Improved early detection of internal infestation by flotation using product-adapted salt solutions

K. Richter and P. Tchalale*

Abstract
The success of stored-pest control strategies depends upon the effectiveness of the methods used for detecting and monitoring. Although many methods have been developed, the early detection of insects feeding inside grain kernels remains difficult. In particular, a method which is effective, rapid, accurate and easy to handle is needed by farmers, grain dealers and millers in developing countries.

Usually, the flotation method is used with standard salt solutions or water. It was found that a salt solution with product-adapted density improves the accuracy of detection (up to 100% of the real infestation) because grain varieties and cultivars differed much more than expected in kernel density. Moreover, adapted solutions permitted a much earlier detection of the hidden infestation after oviposition.

The optimum density of the solution was found to be 12–13% below the average density of sound grain. At this value errors and misinterpretations were minimised.

Using this method, a 75% detection level was reached 10 days after oviposition compared with 22 days with standard salt solutions (example: triticate/Sitophilus oryzae (L.)). The data showed a clear correlation (r = +0.964**) between close adaptation of density of the flotation medium and earliest possible detection.

The method is proposed for the inspection of samples of cereals and grain legumes after harvest, before storage or export, when the sieving method gives negative results for pests causing internal infestation, or when circumstances dictate that the internal feeders need to be detected.

Introduction
Detecting internal insect infestation in cereal and legume grains is important in the prevention of damage during storage, the minimisation of insect fragments in the milling process, and the maintenance of seed quality parameters.

If adults have been removed from the kernels by cleaning or threshing after having laid eggs into grains during transit, storage or in the field under favourable climatic conditions, grain internally infested by larvae, pupae or even newly hatched adults may be mistaken for uninfested grain. A very low population density of adults may lead to a very high level of internal infestation over four to eight weeks by the steady egg-laying process before the hatching of the F_1-generation (Fig. 1).

Tests for the detection of insects feeding inside the kernels include presumptive tests such as staining (Goossens 1949), flotation (White 1956) and cracking flotation separation (Harris et al. 1952), sound detection (Shuman et al. 1993; Vick et al. 1988), CO_2-determination (Bruce et al. 1982), NMR-inspection (Pinniger et al. 1986) and direct visualisation of internal infestation by X-ray analysis (Milner et al. 1950). Most of these methods are expensive, time consuming or require sophisticated equipment.

Although many methods have been developed, the early detection of insects feeding inside the kernel remains difficult. The need for a method which is effective, rapid, accurate and easy to handle is evident for farmers, grain dealers and millers, particularly in developing countries.

The present report suggests the use of product-adapted salt solutions to separate internally infested grain from noninfested grain. This method meets the demand for effectiveness, rapidity, accuracy and simplicity.

Material and Methods
The rice weevil Sitophilus oryzae (L.), and the Brazil bean weevil, Zabrottes subfasciatus (Boh.), were used as the test species in these experiments because their larvae feed exclusively within seeds.

The rice weevil and Brazilian bean weevil were reared on bread wheat and triticate, and on phaseolus beans, respectively.

Developmental stages (from eggs to adults) of known age were obtained by allowing adults to oviposit on uninfested seeds for 48 hours (rice weevil) and four days (Brazilian bean weevil).

Adults were then removed and individual kernels were checked microscopically to identify kernels containing eggs. Selected seeds were then incubated at 26 ±1°C and 65 ± 5% r.h. in darkness. This procedure was replicated in intervals of one to three days to obtain grain infested by larvae at all developmental stages. When the adults began to emerge from the oldest sample, all repetitions of the same series were examined by flotation. All separated grains were then inspected for the presence of internal infestation and for the identification of developmental stages.

Product-adapted salt solutions were prepared as follows: After determining the mean density of the grain lot (healthy), we adjusted the highest density for the salt solution to 12–13% of this value (0.55 kg/L NaNO_3 for density = 1.24 g/cm³). Lower density steps were investigated by using saturated and unsaturated NaCl-solution (density = 1.19 and 1.04 g/cm³), and water.

Results
In all experiments, the recovered percentage of infested grain in relation to the development stage of the larva is well

* University of Leipzig, Institute of Tropical Agriculture, Fichtestrasse 28, D-04275 Leipzig, Germany.
Fig. 1. Calculated increase in the number of internally infested caryopses in relation to the oviposition capacity of one female of *Sitophilus granarius* under different temperatures, using data from Jacobi (1982) and Swatoni (1975a, 1975b).

described by the curves calculated as logistic exponential functions.

The data obtained by flotation with the media in question (salt solutions and water) were significantly different at the 1% level (bars not shown in the figures).

Using the optimum adapted salt solution with a density = 1.24 g/cm³ for triticale and wheat, a 75% detection level could be obtained 10 days and 16 days after oviposition, respectively (Figs 2 and 3). First records of hidden infestation were made possible five and six days after oviposition for the cultivars tested.

In relation to these results, the maximum detection level for triticale and wheat using water averaged 49.5% and 30.0% and for the low-density media 62.4% and 36.7%, respectively.

Moreover, it is worth noting that even a small reduction in density (from 1.24–1.19 g/cm³) may drastically reduce the percentage of detectability from 75% to 55% and the point of earliest traceable infestation from 10 to 22 days in case of triticale (Fig. 2). The analogous results for wheat were 16.5 and 22.5 days (Fig. 3).

To emphasise the relationship between the early detection of infestation in the product and density of the product, a rela-

Fig. 2. The effect of various salt solutions on the detection of internal infestation by flotation (*S. oryzae* triticale).
Fig. 3. The effect of various salt solutions on the detection of internal infestation by flotation (S. oryzae/ wheat).

Fig. 4. Values for possible early detection in dependence of adaptation quotient $Q_a$ ($T =$ triticale; $W =$ wheat).

$tionship for the adaption of the density of flotation medium to the mean density of healthy product was calculated, the quotient of adaption $Q_a$:

\[
Q_a = \frac{\text{Mean density of product}}{\text{Density of salt solution}}
\]

Using this equation, we could find a strong, significant correlation between time after oviposition and adaptation ratio, independent of the product tested (Fig. 4).

Because of the lower feeding percentage in relation to the weight of the kernel, infestation by the Brazilian bean weevil was traceable only at older developmental stages. The point for earliest traceable infestation was found to be nine to ten days after oviposition, with one larva per kernel.

**Discussion**

Usually the flotation method is performed using standard salt solutions of a density = 1.19 g/cm$^3$, fixed salt solutions or plain water (Apt 1950; Peng and Moralilo-Rejesus 1987; White 1956). We found that a salt solution with product-adapted density improves the accuracy of detection to up to
100% of the real infestation, because the density of grains of varieties and even cultivars differs much more than expected. Moreover, adapted solutions allow a much earlier detection of the hidden infestation after oviposition.

For preparing such flotation media, the adaption quotient Qₐ will be a good measure. The closer the Qₐ value to 1, the better the adaption will be. The optimum density of the solution was found to range between 12 and 13% below average density of sound grain, corresponding to an Qₐ value of 1.04. At this value errors and misinterpretations are minimised.

Using this method, a 75% detection level will be reached by 10 days after oviposition compared to 22 days with the standard salt solution (example: Triticale/Sitophilus oryzae L.). The data show a clear correlation (r = +0.964) between close adaption of density of the flotation medium and earliest possible detection.

Comparing the data obtained by flotation with those from X-ray inspection or NMR-technique, it becomes evident that the detectability starts approximately at the same time, around six to ten days after oviposition for both rice weevil and Brazilian bean weevil (Pinniger et al. 1986; Spirina 1982; Milner et al. 1950).

For further investigation, e.g. to distinguish species or developmental stages, the separated kernels can be checked by cutting or X-ray inspection.

The method is proposed for the inspection of samples of cereals and grain legumes after harvest, prior to storage, and before exportation when the sieving method gives negative results for pests or when situations dictate that the internal feeders must be detected.

References


White, G.D. 1956. Studies on separation of weevil infested from noninfested wheat by flotation. USDA, AMS 1, 10 pp.