

# A TEST METHOD FOR FIELD STUDIES OF INSECTICIDAL BAIT FORMULATIONS AGAINST THE ANT *LASIUS NIGER* (HYMENOPTERA: FORMICIDAE)

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**Abstract**—In the early spring *Lasius niger* may be a nuisance indoors. These ants often originate from nests right next to the foundation, below the house or sometimes even directly under the floor. The ants raise the humidity, which may result in severe damage to the woodwork, due to rot. The same applies to an even greater extent to other species: *L. umbratus* and *L. fuliginosus*.

The present method was developed for the evaluation of insecticidal bait formulations in the field. Special emphasis was put on maintaining the colony members' ability to communicate mutually. The study evaluated the response of *L. niger* to seven bait formulations, five containing azamethiphos, one containing chlorpyrifos, and a reference with no insecticide. The primary interest of the experiment was to develop a method where the palatability of the bait formulations, the ability of the baits to attract *L. niger*, and the ants' acceptance of the baits could be investigated.

Thirty-four ant nests were used. When the number of ants on a jam bait indicated high activity, the test was initiated. A new jam bait was put into position for fifteen minutes and then replaced by the test bait for fifteen minutes. This was repeated three times. The number of ants visiting each bait was counted, and a small portion was collected by a sucking device when leaving the bait. The latter was observed for up to 24 hours to estimate the mortality. The results with the reference bait showed that the ants were only slightly affected by the method, while the palatability of the test baits ranged from as good as the controls to complete repellence. This result was confirmed by the percentage of ants killed. And both results indicate that the method could be used to establish comparable data for the efficacy of ant baits.

## INTRODUCTION

In Denmark, as in other North European countries, different species of ants have caused some problems in greenhouses, houses and gardens (Collingwood, 1979). The first time ants were reported to be a problem indoors in Denmark was in 1861 by Meinert. He reported that *L. niger* (*L.*) is sometimes a problem in houses in Copenhagen, but it might very well be a question of a change in the perception of ants as constituting a problem, which is registered in this context, rather than a new development. Based on the past 30 to 40 years of inquiries to the DPIL (Figure 1), there is no doubt that nuisance as well as actual damage caused by ant species such as *L. niger*, *L. umbratus* (Nylander) and *L. fuliginosus* (Latreille) must be considered a problem. Inquiries to the DPIL advisory service about *Lasius spp.* have stabilised at a level between 600 and a 1000 per year or 5 to 12 percent of all inquiries, and ants are always among the top three insect in the advisory service (Figure 1) (T. Hallas *et al.*, 1977; DPIL Ann.Rep. 1980–94).

When ants are considered to be more than a nuisance, it is to some extent linked to changes in the construction of modern houses. In the beginning of the sixties a method was introduced in Denmark according to which the floors in standard houses were constructed by placing a number of joists on top of a concrete deck with insulation in-between the joists and with wooden boards on top. A construction like this provides an ideal nesting site for the above-mentioned ant species (Arevad, 1987). The ants cause an increase of the humidity, which can give rise to severe damage. *L. umbratus* is able to build its nest by using the insulation directly. The queen often starts by invasion of and adoption in *L. niger* nests. The nest is formed into a cell-like structure often as big as a sq. meter. *L. fuliginosus* makes a carton nest out of decaying wood. *L. niger* in the house can originate from nests right outside the house or below the house, but also from decaying, damp wood wherever it appears inside the house or under the floor (Beck, 1991). Both *L. niger* and *L. fuliginosus* need some decaying wood to start with, but then they can keep up the humidity, and exacerbate the original damage. *L. niger* nests in greenhouses have been reported to damage attempts of biological control, as they were attacking eggs of the parasites used for aphids control (Tulisalo and Touvinen, 1975; Liepert and Dettner, 1993).

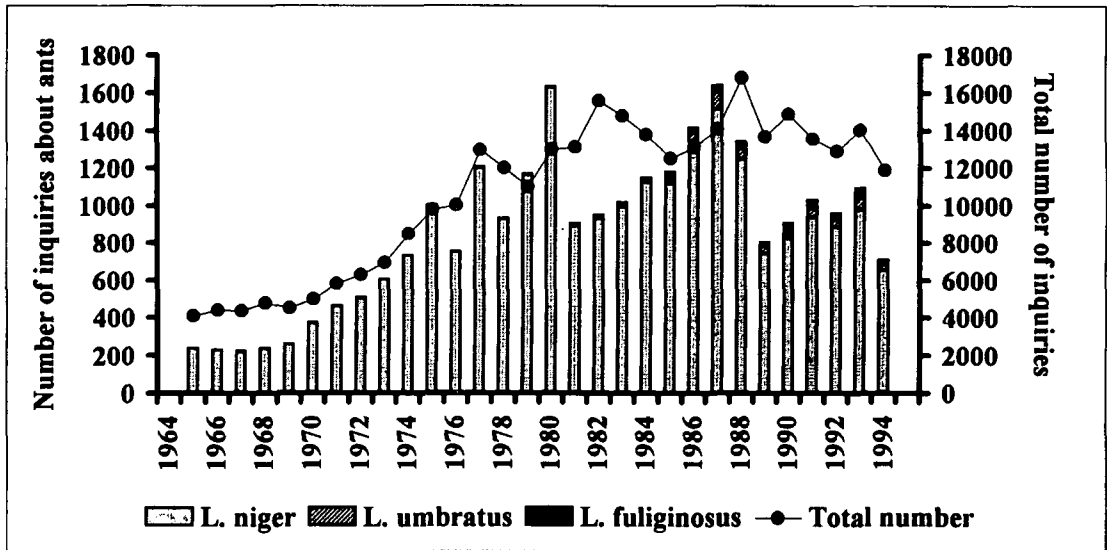


Figure 1. Total number of inquiries from 1965 to 1994 to the Danish Pest Infestation Laboratory, as well as inquiries concerning problems with *L. niger*, *L. umbratus* and *L. fuliginosus*.

In the period 1985 to 1995, 37% of all inquiries to the DPIL about problems with *L. niger* were made between January and April, when it is too cold or too difficult for the ants to find food and to be active outside (Arevad, 1987). The mean number in April was only surpassed by July when the ants are flying (Figure 2). The majority of all inquiries concerned the nuisance caused by ant invasions, while only a few concerned actual damage to constructions.

Nuisance as well as damage calls for some kind of control measures, and it is therefore necessary to be able to make efficacy tests which are as reliable as possible. For the time being it is difficult or probably impossible to do an efficacy tests with *L. umbratus* as it hardly ever deviates from its subterranean life, and is thus only identifiable when the queen and males are swarming in July and August or when dark spots are seen on wooden constructions in the house. Both *L. niger* and *L. fuliginosus* take sweet and fairly liquid food (Collingwood, 1979), and a number of insecticidal baits have been produced to control these ants. Due to the social structure of an ant colony it is difficult to make reliable tests, a fact which has had the unfortunate consequence that a number of efficacy tests of ant-baits have been done either in petri dishes in the laboratory or there have been

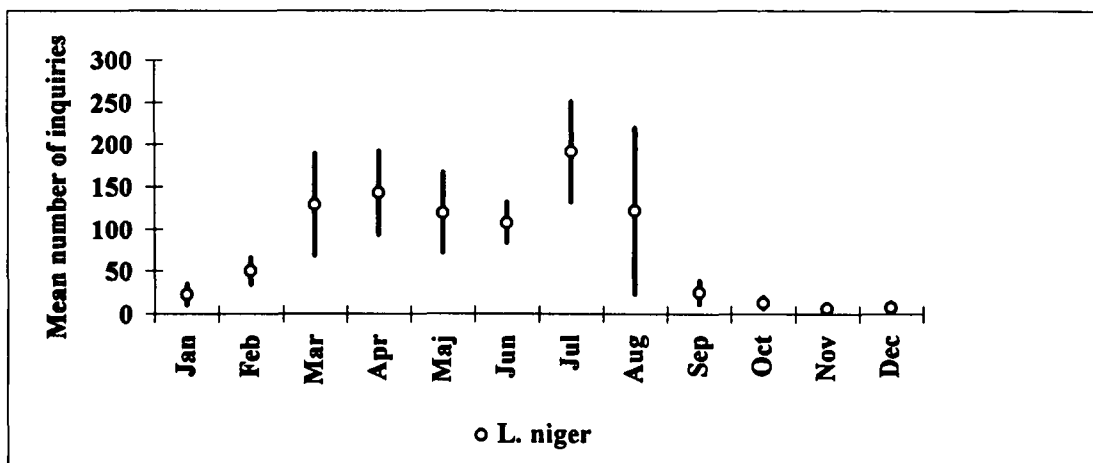


Figure 2. The mean number of inquiries per month to The Danish Pest Infestation Laboratory concerning problems with *L. niger*. The mean is based on the inquiries from 1985 to 1994.

no efficacy tests at all. The present test method relates to *L. niger*, which is by far the most common ant indoors in Denmark.

As other ant species, *L. niger* has a complicated social structure which makes it possible for the ants to communicate information about food, such as quality and distance. In this way they are able to gather a lot of workers in the right place in the shortest possible time (Brian, 1977). To evaluate a bait's efficacy it is therefore necessary not to interfere with the ants' selection of paths or with their trail marks. Trail marks which gives them the possibility to select the richest food source, the shortest path leading to it and to exploit it by intensive recruitment of other workers (Beckers *et al.*, 1992a, 1992b, 1993). The evaluation must avoid interfering with the ants' pheromones and prevent any mixing of ants from different nests, which inevitably would provoke the ants to attack and kill one another (Dumpert, 1978). The following description of a simple test method attempts to take these facts into consideration.

## MATERIALS AND METHODS

Three different insecticidal baits (IB) were used in the trials: a 1% azamethiphos baitbox ("Insectex Ant Bait", Terrasan), hereafter called AZA-B, two 1% azamethiphos scatter-bait formulations (SNIP®, Ciba-Geigy), hereafter called SB1, and SB2 and a 0.5% chlorpyrifos baitbox, hereafter called CHL-B. The latter was an off-label use of a product produced for cockroaches, and was not intended for use against ants (Bayer). The scatter baits were tested in a dry and moist version, due to the fact that from time to time a scatter bait would be moistened by dew or rain, which is not the case with closed bait boxes. The ingredients of the jam used as reference were: strawberries, sugar, glucose, pectin, citric acid and potassium sorbate. 35 g fruit and 53 g sugar in 100 g.

For each test, it was essential to choose days with high ant activity and with a consistently high activity for at least 1½ hours after the trial was initiated. When a particular nest was used, the tests were started by placing a jam bait adjacent to the nest. Jam as well as the IB was offered on clean foil on a circular plywood plate (5 cm in diameter). The plate was kept in position and pressed close to the ground by a 5 inch nail through the middle. When the number of ants on the jam indicated that there was good activity, the real test was initiated by replacing the foil by a new jam bait on foil after shaking off the ants. For the next 1½ hours baits was replaced every 15 minutes, alternating between jam and IB. At the end both bait types had been used three times. In the 15-minute periods, all visiting ants were counted. When the ants were leaving the bait, a number of them were gently sucked up into a test tube. The actual number sucked up was dependent of the number of ants visiting a given bait. The ants were kept in the test tube for up to 24 hours or as long as was needed to get a clear response; in general until 90 to 100% of the ants were dead. Trials like the one described were made at 5 to 9 different nests, except in the tests with scatter bait dry formulations where it very soon became obvious that the ants would not react to the bait. As reference, two more tests were made exactly as the other nine, but this time jam was interchanged with jam, making the second jam-bait act as a highly attractive but non-toxic IB.

There is a constant decrease in the number of ants visiting the different baits throughout the trial. This is not a result of the manipulation in the vicinity of the ant-nest, because when two jam baits are interchanged with one another, there is no decrease in the number of ants collected in each 15-minute period. The decrease when IBs are used, may be explained by the simple fact that some of the ants are killed or otherwise affected. To compensate for this, the three counts of ants visiting the jam baits were used for a linear regressions as were the three counts of ants visiting IB. The regression made it possible to estimate the number of ants on both types of bait at an arbitrarily selected time, in this case 30 minutes after initiation of the trial. It was necessary to estimate the number of ants on both bait types, as, due to the model, there would never be a simultaneous count on both bait types. The slopes between the two regression lines were only significantly different in one case, namely in the CHL-B test, where no ants at all visited the IB (t-test of the differences between the slopes). The palatability of the IB was then compared with that of jam (t-tests,  $H_0: (Jam_{30\text{ minutes}} \div IB_{30\text{ minutes}}) / Jam_{30\text{ minutes}} = 0$ ). The difference between the number of ants killed and the number of ants collected on either the jam bait or the IB, was also compared (t-test,  $H_0: collected \div killed = 0$ ). All statistics were conducted using SAS, ver. 6.08.

## RESULTS

The ants were not interested in SB1 dry, SB2 dry, and CHL-B, while the other bait formulations displayed a palatability as good as or nearly as good as the jam, with no statistical difference on a 5% level in the number of ants visiting the jam bait compared with the number visiting the IB. The two tests with SB1 dry and SB2 dry were only run on two nests before the tests were stopped and later continued with both baits moistened; a change which altered the results significantly towards a higher palatability. The killing of ants collected after leaving the bait was tested. The average difference between killed and collected ants was not significantly different (5% level) when they were collected from SB1 moist, SB2 moist, and AZA-B. This shows that approximately all ants collected were killed on the baits in question. CHL-B could not be tested as no ants were collected on this bait. Concerning the reference it would be expected that only a few ants would be killed, but it turned out that in the first of the collections from one of the reference nests all ants died, undoubtedly due to heat, as they were by accident left exposed to the sun for several minutes. The other eleven collections from the two reference nests gave a total of 163 ants collected out of which only 2 died.

The percentage of ants which were visiting the IBs is presented in Table 1, as well as the percentage of ants killed after they were collected on either jam or IB. The estimated mean percentage of ants on the IBs after 30 minutes is given in Figure.3. Fifty percent or more is in this case ideal because the IB could then be considered as good as or even better than the jam used for comparison.

Only few ants dies among those collected on the jam which indicates that they are not sensitive to the set-up. Apart from the reference, SB1 dry, SB2 dry and CHL-B where only very few or no ants could be collected, the bait formulations obtained a 90 to 100 percent mortality which was used as a criteria for high efficacy in this test.

## DISCUSSION

The primary goal of the field method presented was to make it possible to make a comparison between different bait formulations for *L. niger* control. A method which interferes as little as possible with the communication between the ants. The tests, however, revealed a number of aspects which must be considered. It is essential to be very careful with the handling of the ants after they have been collected in order to prevent additional mortality due to the method; a fact clearly illustrated by the mortality obtained with some of the ants sampled from the reference nests. Likewise is it important to make certain that no ants from other nests are collected in the same tube, which would add to the mortality as the ants would start to kill one another (Dumpert, 1978).

It could be argued that in a test like this, complete control should be obtained among the ants

Table 1. Means of visiting ants are based on the numbers calculated for 30 minutes. Data in italics indicate that the trial series were stopped as it became clear that only a few ants could be collected from the insecticidal baits.

| IB type used   | Number of nests | Number of visiting ants | Mean percentage of ants visiting the IB $\pm$ sdv | Total number of ants collected | Mean Percentage of ants killed $\pm$ sdv |                 |
|----------------|-----------------|-------------------------|---|--------------------------------|--|-----------------|
|                |                 |                         |   |                                | Jam                                      | Bait            |
| Reference      | 2               | 1271                    | 50.8 $\pm$ 3.3                                    | 178                            | 23.6 $\pm$ 33.4                          | 0.0 $\pm$ 0.0*  |
| <i>SB1 dry</i> | 2               | <i>318</i>              | <i>12.3 <math>\pm</math> 12.3</i>                 | 83                             | 3.5 $\pm$ 4.9                            | 86.7**          |
| <i>SB2 dry</i> | 2               | <i>206</i>              | <i>3.11 <math>\pm</math> 7</i>                    | 61                             | 1.6 $\pm$ 2.2                            | 75.0 $\pm$ 35.4 |
| SB1 Moist      | 6               | 1835                    | 46.4 $\pm$ 11.8                                   | 412                            | 2.8 $\pm$ 2.1                            | 90.3 $\pm$ 10.9 |
| SB2 Moist      | 5               | 1051                    | 39.8 $\pm$ 0.2                                    | 303                            | 2.1 $\pm$ 3.3                            | 97.1 $\pm$ 3.2  |
| AZA-B          | 9               | 2053                    | 45.1 $\pm$ 7.2                                    | 527                            | 10.1 $\pm$ 14.6                          | 94.7 $\pm$ 10.5 |
| CHL-B          | 8               | 1132                    | 0.0 $\pm$ 0.0                                     | 230                            | 0.0 $\pm$ 0.0                            | —               |

\*indicates that the method was evaluated by using two jam-baits instead of a jam bait and an IB.

\*\*Ants could only be collected from one of the nests.

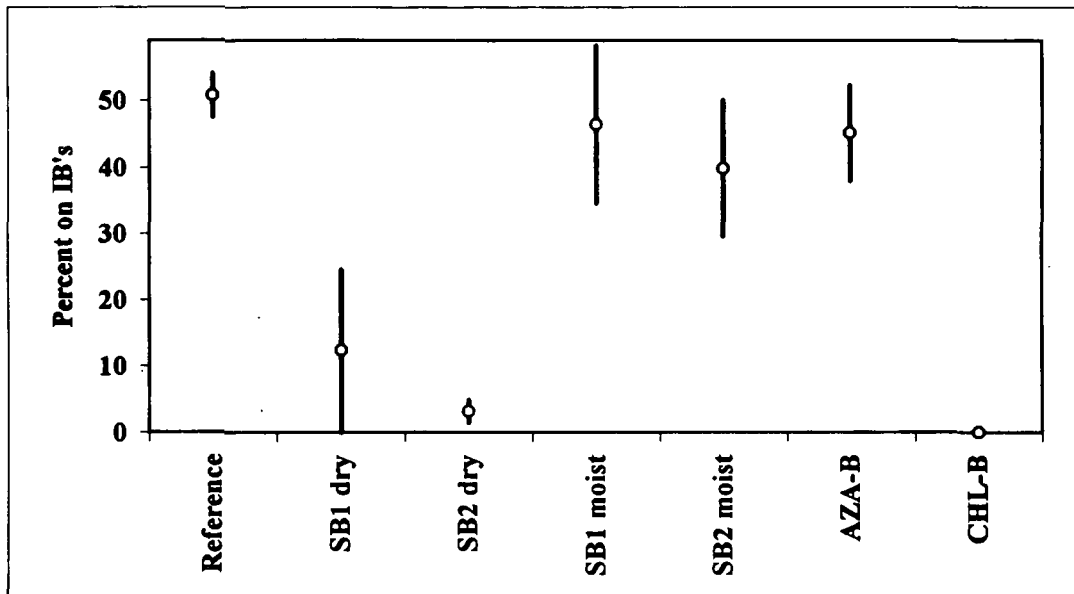


Figure 3. The estimated mean percentage of ants on the IBs after 30 minutes.

collected after leaving the IBs. This is not the case, and in fact a few ants must always be expected to survive. It is not possible in a field trial like the present to determine whether or not an ant has actually been eating or otherwise exposed or for how long it has been on the plate or how much it has been eating. Just the fact that the ant has been on the plate with the IB qualifies it to be collected. Nevertheless the results show that this only gives rise to minor errors, and it is possible, even in some detail, to examine how the ants are affected by the baits.

There is one important question which was not part of this investigation as such, namely the ability of the product to act on the nest as a whole, meaning whether or not the ants were able to bring back the bait material to the nest and feed the larvae and the queen. It is in fact a very important question, as control of the nests must be considered to be one of the essential goals in ant control. This aspect could be investigated by following in detail how the mortality develops after collection of the ants. Due to the set-up it is known when the ants have been eating insecticidal bait, and it will therefore be possible to estimate whether or not the workers would have time enough to reach the nest and feed the larvae or the queen before they are killed themselves. What is not possible is to establish whether or not the ants are affected to such an extent that their co-ordination or ability to follow their trail marks are disturbed. A result which could indicate whether or not this is the case could be obtained by examining the treated nests after a month, to see if they are still active. This was done with a few of the nests used in this investigation. Three nests were examined a month after the treatment with AZA-B, and none of them showed any sign of ant activity, although ant activity in general and in the reference nests was high at the time.

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