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GREEN PEAS DISINFESTATION WITH DELTAMETHRIN AND PHOSPHINE.

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ABSTRACT

In France the infestation level of proteaginous peas by the pea weevil, *Bruchus pisorum* L. (Coleoptera: Bruchidae), is about 13%. In order to satisfy the marketing requirements with regard to sanitation, it is necessary to eradicate this pest before or during storage. The efficiency of phosphine and deltamethrin against this pest has already been shown. Therefore, two series of experiments were conducted using these treatments to increase our knowledge of their insecticidal efficiency.

Deltamethrin applications were carried out after harvest. The treatment followed the French official protocol (CEB n°106) applied to the pea weevil. The application dosage proposed by Bayer Crop Science is 0.25 g/t mixed with 2.25 g/t of PBO (Pyperonyl butoxide) (Protocol CEB 213). .

Phosphine fumigations were applied in September, October, December, January and March during storage. Thus, a possible interactivity between temperature and efficiency of treatment was evaluated. Fumigations concentrations ranged from 200 to 700 ppm.

Results show that the efficiency of the deltamethrin treatment is about 94%, a mortality level that cannot completely prevent the pest's dynamic reproduction. Maximum efficiency of the phosphine treatment against all stages of *B. pisorum* was obtained at 700 ppm for 4 days at 20°C in the laboratory. At 15°C, 100% mortality was obtained in 8 days at 350 ppm. No temperature interaction was measured but the storage period increased the efficiency of the gas on the hidden stages. It is intended to undertake another trial at 700 ppm, at 15°C.

INTRODUCTION

The proteaginous crops (rich in protein) are one of the most important sources of vegetal protein around the world (Gupta and Kashyap 1995). In certain countries, the pea, *Pisum sativum*, has become a substitute for chickpea. In Europe, it is used as a substitute for animal meal and for this reason the demand for peas is increasing. However, this crop is both qualitatively and quantitatively depreciated by the pea weevil, *Bruchus pisorum* L. To satisfy the marketing demands, an absence of live adult insects is crucial during the whole period of conservation.

In order to develop a reliable method to eradicate the populations of beetles from harvest or storage, the efficiencies of two chemical treatments usually used on grain have been tested. The two treatments were: fumigation with phosphine (PH_3) and application of an insecticide, deltamethrin.

The latter was carried out according to protocol CEB N°106 entitled "Method for the study of the practical efficiency of insecticidal treatment under semi-industrial conditions in order to control the granary weevil, *Sitophilus granarius* (L.) and the lesser grain borer, *Rhizopertha dominica* (Fabricius) on harvested corn", which is under validation for the treatment of peas.

These studies were initiated with preliminary preparations: at first, peas of the same variety were gathered to amass a total batch with the same level of infestation. Then, in order to have samples with a significant number of insects (200), the average rate of infestation was determined by radiography. A follow-up of adult emergence was carried out during the 6 months of storage.

BIOLOGY

Bruchus pisorum L. has only one generation per year (monovoltine). In autumn, the adults over-winter in pea storages - under bark,, on the ground - or even inside the seeds (Pears, 1950). Adults only become active in late May or early June when they leave their shelters in search of peas under cultivation (they are able to fly several kilometres). They eat pollen and the pea petals during a few days and then they mate. The yellow 0.6 mm-long, pointed eggs are deposited separately on the pea pods. An average of 400 eggs are laid and incubation lasts ten days. After flowering is over, the hatching larvae traverse the skin of the pods and dig very narrow galleries until a seed is reached. Then, the larva penetrates the seed. Only one larva takes possession of a single pea within which it undergoes several moults.

The larva can continue its development only in peas collected at a mature stage, and its development takes 40 to 45 days. Before pupation, the larva makes a circular cut in the skin of the pea, leaving only a thin film in place. The pupal stage lasts 10 days on average (Bonnemaison 1953). Because oviposition is spread over a few weeks, it is possible to observe various stages of development of the insect in the same batch of pea after the harvest.

Most adults emerge during the first two months of storage and find various places to shelter over winter. Some remain within the peas until the following spring (Whittle 1996).

MATERIALS AND METHODS

Peas

The experiments were undertaken with two 150 kg batches of pea seeds, delivered in August 2002 and consisting of two varieties, "Badminton" and "Atos". For each variety, after homogenisation of the peas, a percentage level of infestation was determined by radiography. Arbitrarily, the "Badminton" variety was used for tests of the insecticidal effectiveness of phosphine and the variety "Atos" was used for the tests with the insecticide (Deltamethrin).

The infestation rate was measured by radiography (X ray apparatus CGR Sigma 60, tube tension - 18KV; filament intensity - 5 my; exposure period - 22 seconds). It was then possible to set up 9 sub-batches containing at least 200 insects.

A sample of about 700 seeds were taken from each sub-batch and these were then spread out over a self-adhesive plate which was radiographed. The number of insects present inside the seeds can be assessed by observing the x-ray film.

Observation of adult emergence

Five 1.5 kg batches of peas containing insects, corresponding to the control of the first 5 treatments, were placed in a cold room to undergo the variations in temperature of storage.

These batches were observed every month in order to count the number of beetles having emerged during the period of storage and to establish a curve of adult emergence.

Phosphine fumigation

Phosphine fumigations are used to protect cereals against stored-product insects. Since this treatment does not leave any residue, it can theoretically be applied to peas.

In order to define the best period of application and thus optimize the treatment efficiency, treatments were carried out at various periods of storage: in September, October, November, December, January and March. The peas were preserved under temperature conditions close to those in commercial storage.

Storage conditions were programmed following the temperature profile of a commercial storage, namely: 15°C from September to November, 4°C until March and from then on, 12°C (Fig. 1).

The fumigation procedure was by treating six 1.5 kg batches of peas with PH_3, each batch containing at least 200 insects. Calculations of efficiency were then corrected to one kg size samples.

Each treatment was repeated (except for sample 3). The exposure times ranged from 12 hours to 16 days. According to preliminary results, the PH_3 dosage was reduced successively from 1 g.m^{-3} to 0.43, 0.08 and 0.04 g.m^{-3}.

The first two series of fumigations were carried out at 1 g.m^{-3} in September and October at 20°C; the next one at 0.43 g.m^{-3} in November at 20°C; and then four series at 15°C and finally two series at 10°C (Fig.1).

In March, the temperature rise in the cold room (from 4 to 12°C) resulted in the emergence of many adult insects. According to the results of efficiency already obtained, it was decided to continue the tests with lower concentrations (0.08 and 0.04 g.m^{-3}), that were applied only to the adult stage present in the batches of seeds.

The batches were placed in an 11 litre leakproof vacuum jar. Each container was connected in parallel by a Rilsan© tube to a 1 m^3 chamber containing a defined PH_3 concentration. This enabled the batches to be subjected to the same gas concentration (Fig.2).

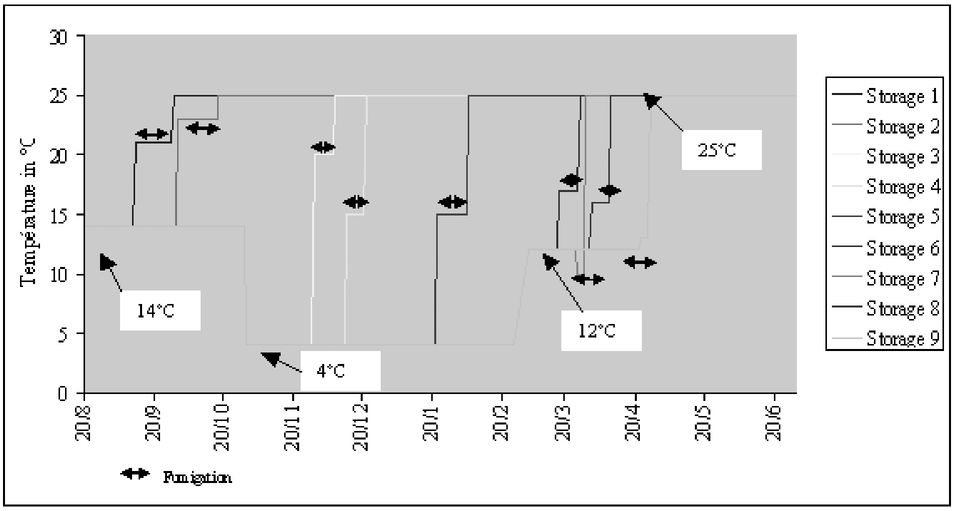

Figure 1. Storage temperature and phosphine exposure for each batch (1 to 9) (<-> phosphine treatment)

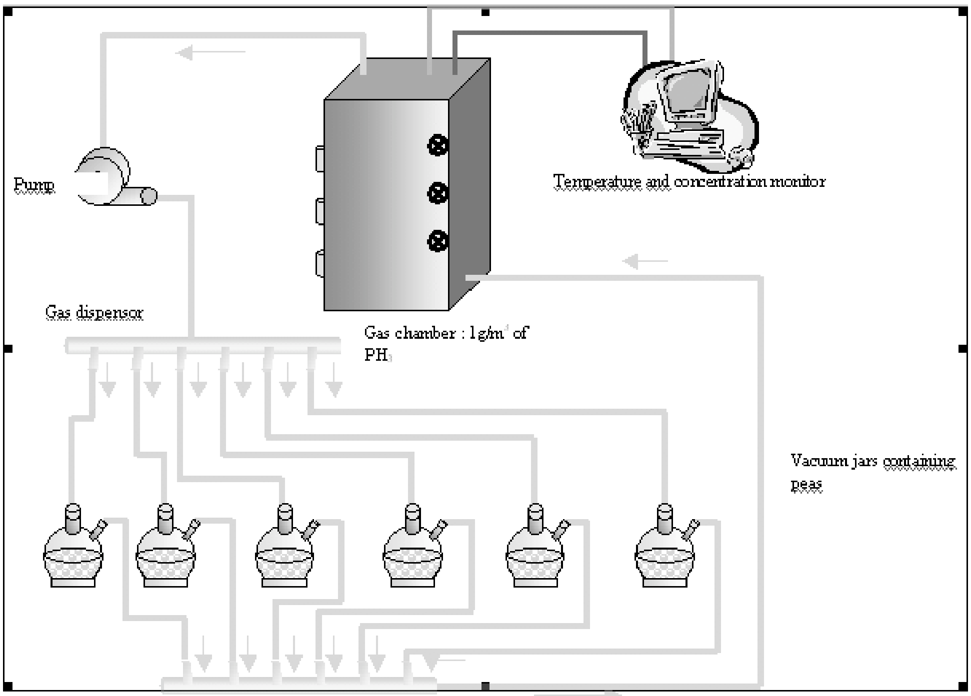

Figure 2. Fumigation system – 6 vacuum jars containing infested peas

Adult mortality counts were 14 days after the end of treatment. After treatment, the batches of peas were placed in plastic boxes (L: 26 cm, W: 13 cm, H: 7 cm). Every month until July, the batches were sifted in order to count the emerged insects. The comparison of emergence between the batches treated and the control batches, made it possible to calculate a rough percentage of reduction in emergence (% RIEB). This was taken as a criterion of the PH_3 efficiency. The formula applied was:

$$\%RIEB = 100 \times \frac{\text{Total insects in the treated b.} - \text{Total insects in the control b.}}{\text{Total insects in the control b.}}$$

Insecticide treatment with Deltamethrin

Two batches of peas (50 kg) were treated with Deltamethrin at a concentration of 0.25 g/T mixed with 2.25 g/t of PBO (Pyperonyl butoxide) (Protocol CEB 213). The same conditions of homogenisation and observation were applied for each batch.

RESULTS

Infestation level

Nine radiographic seed plates were studied in order to measure the percentage of infestation by *B. pisorum*. The results gave an average infestation of 12.7% (±6.6).

Adult emergence during storage

This study was carried out with 5 batches of peas, each batch having been stored over a different time period (Fig. 2). The experiment was repeated to verify the findings. Batches 1 and 2 were repeated with batches 2 and 4 respectively. Only batch 5 was not repeated (Table 1 and Fig. 3).

TABLE 1
Numbers and percentages of *Bruchus pisorum* adults that emerged under different storage temperatures over different time periods.

Batch number	1		2		3		4		5		**Average**	
Storage time	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0-35 days	192	94	151	92	103	77	187	89	190	96	**165**	**90**
36-100 days	9	4	10	6	11	8	23	11	5	3	**12**	**6**
101 days and +	3	1	3	2	21	15	0	0	2	1	**6**	**4**
Average of emerged adults									178 (± 33.2)			

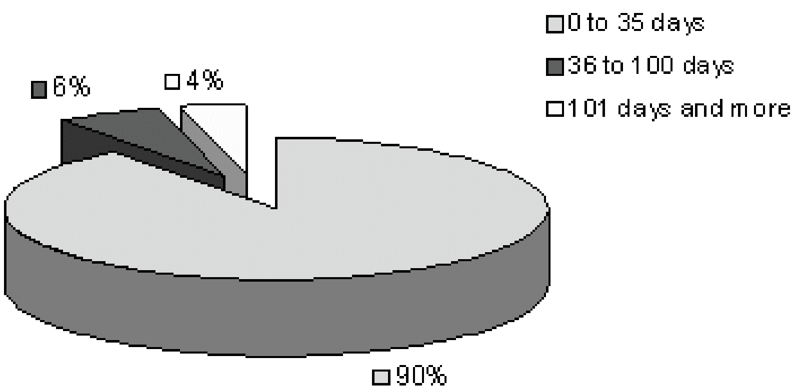

Figure 3. Emergence distribution of *B. pisorum* during storage

Firstly, one can note an average homogeneity in emergence between batches, with 178 (±33.2) adults per kilo of seeds. These results show that rate of emergence of the adult bruchids is induced by temperature variation both before and after treatment.

In the batches stored over a shorter period at low temperature, the emergence of adult bruchids is spread out over time.

The average of emergence was 90.4% during the first 35 days, 6.4% between 36 and 100 days and 3.9% from 101 days onwards. Therefore, it seems that storage at low temperature slows down the emergence of adult insects. In fact, a reheating of seeds reactivates the development of the insects that continue their cycle of development.

Some 90% of the adults emerge at the beginning of the storage, and only a few insects were counted until the following spring whatever the storage temperature.

Deltamethrin treatment

The trial and its repetition were carried out with 25 kg of peas. A sample of 3 kg was taken from each batch and put in a breeding room. These sub-batches were observed once a month until adult emergence ceased in the control batch (Table 2).

TABLE 2
Response of *Bruchus pisorum* to Deltamethrin treatment

Batch	**Number of insects (3 kg of peas)**			**Mortality (%)**
	Live	**Dead**	**Total**	
Control b.	301	6	307	**2**
Trial 1	17	242	259	**93**
Trial 2	17	293	310	**95**
Total (average)			292	

Firstly one can see a homogeneity in emergence between the three batches, with an average of 292 insects per batch. Furthermore, the natural death rate of emerged adults in the control batch was 2%, indicating a healthy condition of the insects in the samples.

In the treated samples the insecticidal efficiency was 93 and 95% respectively. At this high level of control, the remaining insects would require many months before months before population levels would be restored.

The observations spread over a 10 month period show a persistence of action of the active ingredient. The active ingredient had no effect on the hidden infestation but only on the adults after they emerged on to the treated peas. The results are presented in Table 3 below.

TABLE 3
Reduction in emergence of *Bruchus pisorum* to Deltamethrin treatment

Days after treatment	4	14	75	97	124	153	179
No. insects / kg Control batch	28	190	66	13	8	1	1
No. insects / kg Average	2	203	65	10	4	1	1
% RIEB	**96**	**-6**	**1**	**24**	**48**	**12**	**25**

Phosphine treatment

Adults: Results are based on the death rate according to the CTP obtained. The CTP varied according to the gas amount and the duration of the treatment.

TABLE 4
Control efficiency of Phosphine against *Bruchus pisorum* adults at 20°C

a) at a dosage of 1 $g.m^{-3}$, Fumigations 1 and 2

Exposure time (d)	0	4		6	6	8	8	11	12	14	16	14	16
CTP ($g.h.m^{-3}$)	0	47	78	88	119	127	160	220	208	253	282	296	315
% mortality	**1.2**	**3.4**						**100**					

b) at a dosage of 0.43 $g.m^{-3}$, Fumigation 3

Exposure time (d)	0	3	4	5	6	7	8
CTP ($g.h.m^{-3}$)	0	37	49	61	71	83	94
% mortality	**0**			**100**			

The first series of experiments on the adult stage of *Bruchus pisorum* (fumigations 1 to 3, Table 4) indicates a good control efficiency (100%). At 20°C the maximum control was observed with dosages of 37 $g.h.m^{-3}$ and higher, and at exposures of longer than 3 days.

TABLE 5

Control efficiency of Phosphine against *Bruchus pisorum* adults at 15°C and 0.43 $g.m^{-3}$

a) Fumigations 4 and 5

Exposure time (d)	0	0	1	3	5	4	7	5	9	6	7	12	13	8
CTP ($g.h.m^{-3}$)	0	0	0.4	29	29	40	49	50	55	59	69	70	73	81
% of mortality	**1**	**0**	**0**	**100**	**90**					**100**				

b) Fumigations 6 and 7

Exposure time (d)	0	0	1	1	2	2	3	3	4	4	7	8	7	8
CTP ($g.h.m^{-3}$)	0	0	5	7	13	16	20	25	28	34	49	53	58	64
% of mortality	**3**	**22**	**68**	**85**	**97**	**99**	**99**				**100**			

TABLE 6

Control efficiency of Phosphine against *Bruchus pisorum* adults at 10°C and 0.04$g.m^{-3}$ or 0.08$g.m^{-3}$ (fumigations 8 and 9)

Dosage ($g.m^{-3}$)	0		0.04	0.08	0.04	0.08	0.04			0.08	0.04	0.08	
Exposure time(h)	0		13	13	23	24	37	48	62	37	71	48	61
CTP ($g.h.m^{-3}$)	0		0.4	0.6	0.8	1.3	1.3	1.7	2.4	2.6	2.9	3.6	4.7
% mortality	**23**	**55**	**69**	**43**	**91**	**59**	**78**	**92**	**95**	**95**	**100**	**99**	**100**

The second series of experiments (fumigations 4, 5, 6 and 7, Table 5) presents the CTP of the treatment after a temperature reduction from 20ºC to 15°C and for exposure periods from 1 to 8 days. They show no significant difference from those of the first fumigations, namely: for a CTP of about 37 g.h.m-3, the control level was 100%.

The last series of fumigations (Table 6: 10°C at 0.08 or 0.04 $g.m^{-3}$) shows that a 100% mortality was obtained after 61 hours for a CTP of 4.7 $g.h.m^{-3}$ at 0.08 $g.m^{-3}$ and in 71 hours for a CTP of 2.9 $g.h.m^{-3}$ at 0.04 $g.m^{-3}$.

Hidden stages

Results are based on the RIEB according to the CTP obtained. The CTP varies with the gas concentration and the duration of the treatment.

TABLE 7
Control efficiency of Phosphine against hidden stages of *Bruchus pisorum* at 20°C and 1 $g.m^{-3}$ in fumigations 1 and 2.

Exposure time (d)	4		6		8		11	12	14	16	14	16
CTP ($g.h.m^{-3}$)	47	78	88	119	127	160	220	208	253	282	296	315
% RIEB	**100**	**100**	**100**	**100**	**100**	**100**	**99**	**100**	**100**	**100**	**100**	**100**

* Number of insects emerging from control batches = 84 and 59

TABLE 8
Control efficiency of Phosphine against Bruchus pisorum hidden stages at 20°C and 0.43 $g.m^{-3}$ in fumigation 3.

Exposure period (d)	*3*	*4*	*5*	*6*	*7*	*8*
CTP ($g.h.m^{-3}$)	37	49	61	71	83	94
% of RIEB	**100**	**98**	**95**	**100**	**98**	**88**

*Number of insects emerging from control batch = 111

TABLE 9
Control efficiency of Phosphine against hidden stages of *Bruchus pisorum* at 15°C and 0.43 $g.m^{-3}$

a) Fumigations 4 and 5

E	1	2	5	3	3	4	7	8	8	6	7	12	13	8
CTP	0.4	16	25	29	29	40	49	49	59	59	69	70	73	81
RIEB	**16**	**100**	**99**	**99**	**100**	**98**	**100**	**99**	**99**	**99**	**98**	**100**	**100**	**100**

b) Fumigations 6 and 7

E	1		2		3		4		7		8
CTP	5	7	13	16	25	20	28	34	49	58	64
RIEB	**68**	**92**	**100**	**100**	**98**	**100**	**100**	**100**	**100**	**99**	**100**

E = Exposure period in days
CTP = $g.h.m^{-3}$
RIEB = % reduction in emergence

With a dosage of 1 $g.m^{-3}$ (Table 7, fumigation 1 and 2), the results on hidden stages of the bruchid show that there is complete control with PH_3, at a CTP higher than 47 $g.h.m^{-3}$. Indeed, 100% control was found in nearly all the treated batches. Note that the 99% value of RIEB obtained for a 11-day treatment corresponds to the observation of one dead insect.

At the same temperature but with a concentration reduced by half, and after 3 months of storage (Table 8 fumigation 3), an 88% control was obtained after 8 days of treatment (CTP of 94 g.h.m^{-3}). Clearly, the insect is more resistant to phosphine during the hidden stages.

The four treatments (Table 9, fumigations 4, 5, 6 and 7) confirm the results obtained with the previous experiments. The treatment is efficient for a CTP higher than 70 g.h.m^{-3} after 3 months of storage at low temperature. However, the treatment gave complete control more quickly after a 6-month storage period at low temperature. The maximum control efficiency was obtained at a CTP higher than 28 g.h.m^{-3} after 4 days of treatment. Here again, the 99% value of RIEB obtained for a 7-day treatment was due to the observation of one dead insect.

These results indicate that the efficiency of phosphine on the pea weevil increases with the duration of storage in a cold chamber.

DISCUSSION

It would seem that the bruchids that survive several months of storage at low temperatures are in a state of “protective diapause”. At the harvest (mid July), the temperature of the peas is higher than 20°C. Many of the insects finish their development and start to emerge from September. The others find refuge in various recesses waiting for next spring (Pears, 1950). The emergence of adults from batches 1 to 5 are spread out over time due to the different stages of development at harvest.

In fact, it seems that some bruchids continue their development inside seeds during storage. Moreover, it is because winter conditions (temperature and photoperiod variations) slow down the development of insects, that adults emerge after more than 100 days of storage.

A study of the radiographes carried out during storage gives precise information about the kinetics of development of each insect. Furthermore, a thorough study of the mechanisms of resistance to low temperatures could be carried out by measuring the amount of cryo-protective substances produced by the insects during storage. This would enable us to understand the real physiological state of the insect.

With the deltamethrin treatment, the mortality of emerging adults was about 94%, which creates a problem when peas are sold, because phytosanitary inspection services do not allow the presence of live insects in them. Nevertheless, these beetles will quickly die, even if the treatment was applied 6 months earlier.

The amount of PH_3 applied at the beginning (1 g.m^{-3}) corresponds to the standard dose rate. This amount was progressively decreased in order to refine the results of efficiency. Effective control with phosphine against all stages of the pea weevil was obtained after harvest, whatever the ensuing storage period. Adults are very sensitive to phosphine. At 10°C, and at 30 and 60 ppm, a 100% mortality was obtained in 71 and 60 hours respectively.

A 4-day treatment at 20°C with 1 g.m^{-3} was efficient against the beetle under the laboratory conditions. Efficiency of the treatment increased with storage duration.

CONCLUSION

Both methods used to kill *Bruchus pisorum* provided effective control. The deltamethrin treatment at 0.25 g/t (standard concentration) gave an average control efficiency of 94%. Moreover, we note a persistence of action of at least 6 months. However, this treatment was not efficient enough for this type of infestation.

Phosphine treatment provided complete control of all stages at 1 g.m^{-3} and 20°C after 4 days. (Further trials will be carried out with a dosage of 1 g.m^{-3} or more and at 15°C). This fumigation could be used at a low concentration after a deltamethrin treatment in order to kill the remaining insects.

These experiments were carried out in the laboratory on batches of 1.5 kg peas in order to establish the best conditions of treatment (amount of gas used and exposure time). In order to validate these data, the treatment should be carried out at a commercial level, i.e. application on much larger quantities of peas. For this purpose, a study of the sorption of phosphine by peas must first be carried out to determine the exact dosage of fumigant that has to be used.

The temperature and the length of the storage seem to have an effect on the treatment efficiency. This could be confirmed by a study of the physiological stages of the insect during storage at low temperature.

The Table below (Table 10) sums up the efficiency of the various treatments against *B. pisorum.*

TABLE 10
Summary: control efficiency of phosphine and deltamethrin against *Bruchus pisorum.*

	Stages	Temperature	Exposure time	Dosage	CTP (g.h.m^{-3})	Efficiency
Deltamethrin	All stages	Storage	*Efficiency > 6 months*	0.25 g/t	-	94 %
Phosphine	Adults	10°C	60 hours	60 ppm	4.7	100 %
			71 hours	30 ppm	2.9	
		15°C	3 days	0.43 g.m^{-3}	25	
		20°C			37	
	Hidden stages	15°C	8 days		81	
		20°C	4 days	1 g.m^{-3}	47	

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