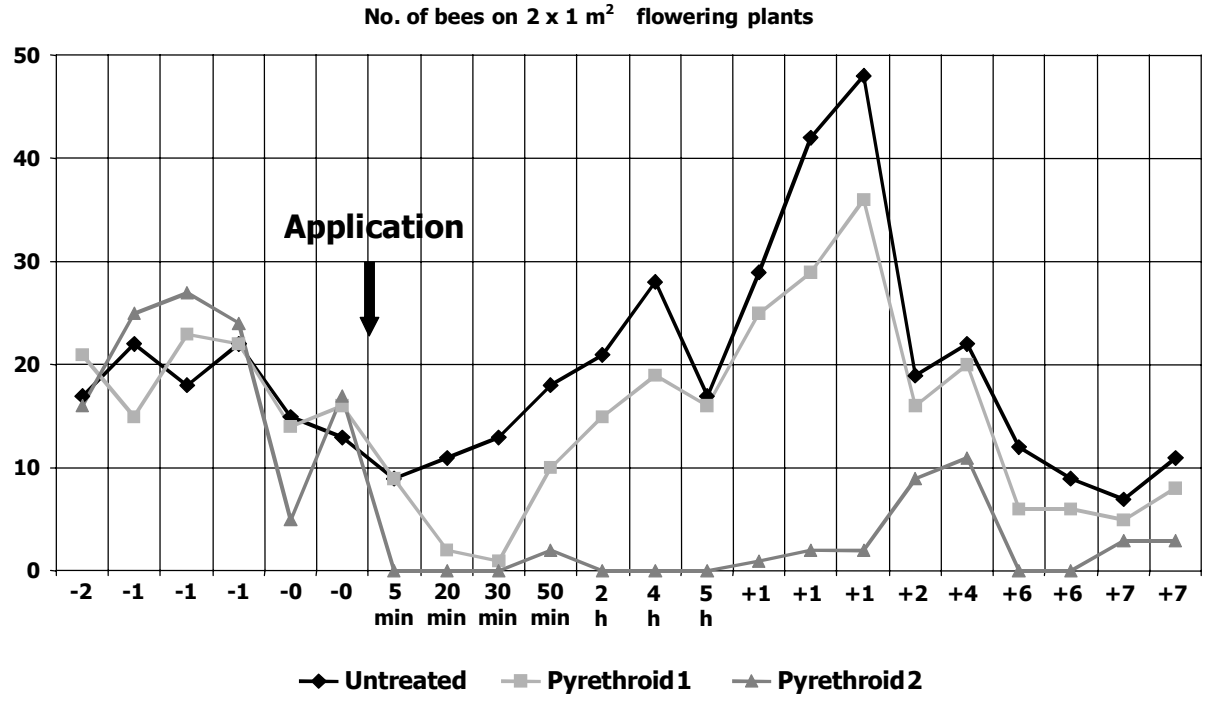

Figure 7. Foraging activity in a tent test sprayed 18.06.1997 on 50 m^2 rape.

Broodnest

It is possible to use the index for the evaluation of the development of the brood. In our trials we determine the size of the broodnest before application. The size is assessed as percent comb area being filled with eggs, larvae and sealed cells (figure 9). It is also possible to assess separately each comb area being filled with eggs, larvae or sealed cells. We would obtain three figures. However, for our example the size of the complete broodnest is assessed. This is done on each side of all combs. In a tent test with a colony on three frames we obtain 6 figures and calculate the average. The assessment of the broodnest starts before application and is repeated 4, 7, 14, 21 and 28 days after application.

This method does not look after the individual fate of a particular egg or larva. We look after the broodnest as a whole. The nest is adjusted by beekeeping techniques to a relatively small size at the beginning of the test. To maintain the harmony in the colony all brood stages should be present. There should be given the possibility to expand the broodnest. This is common practice for the evaluation of brood effects in a bee test.

We obtain the different indices Q_B for the days 4, 7, 14, 21 and 28 after application, when we divide the broodnest size of the respective day after application by the value before application. In our example we look only for such days, when the bee colony is confined in the tent (figure 8, table 3).

$$Q_B = \frac{\text{average area of brood nest on day „y“ after application}}{\text{average area of brood nest before application}}$$

Q is only valid for the test substance and compares the size of the broodnest after application with the size of the broodnest before application

$$I_B = \frac{Q_B}{K_B}$$ (K_B like Q_B, but for control)

I is a clearing quotient and compares test product with the untreated control

Figure 8. Index for brood.

If the index is below 1, the broodnest shrinks. Is the index above 1, the broodnest expands. For a healthy colony not being influenced by the test substance the broodnest should grow under the experimental condition and the index should be above 1. An effect, e.g. a transient depression for a limited period, can easily be detected. A recovering and an expansion of the brood are simply expressed by the index. The index as a relative figure deliberates us also from the problem of different broodnest sizes in untreated and in treated at the beginning of the test. The growth of the broodnest can be recognised by comparing the indices of the test substance with the indices of the untreated. The main emphasis is, that we consider the reaction of the whole colony including the compensation by the queen instead of looking after the fate of single individuals.

Table 3. Brood assessment in a tent test, sprayed 18.06.1997 on 50 m^2 rape.

	Cells filled with brood stages as % comb area (average of 6 comb sides)											
Day	Untreated				Pyrethroid 1				Pyrethroyd 2			
	eggs	larvae	pupae	nest	eggs	larvae	pupae	nest	eggs	larvae	pupae	nest
-2	0.8	8.3	13.3	22.4	11.7	14.2	7.5	33.4	12.5	5.0	23.3	40.8
+12	18.3	6.7	25.0	50.0	10.8	4.2	26.7	41.7	4.2	10.8	15.0	30.0
Index Q after/before				2.23				1.25				0.74
Clearing Index Substance/Untreated								0.56				0.33

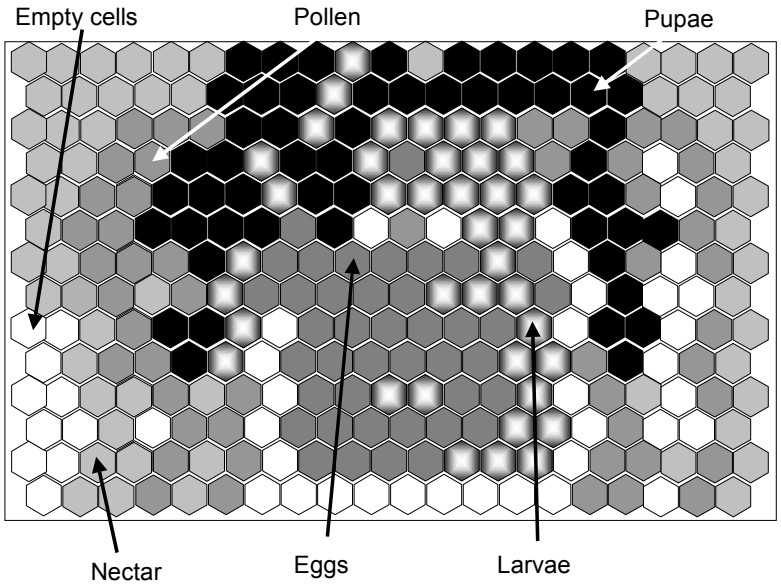

Figure 9. Assessment of the broodnest size as percent comb area being filled with eggs, larvae and sealed cells.

Conclusion

As a contribution to solve the question how to interpret the data obtained in a bee test conducted in the tent or in the field we propose the introduction of indices. Such indices can be applied for mortality, foraging activity and for brood development. It is based on the comparison (= division) of the assessments after application with the assessments before application for the same colony. The base for the mathematical operation are the averages for both sets of assessments. That means the bulk of many data is concentrated or condensed. The index eliminates or flattens the problem of differences between the untreated bee colonies and the treated ones. Differences in the starting situation and between runs or replicates are also balanced. The indices are a useful aid for the interpretation of the test results. As a relative figure the index offers the chance to introduce a threshold for the recognition of an effect or a damage. We propose to consider indices between 0,5 and 2,0 as a tolerance limit. Indices outside this range require investigation of the reason.

If we compare the index for the test substance with the index for the untreated we come to the clearing quotient. This gives a better impression of the deviation of the test substance than the comparison of absolute figures. Although the implementation of the indices requires some experience we recommend them as a standard tool for the interpretation of results obtained in bee tests.

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