Extraction of *Diabrotica* eggs from soil and determining whether *Diabrotica* oviposit in fields of oil seed squash

Extraktion von Diabrotica*-Eiern aus dem Boden und Bestimmung, ob* Diabrotica *in Ölkürbisfeldern Eier ablegt*

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Summary

A soil washing apparatus was constructed and tested in collaboration with colleagues from the grant authority and project partners in Austria and Romania. It is based on a similar apparatus used in the USA but our apparatus can quickly process a large number of samples.

The eggs were extracted by sieving and then floating on $MgSO_4$-solution followed by sedimentation in water. Eggs of *D. virgifera virgifera* were separated from those of other species by studying the structure of egg surface. The efficiency of extraction of the apparatus was checked using samples spiked with blue coloured eggs. In co-operation with Austrian and Romanian colleagues a method was developed for sampling in the field. Eggs of *D. virgifera virgifera* were extracted from soil samples collected from fields in Austria and Romania in spring/summer and autumn 2011. The beetles did not appear to prefer to lay eggs at either the edges or centres of fields. As very few eggs were found at the edges of oil seed squash fields this crop does not appear to be a suitable habitat for egg laying.

Keywords: *Diabrotica virgifera virgifera,* egg laying, extraction from soil samples, oil seed squash field

Zusammenfassung

Eine Eiauswaschanlage wurde zusammen mit Kollegen vom Zuwendungsgeber und von Projektpartnern in Österreich und Rumänien gebaut und geprüft. Sie ähnelt einer Anlage, die in den USA verwendet wird. Unsere Anlage kann jedoch eine größere Anzahl von Proben in kürzerer Zeit verarbeiten.

Die Eier wurden durch Sieben extrahiert und in einer $MgSO_4$-Lösung aufgeschwemmt, um sich dann in Wasser abzusetzen. Die Eier von *D. virgifera virgifera* wurden von denen anderer Arten anhand ihrer Oberflächenstruktur getrennt. Die Extraktionsleistung der Anlage wurde mit Hilfe von Proben, die mit blau gefärbten Eiern versetzt wurden, geprüft. Zusammen mit den österreichischen und rumänischen Kollegen wurde eine Probenahmemethode für das Freiland entwickelt. Eier von *D. virgifera virgifera* wurden aus den Bodenproben von Feldern in Österreich und Rumänien im Frühjahr/Sommer und im Herbst 2011 extrahiert. Die Käfer bevorzugten offensichtlich weder den Feldrand noch die Feldmitte für die Eiablage.

Stichwörter: *Diabrotica virgifera virgifera*, Eiablage, Extraktion aus Bodenproben, Ölkürbisfeld

1. Introduction

In order to forecast the damage caused by and control the pest *Diabrotica virgifera virgifera*, which was introduced from the US into Europe, it is important to have more detailed information on where and how many eggs it lays.

The numbers of eggs laid in the soil are directly related to the subsequent number of damaging larvae. Although it is difficult to accurately determine the number of eggs in soil because it takes a long time to process the large volumes of soil needed to do this, it is possible to forecast the expected abundance if one can measure egg density. In addition, by extracting its eggs from soil it is also possible to answer questions about the egg laying behaviour of *D. virgifera virgifera*.

Studies in the USA and Europe reveal that it is possible to obtain quantitative data on the number of eggs laid in a defined volume of soil by washing the soil through sieves of different mesh sizes and separating the eggs from the residue by a process known as floating (e.g. Foster *et al.,* 1979; Matteson, 1966; Park and Tollefson, 2006; Shaw *et al.*, 1976; Ruesink, 1986; Ujvari *et al.*, 2004). But whether the methods used in the USA and South-East Europe are suitable for being used elsewhere needs to be checked. For example, fields in the USA are much larger than in Europe and therefore local soil samples vary less in terms of soil types and soil structures.

Currently it is also unknown if differences in the fauna will result in the presence of eggs of other taxa that are similar to those of *D. virgifera virgifera*, which makes it difficult to identify its eggs. The objective of this project was to develop a method for extracting eggs of *D. virgifera virgifera* from soil samples suitable for determining whether *D. virgifera virgifera* oviposits in fields of oil seed squash.

2. Material and methods

To determine the number of eggs laid by females of *D. virgifera virgifera* in fields a soil washing apparatus was constructed and tested (Fig. 1-4). The apparatus had to be transportable and capable of quickly processing a large number of samples. In principal, the construction is based on equipment used for this purpose in the USA in terms of water pressure, rotation speed and position/angles (Shaw *et al*., 1976). The extraction of samples is done by washing soil trough two sieves, the first a fixed 550 µm mesh sieve followed by a rotating drum fitted with a 250 µm mesh sieve (Fig. 2). In order to determine the appropriate mesh sizes eggs of *Diabrotica* of different ages and developmental stages were measured.

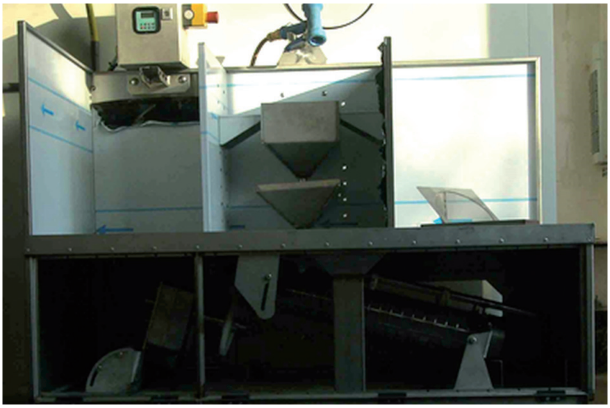

Fig. 1 Apparatus used for extracting eggs from soil.

Abb. 1 *Apparat zur Auswaschung von Eiern aus Bodenproben.*

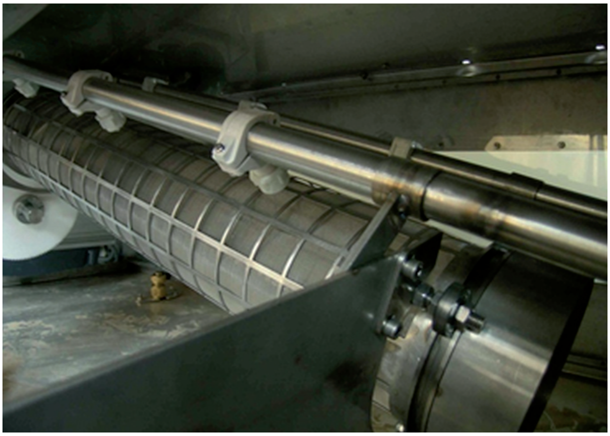

Fig. 2 Drum sieve.

Abb. *2 Trommelsieb.*

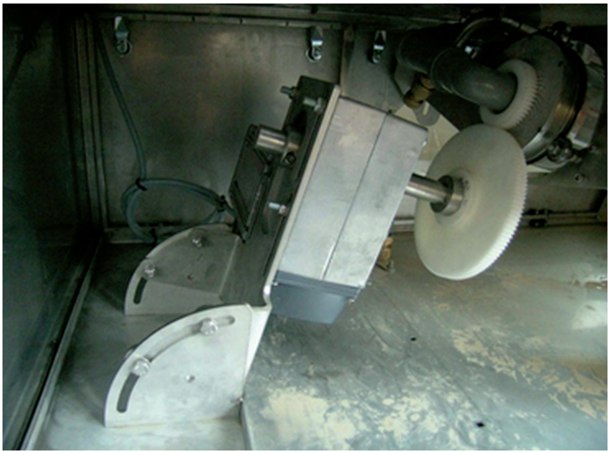

Fig. 3 Details of motor suspension.

Abb. 3 *Detailaufnahme des Motorgetriebes.*

Fig. 4 Speed control.

Abb. 4 *Geschwindigkeitskontrolle.*

The eggs were extracted by transferring the residue on the sieves to funnels filled with 1 liter $MgSO_4$-solution (2 mol/L) on which the eggs float (Fig. 5) and then replacing this solution with water (Fig. 6), which results in the sedimentation of the eggs (Palmer *et al*., 1976). Counting the eggs was done under a microscope. Eggs of other insects, which were not removed during the washing procedure because they were the same size and specific gravity as those of *D. virgifera virgifera,* were identified by checking the structure of egg surface (Fig. 7). The eggs of *D. virgifera virgifera* are characterized by sculpturing consisting of bold ridges forming polygons (Atyeo *et al*., 1964; Krysan, 1986).

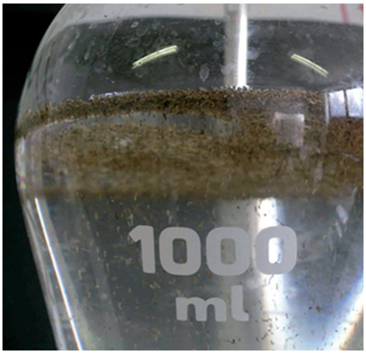

Fig. 5 Eggs floating on $MgSO_4$-solution.

Abb. 5 *Aufschwemmung der Eier in $MgSO_4$ Lösung.*

Fig. 6 Sedimentation of eggs in water.

Abb. 6 *Sedimentation der Eier in Wasser.*

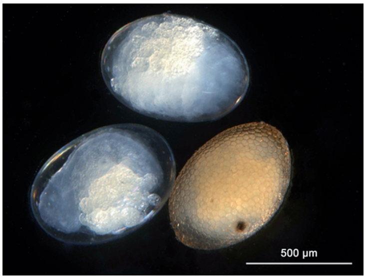

Fig. 7 Eggs of same size and density extracted from soil samples. The chorion of the egg of *D. virgifera virgifera* (*right*) has a characteristic polygonal sculpturing (Atyeo *et al*., 1964).

Abb. 7 *Aus Bodenproben extrahierte Eier gleicher Größe und Dichte. Das Chorion der Eier von* D. virgifera virgifera *(*rechts*) hat eine charakteristische polygonale Skulpturierung (Atyeo* et al.*, 1964).*

To verify the efficiency of this method every fifth sample was spiked with 10 eggs coloured with Giemsa and the number coloured eggs recovered after washing and floating was determined. These eggs were produced by a *Diabrotica* culture that originated from Hungary and was reared in the laboratory.

To optimize the sampling in the field the soil samples were collected using different equipment (spade, shovel, soil core borer) and transferred in different quantities to the egg-washing apparatus.

After different durations of washing the composition of the different sized pieces of debris on the different sieves was analysed. Both published and our results were used to finalize the adopted design of the sampling method. This design was used in 2011 in field trials in Austria and Romania. These trials were done in close co-operation with colleagues, Grabenweger in Vienna and Foltin in Wulkaprodersdorf, Austria and Lauer/Gräpel in Timişoara, Romania.

3. Results

The apparatus we constructed was used to obtain qualitative and quantitative data on the abundance and distribution of *D. virgifera virgifera* eggs in the field with the aim to use this data to forecast the expected future abundance of this pest. In addition, this information will be used to address a number of questions about the egg laying behaviour and model the population dynamics of this pest.

The choice of the mesh sizes of stainless steel sieves was determined by the size of the eggs of *D. virgifera virgifera,* which originated from Hungary (length 0.63 ± 0.03 mm (min. 0.54 mm, max. 0.69 mm); diameter 0.40 ± 0.03 mm (min. 0.34 mm, max. 0.46 mm)). The fixed primary sieve has a mesh size of 550 µm and the rotating drum sieve has a mesh size of 250 µm. The efficiency of the soil washing apparatus was tested by using soil samples containing known numbers of eggs of *D. virgifera virgifera* obtained from a culture of this species reared in-house since 2003. The efficiency with which blue coloured control eggs were recovered was similar to that reported in the literature (e.g. Shaw and Hummel, 2003; Takács *et al*., 2005).

There was a negative relationship between the volume of soil sample placed in the egg-washing apparatus and number of eggs extracted (Fig. 8). This indicated that a soil sample should not be larger than 750 ml otherwise the number of eggs recovered is less than 88%.

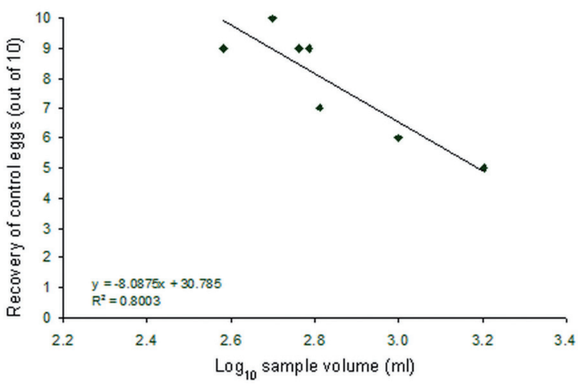

Fig. 8 Effect of soil sample size on the recovery of blue coloured eggs.

Abb. 8 *Einfluss der Bodenprobengröße auf die Wiederfindung von blau gefärbten Eiern.*

During the counting of eggs under a microscope it became clear that there were also eggs laid by other species. If these eggs were of the same size and density as those of *D. virgifera virgifera* then they would not be separated from those of the target species during the washing process. The eggs of *D. virgifera virgifera,* however, can be reliably identified as the sculpturing on the chorion is very characteristic (Atyeo *et al*., 1964; Rowley and Peters, 1972).

In collaboration with colleagues from the grant authority and project partners in Austria and Romania a method of collecting soil samples in the field was developed and used to obtain the soil samples collected in Austria and Romania, which were processed in the soil washing apparatus.

The first set of field samples was collected in spring/summer 2011. In total 36 samples were collected in Austria (locations in Styria and Burgenland) and 14 in Romania (locations in Banat).

The second set of field samples was collected in autumn 2011. In total 99 samples were collected in Austria (locations in Styria and Burgenland) and 62 in Romania (locations in Banat).

To obtain data on (i) the distribution of eggs from the edge to the centre of a field and (ii) whether eggs are laid in oil seed squash fields near a maize field, 5 sample points per area were selected (Fig. 9). Analyses of soil samples from oil seed squash fields were carried out only in Styria and only for fields close to maize fields infested with *D. virgifera virgifera*. Each sample consists of 3 subsamples of soil collected down to a depth of 20 cm using either a spade or core borer. 2 l of soil were collected from the base of plants, 2 l within a row and 2 l between rows. After carefully sieving (mesh size 1 cm) and sorting to remove stones and roots 3 subsamples 2 x 500 ml were isolated and transferred to the laboratory for extraction and the numbers of eggs of *D. virgifera virgifera* – eggs extracted are given in Table 1.

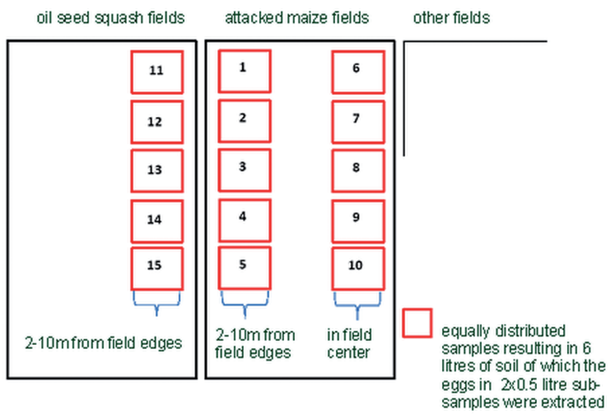

Fig. 9 Number and location of the samples collected in fields in Austria in autumn 2011. Soil samples from oil seed squash fields were collected only in Styria.

Abb. 9 *Anzahl und Platzierung der Bodenprobenahmen in Feldern in Österreich im Herbst 2011. Proben aus Ölkürbisfeldern wurden nur in der Steiermark gesammelt.*

In spring/summer 2011 the maximum number of eggs per litre of soil was 8.1 in Austria and 3.1 in Romania. The samples collected in autumn 2011 contained a higher maximum number, 69.2 eggs per litre in Austria and 45.0 eggs per litre in Romania (Tab. 1). The analyses show that in the soil samples collected during spring-summer there were more eggs recorded at the edges of 4 of the fields and more eggs from the centre in 1 field. In the former there was no significant difference in the number of eggs recorded at the edges and centres of the fields.

Tab. 1 Mean number (± SD) of *Diabrotica* eggs per litre of soil extracted from samples collected in 2011. (position: C center, E edge, M maize, OSS oil seed squash; crop rotation: B barley, M maize, OSS oil seed squash, P potato, R oilseed rape, S soya, SB sugar beet, W wheat).

Tab. 1 *Durchschnittliche Anzahl (± SD) von* Diabrotica-*Eiern je Liter Boden aus Proben gesammelt in 2011. (Lage: C Zentrum, E Rand, M Mais, OSS Ölkürbis; Fruchtfolge: B Gerste, M Mais, OSS Ölkürbis, P Kartoffel, R Raps, S Soya, SB Zuckerrübe, W Weizen).*

Country		Location	Position	Crop rotation	Sample (n)	Eggs (n)	Mean		Max/ Ltr	Min/ Ltr
Austria	May	Bruckneu-dorf	E-M	P-WW-M-M	2	0	0.0	± 0.0	0.0	0.0
			C-M	P-WW-M-M	2	0	0.0	± 0.0	0.0	0.0
	May	Dedenitz	E-M	M-M	4	0	0.0	± 0.0	0.0	0.0
			E-OSS	WW-OSS	2	0	0.0	± 0.0	0.0	0.0
	May	Deutsch Jahrndorf	E-M	M-	2	6	4.8	± 4.7	8.1	1.5
			C-M	M-	2	3	1.5	± 0.4	1.7	1.2
	June	Deutsch Jahrndorf	E-M	M-	2	1	0.7	± 0.9	1.3	0.0
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
	May	Dornau	E-M	OSS-M	4	0	0.0	± 0.0	0.0	0.0
			E-OSS	M-OSS	2	0	0.0	± 0.0	0.0	0.0
	May	Raabfeld	E-M	M-	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
	May	Wallern	E-M	M-	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
	May	Zuberbach	E-M	M-	2	3	2.0	± 1.2	2.9	1.2
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
Romania	May	Grabatz	E-M	M-M-M-WW	2	3	1.1	± 0.2	1.3	1.0
			C-M	M-M-M-WW	2	2	0.8	± 1.2	1.7	0.0
	May	Iecea Mare I	C-M	M-M-WW	2	0	0.0	± 0.0	0.0	0.0
		Iecea Mare II	E-M	M-M-WW	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-M-WW	2	5	2.0	± 1.6	3.1	0.8
	May	Lenauheim	E-M	M-M-M-WW	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-M-M-WW	2	0	0.0	± 0.0	0.0	0.0
Austria	Oct.	Antau	E-M	M-W-M-R	5	74	12.9	± 12.9	26.4	0.0
			C-M	M-W-M-R	5	26	4.6	± 5.1	12.7	0.0
	Sept.	Badersdorf	E-M	M-S-WW-M-S	5	49	15.3	± 19.5	48.4	0.0
			C-M	M-S-WW-M-S	5	10	3.1	± 3.7	8.3	0.0
	Sept.	Kukmirn	E-M	M-	5	0	0.0	± 0.0	0.0	0.0
			C-M	M-	5	8	2.6	± 3.7	7.9	0.0
	Oct.	Neutal	E-M	M-WW-R-M	5	2	0.7	± 1.0	2.0	0.0
			C-M	M-WW-R-M	5	13	3.6	± 5.0	12.0	0.0

	Oct.	Siegendorf 1	E-M	M-M-Durum-WW	5	11	3.2	± 2.8	7.1	0.0
			C-M	M-M-Durum-WW	5	150	43.1	± 30.6	85.1	0.0
		Siegendorf 2	E-M	M-M-WW-SB	5	52	15.8	± 16.3	42.9	0.0
			C-M	M-M-WW-SB	5	67	20.2	± 20.9	47.7	0.0
	Oct.	Stoob	E-M	M-WG-R-M	5	1	0.3	± 0.7	1.6	0.0
			C-M	M-WG-R-M	5	9	2.8	± 2.6	5.0	0.0
	Oct.	Wallern	E-M	M-	5	17	5.2	± 7.5	17.6	0.0
			C-M	M-	5	32	10.1	± 6.7	21.7	5.7
	Oct.	Wulkapro-dersdorf 1	E-M	M-WW-SB-R	5	10	2.2	± 4.4	10.0	0.0
			C-M	M-WW-SB-R	5	28	6.7	± 7.5	18.3	0.0
		Wulkapro-dersdorf 2	E-M	M-WW-SB-R	5	4	1.3	± 2.4	5.5	0.0
			C-M	M-WW-SB-R	5	2	0.7	± 0.9	1.7	0.0
	Oct.	Halbenrain East	E-M	M-OSS-M-OSS	5	27	8.5	± 12.0	29.5	0.0
			C-M	M-OSS-M-OSS	5	15	4.8	± 5.0	11.9	0.0
			E-OSS	OSS-M-S-M	5	0	0.0	± 0.0	0.0	0.0
	Oct.	Halbenrain West	E-M	M-OSS-M-OSS	5	12	3.9	± 6.5	15.3	0.0
			C-M	M-OSS-M-OSS	5	20	6.7	± 9.5	23.3	0.0
			E-OSS	OSS-M-S-M	5	2	0.6	± 1.3	2.8	0.0
	Oct.	Poppendorf East	E-M	M-OSS-M-OSS	5	8	2.3	± 1.7	4.5	0.0
			C-M	M-OSS-M-OSS	5	26	8.7	± 7.4	20.7	1.7
			E-OSS	OSS-M-OSS-M	5	3	0.9	± 1.4	3.2	0.0
		Poppendorf West	E-M	M-OSS-M-OSS	5	1	0.3	± 0.7	1.7	0.0
			C-M	M-OSS-M-OSS	5	25	8.1	± 6.5	18.3	1.9
			E-OSS	OSS-M-OSS-M	5	2	0.6	± 0.9	1.6	0.0
	Oct.	Zelting 1	E-M	M-M-WG	5	163	48.3	± 25.4	69.2	4.2
			E-OSS	OSS-M-M	5	7	2.0	± 1.9	4.3	0.0
		Zelting 2	E-M	M-M-M-M-M	9	37	9.1	± 11.8	33.3	0.0
			E-OSS	OSS	10	1	0.2	± 0.7	2.3	0.0
Romania		Grabatz I	E-M	M-M-M	8	17	3.5	± 6.1	13.3	0.0
			C-M	M-M-M	8	1	0.2	± 0.6	1.7	0.0
		Grabatz II	E-M	M-M-M	8	0	0.0	± 0.0	0.0	0.0
		Grabatz III	E-M	M-M-M	2	0	0.0	± 0.0	0.0	0.0
		Iecea Mare II	C-M	M-M-M-WW	10	9	1.5	± 3.7	11.7	0.0
		Lenauheim I	E-M	M-M-M	8	69	15.0	± 18.9	45.0	0.0
			C-M	M-M-M	8	0	0.0	± 0.0	0.0	0.0
		Lenauheim II	C-M	M-M-M	10	13	2.1	± 2.1	6.1	0.0

For the soil samples collected in autumn there were more eggs at the edges of 5 fields and more eggs in the centre of 9 fields. If the results for the samples collected in both periods are combined, then more eggs were recorded at the edges of 10 fields and more eggs at the centres of 11 fields. That is, we did not find a preference for laying eggs either at the edges or centres of fields.

Only very few eggs were laid in oil seed squash fields close to maize fields. For the 8 oil seed squash fields sampled the number of eggs/litre of soil varied between 0.0 (± 0.0) - 0.9 (± 1.4). In only one field (located near Zelting, Austria) were 2.0 (± 1.9) eggs/litre and that was close to a maize field that was heavily infested with *Diabrotica* and in the soil of which there were 48.3 ± 25.4 eggs /litre.

4. Conclusions

In order to determine the number of eggs laid by females of *D. virgifera virgifera* in fields a soil washing apparatus was constructed and tested in collaboration with colleagues from the grant authority and project partners in Austria and Romania. This apparatus is transportable and can be used to quickly process a large number of samples. It is based on a similar apparatus used in the USA. The soil is first washed through a 550 µm sieve and then in a rotating sieve drum with a mesh size of 250 µm.

The eggs were extracted from the residue by first floating them in $MgSO_4$-solution followed by sedimentation in water. The numbers of eggs were counted under a microscope. Eggs of *D. virgifera virgifera* can be easily identified as the surface of its eggs has a characteristic reticulated structure. Thus it was possible to separate its eggs from those of other insects, which were not removed during the washing procedure because they are the same size and specific gravity as those of *D. virgifera virgifera*. The extraction efficiency of this apparatus was checked by extracting soil samples spiked with blue coloured eggs. In co-operation with Austrian and Romanian scientists a method was developed for sampling in the field that could be quickly used to determine both the horizontal and vertical distribution of eggs of *Diabrotica* in the soil.

The beetles did not appear to prefer to lay their eggs at the edges or centres of fields and only very few eggs were recorded at the edges of oil seed squash fields.

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