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ADVANCES IN MODIFIED ATMOSPHERES; NOVEL APPLICATION METHODS AND RESEARCH NEEDS

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ABSTRACT

The economics involved in the application of MAs prevent their full replacement of conventional fumigants. Novel approaches to the use of MAs are reviewed in this presentation. A method of treatment based on hermetic storage against the large narcissus fly (*Merodon eques*) was developed. The MA is created due to the respiration of the bulbs that deplete the O_2 , thus controlling the maggots of *M. eques*. Nitidulid beetles were shown to be sensitive to low O_2 or high CO_2 concentrations for disinfestation of dry fruits. This method is being used for the disinfestation of dates. A combination of high temperature and CO_2 also offers an alternative for particular applications such for the control of Mediterranean Fruit-Fly in persimmons. Another development is the use of MAs in a low-pressure environment. Special portable chambers made of flexible tarp-like sheeting provide the benefit of treatment in any location with low pressure treatments. In addition they are suitable for MA or for hermetic storage. Use of these flexible chambers for dry fruits or museum artifacts seems to be most suitable. High pressure carbon dioxide is another area that needs to be further explored for specific applications.

INTRODUCTION

The only technology that retains the special capacity of fumigation for in-situ treatment of stored commodities, as well as offering a similar diversity of application technologies, is the modified atmosphere (MA) method. MAs offer an alternative that is safe and environmentally benign to the use of conventional residue-producing chemical fumigants for controlling insect pests attacking stored grain, oilseeds, processed commodities and packaged foods.

Although the economics involved in the application of MAs prevent their full replacement of conventional fumigants, novel approaches to the use of MAs indicates their suitability for niche applications. In this paper we review the developments and applications of these technologies.

<u>Control the large narcissus fly:</u> The large narcissus fly <u>Merodon eques</u> F. attacks narcissus bulbs and also bulbs of other geophytes. This species has not been recorded in the USA; and is therefore included within quarantine requirements that demand total mortality prior to export to the USA (Donahaye *et al.* 1997). Fumigation with methyl bromide (MB) has been used to eliminate narcissus fly infestation in flower bulbs due to its rapid killing time (4 hours). However, MB is also known to cause damage to the bulbs. Therefore, our initial trials were aimed at finding alternative treatments to MB so as to eliminate phytotoxicity. These trials were carried out in flexible plastic chambers that replaced the rigid fumigation chambers (rooms) previously used.

In experimental procedures, Navarro *et al.* (1997) found that there was an extremely rapid increase in CO_2 due to the respiration of the newly harvested bulbs. This procedure also revealed the significant toxic effect of CO_2 during treatment and the possibility of using it alone as a control measure. By this time the limitations on MB fumigations were already being defined in meetings of the Montreal Protocol (UNEP, 1998) and required the growers to find a permanent non-chemical solution that would also be economical and easy to implement in the field. Consequently, in this paper we report results of three treatments of MAs: high CO_2 concentration (95 percent), vacuum (low pressure of about 50 mm Hg), and storage under hermetic conditions alone (Rindner *et al.*, 2003). Although high CO_2 and vacuum treatments provided a shorter treatment time than storage under hermetic conditions alone, the possibility of obtaining a bio-generated modified atmosphere utilizing the bulb respiration was too tempting to ignore and the hermetic storage was adopted as a practical approach at the commercial level (Finkelman *et al.*, 2002a).

Combination of high temperature and CO2: MA technology can fulfill a specific

niche where use of other fumigants is unacceptable such as treating organic foods. MAs are limited by the long exposure times required to produce complete mortality (Navarro and Jay, 1987), and are similar to those required for phosphine (PH₃) fumigations (Navarro and Donahaye, 1990). In cases where rapid disinfestation of commodities is required, the possibility of using CO_2 at temperatures raised to levels that will not adversely affect the commodity should be considered. It has been recognized that insecticide treatments, particularly those affecting the respiratory system are more pronounced at higher temperatures (Navarro and Calderon, 1980). Respiratory metabolism has also been observed to increase with temperature up to a maximum and then sharply declines at the upper lethal temperature (Chapman, 1982). A number of laboratory studies on the combined effects of high CO_2 and optimal elevated temperatures against insects have shown a corresponding increase in insect mortality with increase in temperature (AliNiazee, 1971; Bell *et al.*, 1980;

Donahaye *et al.*, 1996; Navarro and Jay, 1987; Soderstrom *et al.*, 1991). The objective of this work is to report on the mortality of different life stages of stored product insect pests exposed to increased temperatures under CO_2 enriched atmospheres to enhance the controlling effect on the treated pests.

<u>Application of vacuum technology:</u> Investigations on effects of low pressures on storage insects were carried out by Back and Cotton (1925), Bare (1948), and later on by Calderon *et al.* (1966), Calderon and Navarro (1968), and Navarro and Calderon (1969; 1972a; 1972b). Recently Mbata and Phillips (2001) investigated the effects of temperature and exposure time on mortality of three stored product insects exposed to low pressure. Insect mortality under low pressure is predominantly a result of oxygen deficit and not due to physical pressure effects (Navarro and Calderon 1979).

In a first attempt to use low pressures to preserve cacao beans quality, Challot and Vincent (1977) used polyethylene bags applying a low pressure of 600 mm Hg. Although 600 mm Hg may be effective in maintaining the product quality and prevent ingress of insects, storage insects can tolerate this pressure. For mortality of storage insects, low pressures below 100 mm Hg are required.

Gas tight flexible structures using the hermetic storage method have been developed and are in use on an industrial scale (Navarro *et al.*, 1994; Silberstein *et al.*, 1998). These structures enable treatment with modified atmospheres or fumigation (Navarro *et al.*, 1995), and they are termed "Volcani CubesTM" or "GrainPro CocoonsTM" (Navarro *et al.*, 1999). The use of these flexible storage facilities to maintain low pressures of 25-30 mm Hg was reported in two recent works (Phillips *et al.*, 2000; Navarro *et al.*, 2001). The objective of this section of the paper is to report on the application of transportable systems for the vacuum treatment for control of storage pests.

APPLICATION OF BIOGENERATED VACUUM-HERMETIC FUMIGATION (V - HF) PROCESS TO CONTROL THE LARGE NARCISSUS FLY

Materials and Methods

The special V-HF Cocoon consisting of a 5.5 m long, 2.6 m wide and 2.4 m high plastic reinforced PVC enclosure was used to accommodate 10 pallets containing the bulbs. The special V-HF Cocoon consisted of two sections; the upper section that was used to cover the top and sides 1.4 m from the top, and the bottom section that had a wall 1 m high. The bulbs were loaded into the bottom section of the module on their original shipping pallets using a forklift (Figs 1 to 3). An air circulation fan and a heater (controlled by a thermostat) were added to assist distribution of heated air from the top layers of the pallets to the lower layers (Figs 4 to 6). Then the top and the bottom sections were zipped together to create a sealed structure (Figs. 7 and

8). Each pallet consisted of 920 kg of narcissus bulbs stored in 40 crates stacked in 8 rows. In each trial, 10 pallets of the commodity were arranged inside the special V-HF Cocoon. At the start of the trial, a slight vacuum of 200 Pa, using a vacuum cleaner was applied solely to adhere the V-HF Cocoon liner to the crates, thus minimizing the free space within the V-HF Cocoon (Figs 9 and 10). Before each trial, paper bags containing 50 bulbs taken from lots infested by *Merodon eques* maggots were placed on the top and bottom, inside the special V-HF Cocoon. At the end of the trials bioassays were checked for presence and mortality of the maggots. Data loggers to measure temperature and air relative humidity were also placed in each trial on the top and bottom inside the V-HF Cocoon.

Results and Discussion

After applying a slight vacuum to adhere the liner to the crates to reduce the free air space in the Cocoon, the desired modified atmosphere was obtained taking advantage of the respiration of the narcissus bulbs. The hermetic seal of the V-HF MA system resulted in a rapid O_2 depletion. In eight trials carried out, the O_2 concentration was reduced to levels of 0.1% within 48 h, while the CO₂ concentration increased up to 25% (Figs 11 and 12). During the narcissus bulbs harvest season (July 2003) the natural temperature of the bulbs fluctuated between 24° and 32°C. Since temperatures lower than the set temperatures of 30°C were observed at the lower layers of the pallets, there was a risk for survival of larvae. Therefore, artificial heating of the bulbs to reach 30°C was assayed. An air circulation fan was added to assist distribution of heated air from the top of the pallets to the lower layers (Figs 5 and 6). The demonstration quarantine application was carried in the packinghouse of S. Z. Segal Company, a narcissus bulb grower. Although, according to our laboratory work an exposure time of 34 h was needed to obtain 99% mortality of the pest, the system was kept sealed for an additional 48 h after the O2 concentration level reached 0.1%, to ensure the successes of the treatment in the V-HF system. Infested narcissus bulbs were examined after each trial. Infested samples placed at the top of the stack and below the V-HF Cocoon liner revealed no live larvae at the end of the eight treatment trials. Although infested samples placed at the bottom, revealed occasional moribund larvae before the artificial heating was added, extension of exposure time to 48 h after the O_2 concentration level reached 0.1% and increasing the temperature to 30°C at the bottom by re-circulating the internal atmosphere, ensured complete mortality.





Samples of narcissus bulbs were taken before and after the eight trials carried out with the V-HF Cocoon. The treated non-infested bulbs were evaluated at the end of each treatment and bulbs from all pallets passed the requirement needed for export approval. Samples of non-infested treated bulbs and control bulbs were planted under controlled green house conditions in October 2003 to ensure that there were no phytotoxic effects and to evaluate the quality of the narcissus bulbs. In all cases, no

phytotoxic effects were observed and the quality of the treated bulbs was as good as the control bulbs.



EFFECTS OF TEMPERATURE AND CO₂ COMBINATIONS

Materials and Methods

For CO_2 treatments, concentrations varying from 60% to 90% of CO_2 in air at temperatures ranging from 30° to 45°C were tested. Diapausing larvae of Khapra beetle (Trogoderma granarium) were obtained by removing active larvae from cultures and placing them in groups of several hundred without food for one month at 28°C (Lindgren and Vincent, 1960). Adults of Tribolium castaneum, Oryzaephilus surinamensis, Ephestia cautella and Plodia interpunctella were taken from laboratory cultures maintained at the Department of Food Science, Volcani Center, Agricultural Research Organization, Bet Dagan, and mass reared on a standard artificial diet. Eggs, pupae and adults (1-2 days old) and larvae (4-15 days old) were taken from the same batch.

Eggs of tested species were used within 0-2 days of oviposition. The eggs were obtained by placing 500-1000 adults' beetles in 500 g of wheat flour containing 5 g of brewers' yeasts. To obtain eggs from moths, E. cautella and P. interpunctella were placed on a mesh covered inverted jar, overnight and the females laid the eggs in a Petri dish. Two Perspex slides each with 50-drilled "wells" were used to individually place 100 eggs from each of the studied species. The slides were then covered with a cover glass to retain the eggs (Navarro and Gonen, 1970).

Following treatment, larvae, pupae and adults were transferred to small jars (50 ml) and maintained at $28\pm1^{\circ}$ C and $65\pm5\%$ R.H. The larvae were provided with food. The eggs were transferred to watch glasses and incubated under the same conditions as the other developmental stages. Mortality counts for larvae were carried out after two weeks of exposure; for pupae after one week, for adults after one day, and for eggs after 4-5 days of exposure. Mortality for larvae was based on those that failed to pupate, for pupae, those that failed to emerge as adults, for adults, those that were dead or moribund, and for eggs, those that failed completely to hatch.

Results and Discussion

Table 1 shows the influence of CO_2 concentrations at different temperatures as expressed in LT_{99} mortality values for diapausing larvae of *T. granarium*. At 45°C, by increasing the CO_2 concentration to 90% the LT_{99} value decreased to 10 h, whereas at 35°C the LT_{99} value was 29 h. *T. granarium* is one of the most serious pests of stored cereal grains and oil seeds, and is subject to strict quarantine regulations in the US, Australia and several other countries. It is a member of the dermestid family and is a voracious feeder of grain products. The larvae can hide in cracks of the storage structure in a state of facultative diapause and can remain in this condition for years. It is particularly difficult to control with insecticides. Consequently, many quarantine treatments are mandatory when products such as rugs, spices and cereal products are imported from infested countries. In such situations, MB is still the only effective fumigant against this pest. Distribution of *T. granarium* includes Western Africa through the Northern Indian subcontinent (Cuperus *et. al.*, 1992). Results shown in Table 1 may serve as guidelines to the possibility of applying slightly elevated temperatures for control of the most resistant diapausing larvae of *T. granarium*.

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Influence of CO₂ concentrations expressed in LT₉₉ (hours to obtain 99% mortality) values for *Trogoderma granarium* diapausing larvae at three different temperatures.

Temperature (°C) –	CO_2 concentration (%)				
	60	70	80	90	
35	38	29	28	28	
40	28	24	20	18	
45	17	15	14	10	

A similar approach of applying various CO_2 concentrations at different temperatures was investigated for four developmental stages of *E. cautella*. Results in Table 2 summarize the effectiveness of the combination of CO_2 at temperatures in the range of 35°C to 45°C. Tests with *Ephestia cautella* showed that the pupa was the most resistant stage when exposed to 90% CO_2 with an LT_{99} value of 17 h at 35°C, and only 3 h when exposed at 45°C. The adult was the most sensitive stage of *E. cautella* requiring only 4 h of exposure to 90% CO_2 at 35°C.

Results on the influence of various CO_2 concentrations at different temperatures on *O. surinamensis* development stages are shown in Table 3. For this species as well, an increase in CO_2 concentration resulted in a decrease in the LT_{99} value. Generally, the eggs were the most resistant stage; at 40°C and 90% CO_2 a six h exposure was required to obtain an LT_{99} value.

TABLE 2

Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Ephestia cautella* various development stages exposed to CO_2 concentrations in air at three different temperatures.

Temp. (°C)			35		40			45				
CO ₂ (%)	60	70	80	90	60	70	80	90	60	70	80	90
Eggs	23	23	17	9	16	12	8	5	9	5	3	2
Larvae	60	27	20	12	17	9	6	6	5	4	2	2
Pupae	56	37	17	17	36	10	8	4	7	4	4	3
Adults	20	14	6	4	6	5	3	2	3	2	2	2

TABLE 3

Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Oryzaephilus surinamensis* various development stages exposed to CO_2 concentrations in air at three different temperatures.

Temp. (°C)	Life Stage —		CO_2 conce		
		60	70	80	90
30	Eggs	-	-	38	22
	Adults	21	-	22	9
35	Eggs	29	25	21	9
	Adults	26	11	8	4
	Eggs	15	7	6	6
40	Larvae	8		2	2
	Pupae	-	-	-	5
	Adults	12	11	6	3

APPLICATION OF VACUUM TECHNOLOGY

The tested transportable system was made of flexible PVC, which has been in use commercially for hermetic storage of commodities to control insect disinfestation by modified atmospheres (Navarro *et al.*, 1999). Experiments were carried out using a 15-m³ capacity plastic container termed the "Volcani CubeTM" or "GrainPro CocoonTM".

Navarro and Calderon (1979) compared the influence of low pressure on *Ephestia* cautella pupae with that of low oxygen concentrations, and deduced that the partial pressure of oxygen has a decisive effect on insect mortality, while no significant

function could be attributed to the low pressure itself. At 50 mm Hg the partial pressure of oxygen is equivalent to 1.4%, this being similar to the target oxygen concentration under a modified atmosphere obtained by nitrogen flushing. Table 4 shows the various units used in the literature for describing the equivalent values of various low pressures and equivalents in oxygen % at normal temperature and pressure of the atmosphere. In the process of evacuation, the main significant effect is the reduction in the partial pressure of the oxygen. The drop in the partial pressure of oxygen concentration is proportional to the atmospheric pressure and is almost linear. However, the commodity moisture largely dictates the humidity within the treated enclosure. Humidity response is linear if the treated enclosure is empty and no other gas then air is present in the enclosure. To demonstrate the effect of commodity moisture, Fig. 13 was prepared and tested for cocoa beans of 6.2% moisture content. According to Gough (1975), the equilibrium relative humidity (ERH) of cocoa beans is 60%. Although an evacuation process may reduce the humidity of the enclosure for a short time, the commodity gives off moisture to the interstitial space until the commodity moisture equilibrates with the humidity of the atmosphere of the interstitial space. For cocoa beans with a fill ratio of 63% in desiccators, this equilibration process lasted less than one hour at 30°C.

					-			
mmHg	atm	kg/cm^2	inches Ha	kPa	mbar	mm Hg	%	
(≈torr)	atili	Kg/CIII	menes rig	KI a	moai	oxygen	oxygen	
760	1.00	1.03	29.92	101,325	1,013	159	20.9	
600	0.79	0.82	23.62	79,993	800	125	16.5	
500	0.66	0.68	19.68	66,661	667	105	13.8	
400	0.53	0.54	15.75	53,329	533	84	11.0	
300	0.39	0.41	11.81	39,997	400	63	8.3	
200	0.26	0.27	7.87	26,664	267	42	5.5	
100	0.13	0.14	3.94	13,332	133	21	2.8	
50	0.07	0.07	1.97	6,666	67	11	1.4	
0	0.00	0.00	0.00	0	0	0	0.0	

TABLE 4 Units used to express atmospheric pressure and their equivalent partial pressure of oxygen expressed in mmHg and in percentage.

Materials and Methods

Field trials in Israel

The field trials were conducted on March 2001 in Israel, at the Agricultural Research Organization (ARO) campus. Two vacuum cubes of 15 m³ capacity and adapted to facilitate low pressure were used. The pressure in the cubes was established using a rotary vane oil-lubricated vacuum pump (3 hp Becker model U 4.70, Germany) to within the range of 23 and 75 mm Hg for duration of 3 days in one cube and 7 days in the other. The commodity was cacao beans originating from the Ivory Coast. Each cube contained 100 jute bags each weighing 65-kg (total 13,000 kg) (Fig. 14).

Five sets of bioassay replicates were placed in each of the cubes, each set containing all life stages of *E. cautella* and *T. castaneum*. Four of the bioassay sets were located, one on each side of the four cube walls at mid center height, and one at the top center. The control bioassay was placed on the top, above the liner of the 7-day cube in an open plastic container filled with cacao beans. Temperatures at the top and at the four side faces of the cubes were recorded during the trials using data-loggers (HOBO Pro Series).



Figure 13- Linear relationship between atmospheric pressure (mm Hg) and air humidity (g/kg) at 30°C.

Field trials in Ivory Coast

The field trials were conducted in November 2001 in Abidjan, Ivory Coast at an exporter's storage warehouse. The warehouse was 75 m in length by 60 m width by 6-8 m high. On the walls near the roof there was a web of brick size holes providing ventilation of the storage warehouse when the doors were closed.

Each vacuum cube contained 87 jute bags, each weighing 65 kg (total 5,650 kg per cube) (Fig. 15). A control a stack consisting of 4 pallets, 25 jute bags per pallet, for a total of 100 jute bags was used without applying vacuum.

Three sets of bioassay replicates in bags containing insects in tubes and eggs in pitted slides were placed in each cube and in the control stack. Each bioassay set contained, separately: 50 eggs, 30 larvae and 30 pupae of *E. cautella* and *P. interpunctella*, and 50 eggs, 50 larvae, 50 pupae and 50 adults of *T. castaneum*. About 1 g of culture medium was added to the tubes containing adults and larvae. All bioassay replicates were placed at the center of each test cube one at the top, one at center and one at the bottom of each cube. In each of the locations of the bioassays the equivalent r.h. of the cocoa beans were measured (by Defensor[®] Novasina model MSL, Switzerland) at the start and at the end of the treatment.

Two data loggers (HOBO Pro Series) were inserted in each cube and in the control stack, one at the bottom and one at the top to record the temperatures and r.h. during the trials. One data logger was placed outside the cube destined for seven days vacuum exposure (together with the bioassay) to record the ambient conditions in the storage area.

Results and Discussion

Field trials in Israel

Pressure within the two test cubes was regulated at 23-75 mm Hg for the two exposure time-durations. Subsequent bioassays revealed complete mortality within three days of exposure for all life stages of the two insect pest species, *E. cautella* and *T. castaneum*.

The pump required 55 minutes to reduce the pressure in the two cubes to 23mm Hg. The "time-out" interval between pumping was 10 min on the first day of the trial. For the three-day exposure cube, temperature at the top of the cube was $28.0 \pm 0.5^{\circ}$ C and the relative humidity stabilized at 65%. At the northern cube-wall face the temperature was $27.9 \pm 1^{\circ}$ C and the relative humidity stabilized at 69.5%. For the seven-day exposure cube, temperature at the top was $27.9 \pm 0.5^{\circ}$ C and the relative humidity stabilized at 69.9 ± 0.5% at the top was $27.9 \pm 0.5^{\circ}$ C and the relative humidity stabilized at 69.9 ± 0.5% at the top and the northern cube-wall face of the cube.

In conclusion the low-pressure/vacuum treatment was successful in providing total mortality of the insect pests and in protecting the commodity from re-infestation. Furthermore the cube provided protection for the cocoa beans from loss or increase in moisture during storage. These results indicate that effective control can be obtained in less than three days.

The transportable system was made of flexible PVC, which has been in use commercially for hermetic storage of grain and other commodities to control insect disinfestation by naturally obtained modified atmospheres (Navarro *et al.*, 1999). For the disinfestation of durable commodities, these flexible storage containers can be considered for the application of vacuum as an alternative to treatments with methyl bromide and other toxic fumigants.

Field trials in Ivory Coast

The system preformed flawlessly throughout the demonstration trial in the Ivory Coast. At the start of the treatment only 20 min were needed to achieve the pressure of 25-mm Hg within the cubes. "Time-out" intervals between pumping were 15 min on the first day of the trial and 35 min on the third day. The pressure within the preset range of 25 and 50 mmHg was maintained in one vacuum cube for 3 days and in the other for 7 days.

The ambient temperatures of the storage area ranged from 30°C to 35°C during the days and cooled during the nights to about 26°C. The r.h. of the storage area ranged from 50% to 63% during the days and from 70% up to 90% during the nights. The temperature within the cubes measured by the data loggers at the top and at the bottom of both cubes was constant at 30 ± 0.3 °C. The r.h. within the cubes at the bottom of both cubes was constant at $67.2 \pm 0.3\%$ and at the top of the cube the r.h. increased only slightly to $68.5 \pm 0.5\%$. In the first hour of the treatment in both cubes the humidity decreased to 60% but after 1.5 to 2 h it stabilized in the range of 67.2% and 68.5%. Similar r.h. ranges were recorded also in the control stack and therefore cannot be attributed to the use of vacuum in the cubes. The temperature at the top of the control stack was influenced by the ambient conditions although at nights it did not decrease below 30°C and during the days it reached 32 to 35°C. The temperature in the bottom of the control stock was constant at 30 ± 0.7 °C. The air r.h. measured at the top of the control stack ranged from 49 to 70 % following the changes in the ambient conditions. At the bottom of the control stack the changes in air r.h. were less variable and ranged between 61 and 68 % but as constant as measured in the vacuum cubes. Therefore, we conclude that the commodity was the main factor in determining the temperature and the humidity inside the cubes as well as in the control stack.



Figure 14. Two Volcani Cubes[™] under a pressure of 50 mm Hg (3 days exposures on the right and 7 day exposures on the left) connected together to the pump at the trial site in Israel.

The cocoa beans selected for this trial were of good quality as revealed by the entomological inspection. In each of four cocoa bean samples collected before the treatment 1 to 2 live mites were found and in one sample a live adult and larva of *Cryptolestes sp.* beetle was recorded. In the control stack no infestation was found. No live mites or insects were found in all the samples collected after the treatment.

Currently, cocoa beans are treated in the Ivory Coast with phosphine for five days and this treatment is being used to replace the conventional methyl bromide fumigation (Finkelman *et al.*, 2002b). The option of low pressure therefore is a promising additional solution that can provide the required insect control in less time than phosphine and without the need to use environmentally harmful chemicals.

In conclusion the system at this demonstration trial preformed as expected maintaining the required low pressures and therefore achieving the objective of total mortality of the insect pests. At present additional work is being carried out using the same methods on other commodities such as chickpeas, peanuts, sunflower seeds and semolina. Furthermore as a result, of this study, loading the cube with manual labor has been abandoned and the commodity is now loaded into a new system termed V-HF[™] (vacuum hermetic fumigation) system on their original pallets by forklift.



Figure 15. GrainPro Cocoon[™] containing cocoa beans used as vacuum cube during the trials in Ivory Coast. The rotary oil-lubricated vacuum pump is shown on the left of the picture.

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