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## **Session 5 : Fumigation, Hermetic Storage and Modified Atmospheres**

### **Hermetic storage for those who need it most - subsistence farmers**

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#### **Abstract**

Farmers in Africa, especially subsistence farmers and those of low economic means, badly need good storage technology for their harvested grain. Such farmers are the ones who suffer the most from postharvest insect pests and resulting losses of food and monetary value. Any storage technology they use must be effective, low-cost, not use insecticides, available in local markets, easy to learn, culturally acceptable and sustainable. Hermetic storage fits many of these criteria. One hermetic technology, the Purdue Improved Crop Storage bag, has found wide acceptance in some parts of the world, especially Africa. An overview of this technology, lessons learned while developing it, and challenges remaining to our understanding of hermetic technology in general are presented.

Keywords: postharvest, grain, bruchids, hermetic, PICS, extension

#### **1. Hermetic storage – an ancient technology**

Neolithic farmers—the pioneers of subsistence farming—were the first to have to deal with the problem of storing large amounts of grain for long periods of time. The grain they obtained by agriculture was their major food supply for the entire year, and it arrived almost all at once, at harvest. To ensure that food was available for the many months ahead—ideally until the next harvest—they had to store their grain safely. They knew that unless they took proper precautions, insects and rats might eat it up or molds ruin it. In time, these early peoples discovered a form of hermetic storage that involved digging a hole in the ground in a place well above the water table and putting their grain in. They covered the grain over carefully and sealed and camouflaged it to hide it and voila! It was now safe not only from thieves and marauders but from insects and rats, too.

How does underground storage work its protective magic? The ancients knew the answer. The Roman farmer and writer Marcus Varro (47 B.C.) spelled it out two thousand years ago in his *De Re Rustica*, written in 47 B.C., during the reign of Julius Caesar. He observed that insects do not breed where fresh air does not reach, and cautioned that farmers should wait after opening their storage pits before entering them because people had suffocated when they went into the pits too soon, i.e., right after opening them. Implicit in his remarks was the idea that sealed underground storage somehow caused the air surrounding the grain to change such that it was unable to sustain normal insect or human life. Prior to Roman practices, conical, easily sealed pits were in use by Celtic tribes in Great Britain, as at Danebury (Cunliffe, 2013). In modern northwest China, where underground storage pits are still used on farms,

farmers take lighted candles into the pits after opening them. If the candle flickers out, they leave, knowing that the air won't support life (Yan Yan, personal communication).

## **2. Hermetic storage today**

There are many different forms of hermetic storage in use today. They work according to the principles articulated by Prof. Schlomo Navarro (1978); whose work helped lay the foundation for the use of gas impermeable plastic containers ranging in capacity from small storage bags to giant cocoons promoted by the commercial company GrainPro. Under hermetic (air-tight) conditions respiration by insects initially present in the grain as well as by the grain itself releases CO<sub>2</sub> into the surrounding enclosed airspace while depleting the O<sub>2</sub>. This biologically-modified atmosphere arrests the development of pest insect populations. Several forms of hermetic storage that depend upon biological modification are currently in use: (1) sealed drum storage—which came into widespread use in Senegal in the 1980's (Seck and Gaspar, 1992; Seck et al., 1996); (2) GrainPro Super bags<sup>TM</sup> and cocoons that rely on high-tech highly O<sub>2</sub> impermeable plastics (Villers et al., 2008; DeBruin et al., 2012); (3) metal silos as now being promoted by CIMMYT and others (Tefera et al., 2011); (4) jerrycans and water bottles (Walsh et al., 2014); (5) triple layer plastic bags (Murdock et al., 1997) consisting of two inner polyethylene bags surrounded by a woven polypropylene bag for strength (PICS<sup>TM</sup> bags—Purdue Improved Crop Storage bags; Baoua et al., 2012). The authors of the present paper owe most of their practical experience with triple layer PICS bags while working with low resource farmers in Africa over many years, and necessarily draw mainly on that experience for the current paper.

## **3. Poor people today need storage technology more than ever**

The world faces a food supply problem. In the next 35 years, by 2050, the earth's population will cross 9 billion. That's an increase of about 2 billion over today—in just one short generation. Everyone is asking where that additional food will come from? Certainly we'll grow more. There will be improvements in plant and animal genetics and in agronomy. Biotechnology will likely make a contribution. The most obvious way of all to increase food availability would be to put more land into production, i.e., simply grow food on more land area. Unfortunately, we have nearly exhausted the supply of new arable land in places where there is adequate water. In Africa the situation is even worse, in that a major part of the soils available for agriculture are degraded to the point of being unsuited for sustained food production (cf., Montpellier Panel report, 2014). Of course there IS a way to make more food available without finding additional land and water—through better storage. Depending on the crop, the location and the year, storage losses range up to 25% (Zorya et al., 2011) and may even go higher. These major losses can be stopped safely by better storage technology.

## **4. Who needs storage technology the most?**

So who needs good storage technology the most? It isn't economically advanced nations, where technical know-how and the capital needed to implement sophisticated technology is available. The people who need it most are the low-resource and the subsistence farmers of the world. Perhaps the greatest challenge of all lies in Africa, where much of the population increase of the next 35 years is going to occur.

It may be helpful for those readers who have not experienced subsistence farming to describe the living conditions of the people who are most in need. They live off the main roads, indeed, there may not be a road leading to where they live at all, only a hard-packed path beaten by countless thousands of feet over a great many years, by carts, bicycles and motorbikes. They are often illiterate, or have at best minimal education. They build their dwellings with their own hands with materials they have gathered up from the fields and savannahs. All too often they struggle through life from hand to mouth. Their crops are mostly traditional ones. They plant little fields with primitive methods, by hand or with some animal traction. They plant mostly unimproved traditional cultivars using seed they save themselves or barter from neighbors. They use few yield-enhancing inputs except perhaps animal manure. The family eats what it grows or can buy or barter at the local market or at the roadside. In the best of circumstances their food store furnishes food from one harvest to the next. But if their store is used up, or has been destroyed by insects or rats or sold because of a pressing family need for cash—like a wedding or payment of a debt or school fees—they suffer through a situation they know so well they have given it a name: the hungry time, called in French the “*periode de soudure*”, the weeks and months between the exhaustion of their food supply and the start of the next harvest. Now and then they earn a little cash money, by gathering firewood, selling their unskilled labor or trading some hard won grain at local markets. It’s a hardscrabble life when things are going well and the weather is good. But when the rains don’t come and the food runs out, and the baby gets glassy-eyed with its ribs showing and its belly swollen and its hair reddens from protein deficiency, there’s nowhere to turn. There’s nothing to do then but suffer through the hunger and the complaints of the children and try to hang onto some frayed threads of hope. These farmers—a great many of whom are women—and their families have not much in the way of possessions and little hope for betterment. They are the ones who starve to death when famine comes. Against the backdrop of this gloomy picture there is a tiny ray of hope: wretched as their lives may sometimes be, these people can benefit enormously if technology—truly useful, practical, affordable and sustainable technology—reaches them. Anyone who goes among these poverty-stricken folks learns that they are hungry not just for food but for a better, less precarious life. They are eager to learn—will walk miles to see a technology or useful skill demonstrated—and on average are just as intelligent as any typical educated person you ever met.

### **5. Trying to help low-resource farmers store their grain**

More than thirty years ago we at Purdue began looking for ways to help low resource farmers in Africa—beginning in the early 1980’s we were concerned with Niger and in 1986 began to be involved with northern Cameroon thanks to support from USAID’s Bean/Cowpea Collaborative Research Support Program (CRSP). Resource-poor farmers in the semi-arid north of Cameroon faced the problem of how to store their cowpea grain after harvest in ways that avoided losses to seed beetles called cowpea bruchids or sometimes cowpea weevils. With the help of Cameroonian colleagues we devised several technologies farmers could use to safely store their grain after harvest (Murdock et al., 2003).

When we began our work we asked ourselves what a really good technology for poor, uneducated farmers with little means should be like? We determined that it should be:

1. low-cost—not only economical but affordable by poor people
2. available—where the people who need it are located, not in some distant city
3. simple—and easy to learn
4. durable—useable for two or more storage seasons, lowering the cost
5. locally manufactured, or at least regionally—creating needed local jobs
6. culturally acceptable—similar to existing storage methods used by farmers
7. not use chemicals—no fumigants or insecticides, increasing safety, lowering cost
8. scalable in quantity—can be used to store small or large quantities of grain
9. sustainable—not given away but sustained by profits along the value chain
10. innocuous to use—doesn't affect subsequent uses of the grain for food, fodder or seed

We also decided at the time that the best test—indeed, the only truly substantive one—of the success of our project should be the number of people who adopted it. We determined not to count papers published in scientific journals, project reports, grants received, lectures given, etc. in our assessment of success. All of those academic measures, in our eyes, paled in comparison to the one really significant measure: the degree to which low-resource farmers adopted our technology and continued to use it.

Here we mention briefly only two of the five technologies we developed that met most if not all of the above criteria: (1) disinfestation using the sun's rays to heat the grain to the point that killed the insects living in it (Murdock and Shade, 1991; Kitch et al., 1992), and; (2) triple bagging in polyethylene sacks purchased at local markets and combined into one compound bag (Kitch and Ntougam, 1991). Both worked well, and farmer's liked them. They told us so and immediately after watching them demonstrated asked where they could get the materials so they could try them.

These were good technologies. They performed well, preserved the grain nearly perfectly, at least in terms of controlling insects attacking cowpeas in storage. But in working with low-resource farmers in the latter '80's and early 90's we soon came to realize that a good technology alone is not enough to have impact on people's lives—not nearly enough. If we want to have big impact, i.e., big adoption rates—our definition of project success—we must not only have an excellent technology, we must also: (1) make people aware of that technology and we must; (2) build a supply chain to be sure that the necessary inputs reach those who need them.

Within a few years of the turn of the present century it was clear that Bean/Cowpea CRSP technologies had experienced substantial adoption across West and Central Africa. A study by economists (Moussa et al., 2011) stated that "... the net present value of the investment [by the Bean/Cowpea in cowpea storage technologies] amounts to more than 295 million US dollars ... with an annual return of 17 million in 2011". While the CRSP research was clearly having impact (cf. Boys et al., 2007), surveys revealed that adoption was variable from country to country and rarely reached more than 20 percent. The big and important point of this and a still earlier adoption study (Moussa, 2006) was that only a small fraction of potential cowpea storers were using any of the CRSP technologies.

## **6. The PICS Project**

Purdue scientists and their partners initiated in 2007 a major effort focused on cowpea storage in West and Central Africa. Goal was to bring the practical and useful CRSP-developed

triple bagging technology (named PICS, for Purdue Improved Cowpea Storage) to large numbers of poor families. We set out to disseminate the technology to millions of farm families encompassing about 50 million people living in ten cowpea-growing countries, namely Cameroon, Nigeria, Niger, Benin, Togo, Chad, Burkina Faso, Mali, Ghana and Senegal. Project objective was to have half of on-farm storage of cowpea in those countries stored in PICS bags or other hermetic containers. By 2012, the project had reached 1.7 million farm families who gained an average of \$150 by storing their cowpea grain in PICS bags for a net gain in the region of \$255 million (<https://docs.gatesfoundation.org/Documents/profiles-of-progress-cowpea.pdf>).

Given the substantial adoption of PICS bags for storing cowpeas after only a few years of effort, the Gates Foundation invested in new research to determine: (1) whether the hermetic PICS technology would protect other crops from losses due to insects, and; (2) whether the return on investment to introduce PICS bags (now PICS = Purdue Improved Crop Storage) for those other crops would be enough to justify a new extension and value chain effort. This was the PICS2 project (<http://www.entm.purdue.edu/PICS2/>). PICS2 research showed that nearly all crops or commodities tested (Table 1) were protected against insects when they were stored in PICS bags (cf. Special Issue of the Journal of Stored Products Research, Murdock et al., 2014). In addition, preliminary studies indicated that PICS bags are successful at retarding *A. flavus* growth and aflatoxin spread during grain storage (Williams et al., 2014)

**Table 1** Commodities stored in PICS bags with good insect protection.

Cowpea	Sorghum
Common bean	Millet
Chickpea	Bambara groundnut
Pigeon pea	Peanut
Mung bean	Hibiscus seed
Maize	Sesame
Rice	

Economic analyses of selected crops revealed that many of them, e.g., common beans in Tanzania, can be profitably stored in PICS bags (Jones et al., 2011). Hermetic storage can have many benefits besides financial ones. It can reduce or make unnecessary the use of insecticides on the stored crops, protecting both applicators and those exposed to the chemical directly or indirectly, as through food residues. Hermetic storage can be cost-effective compared to insecticides, which often have to be applied two or more times for longer periods of storage. Further, by making it possible for the grain to be stored for weeks and months, the farmer can wait for the normal price rises that generally occur after prices hit their lows at harvest time. Hermetic storage can also reduce undesirable changes in the flavor or processing properties of the stored grain. Moldy, musty, insect-infested grain is distinctly unpleasant in smell and taste, loses value in the market and is best avoided. The early success of the PICS project owed greatly to its simplicity and effectiveness, innovative extension and value chain development, work that will be described elsewhere.

## 7. How to reach smallholder farmers

Training and capacity building have been integral parts of PICS technology dissemination. To build awareness among smallholder farmers, Purdue and its partners have relied on local government and NGO extension services. Helping extension agents to understand the importance of storage pest management and deal with it through technology use and technology transfer have proven effective ways to reduced grain storage losses at the farm level. Given the complexity of issues faced by small-holder farmers and the challenges in reaching them with new technologies, we used both traditional and non-traditional extension approaches to implement PICS activities. These included demonstrations of the technology in villages as well as via radio, television, and cellphone videos. Village training activities served as marketing tools to convince farmers about the PICS technology, its effectiveness and benefits.

Village demonstrations helped us reach millions of farmers in rural areas. These public demonstration involved volunteer farmers storing their own grain in PICS bags for at least 4 months after which most farmers were convinced of the effectiveness of the technology, literally “seeing is believing”. Once they were convinced that the technology worked, those with grain to store began purchasing bags. Media, especially radio, served to reinforce PICS training activities. Radio and TV messages supported extension agents’ efforts by building awareness of the PICS technology and making people aware of its availability in rural areas. We used cellphone videos as visual tools to help farmers learn how to use the PICS bags. These were especially useful for those who did not attend demonstration activities.

**Table 2** Countries with PICS activities (2007-2014).

<b>Project</b>	<b>Region and Countries</b>	<b>Activities</b>
Purdue Improved Cowpea Storage (PICS1)	<u>West and Central Africa:</u> Senegal, Mali, Burkina Faso, Ghana, Niger, Nigeria, Togo, Benin, Cameroon, Chad	Large-scale dissemination of PICS bags for cowpea storage (2007-2014)
Purdue Improved Crop Storage (PICS2)	<u>Sub-Saharan Africa</u> Senegal, Mali, Burkina Faso, Ghana, Niger, Kenya, Rwanda, Malawi, Mozambique, Uganda, Benin, USA	Research activities focusing on evaluating the use of PICS bags to store crops other than cowpea (2011-2013)
Leveraged activities	Kenya, Rwanda, India, Afghanistan	Dissemination of PICS bags for storage of crops other than cowpea (2012- 2014)

By 2014, more than 2.5 million farmers had been trained in the proper use of the PICS bags through activities involving at least 32,000 villages in Sub-Saharan Africa. Building local capacities in research and extension led to sustained reduction of storage losses among smallholder farmers. In addition, government and NGOs (local and international) programs have taken advantage of existing trained extension personnel to expand the reach of the PICS technology among smallholder farmers.

## **8. A primer on supply chains**

It does little good to train farmers in the use a technology if they cannot find the materials they need to use it in local markets. Sustaining the availability of improved storage technology requires the participation of key stakeholders including the private sector. Without interest, commitment, and investments from the private sector in manufacturing, distribution and sale of a technology, storage losses will continue to be high, even after major interventions. Creating demand through training and product promotion helps provide incentives for the private sector to invest in developing the supply chain necessary for sustainability. Scaling-up storage innovation in term of extension activities is a prerequisite for building demand that is large enough to attract the private sector.

The PICS approach has been to develop local manufacturing capacity in the country or region where the bags are used. Purdue worked with local manufacturers and distributors/vendors to produce and sell PICS bags. Partnering with local manufacturers provides business opportunities, reduces the cost of the bags, and improves logistics by reducing the lead time required to order and receive the bags. Timely supply of PICS bags is critical when dealing with smallholder farmers. PICS efforts have also focused on building awareness among private sector partners to help them understand the technology, quality requirements, potential market, and challenges in reaching smallholder farmers with a new product. Meetings and training sessions are organized for manufacturers, distributors and vendors. Extension activities help build the customer base required to attract investments by the private sector. Unless demand is created, the private sector is often reluctant to invest in developing the supply chain. To be commercially viable, the technology needs to be effective, cost-effective and meet the needs of the end-users.

Purdue and partners successfully transferred the PICS supply chain from the project to the private sector. Despite some challenges at the lower-level of the supply chain, PICS bags have been produced and sold by the private sector since 2010 without investments of project monies for this purpose. By 2014, more than 4.5 million PICS bags have been sold in Sub-Saharan Africa. Currently, there are 16 plastics manufacturers producing PICS bags in Sub-Saharan and Asia [Mali (2), Senegal, Ghana, Nigeria, Burkina Faso, Ethiopia, Tanzania, Kenya, Rwanda, Uganda, Zambia, Malawi, Afghanistan, Nepal, and India]. Some of the manufacturers supply PICS bags to more than one country. Scaling-up extension activities as well as manufacturing of PICS bags is critical for sustaining the availability of PICS bags among low resource farmers.

## **9. Observations on how hermetic storage works**

The Oxford English dictionary definition of “hermetic” encompasses the concepts of “airtight” and “sealed” and the term is sometimes used to mean “gas tight.” More broadly, it refers to the notion of “sealed off from external influences”. As Navarro and colleagues have pointed out, hermetic storage can take multiple forms, the most natural and common one being that in which biological activity (respiration of insects in the grain, the grain itself, and in some cases molds) change the composition of the gases surrounding the stored commodity. The level of CO<sub>2</sub> rises and that of O<sub>2</sub> falls. If the container does not allow gas exchange with the outside to occur, i.e., if it is a hermetic container, the changed gas composition inside ultimately leads to arrest of insect population growth, development and reproduction. Since insect problems in storage typically become serious only after the insects reproduce through two or more generations, early arrest of feeding, growth and development—before major population expansion occurs—prevents the problem from becoming serious.



The nature of the container wall that serves to retard or prevent changes in the composition of gases within the container is important. Hermetic containers can take many forms, the simplest being the underground pits mentioned already. More sophisticated yet is the use of plastic or metal containers, also mentioned earlier. These include metal drums, metal silos, triple-layer plastic bags, high tech plastic bags and cocoons as well as simple jerry cans and water bottles suitable for small amounts of seed. All of these work well. The one selected for use by farmers depends upon cost, availability, durability and cultural and other factors.

While it has long been known that prolonged exposure to low O<sub>2</sub> and elevated CO<sub>2</sub> leads to the death of insects within the hermetic container, how these conditions cause death has not been fully recognized until recently. The crux of the issue of insect survival in stored grain is their water supply. Because insects that live in dry grain and other dry materials have no external water source, they rely heavily on metabolic water to survive (Fraenkel and Blewett, 1944). But insects living in dry grain rely in turn heavily on their carbohydrate rich diets for their energy supply and their water supply (Murdock et al., 2012). In other words, depriving insects of O<sub>2</sub> deprives them both of energy and of water. Insect physiologists have long known that the single greatest danger faced by terrestrial insects living in air is desiccation. We suggested that the ultimate mode of action of hermetic conditions for insects is death by desiccation (Murdock et al., 2012). Even when it is not the direct cause of death, it is a contributing factor. It is useful to remember that terrestrial insects have relatively water-rich bodies and that they typically live in an air environment poor in water content. The result is a continual loss of water from the insect body into the environment. The small size of insects makes the problem even worse, since the surface-to-volume ratio of very small bodies is relatively high compared to larger ones, and thus favors disproportionate water loss. Insects have evolved mechanisms to minimize water loss like waterproofing their cuticle and recovering water efficiently from the digesta, but these mechanisms are never absolutely effective, and some loss always occurs.

## **10. Future challenges**

We have learned a lot about hermetic storage and how it works (cf. Navarro, 2006; Navarro, 2012) but our knowledge is still incomplete and in some neglected areas it is sketchy indeed. In the hope of stimulating needed research, we here explore some areas that might benefit from further studies.

### *10.1. Effects on insect behavior of changes in ambient CO<sub>2</sub> and O<sub>2</sub>*

Those studying stored products entomology have long been dominated by the idea that you have to kill insects to control them. This is likely the legacy of decades of the use of fumigants and insecticides to control storage pests. If you didn't kill the insects, they remained to reproduce and your effort at control was wasted time and energy. In fact, in a great many cases, storage insects are not sufficiently numerous to be a problem when the grain is first put into storage. A few insects do little harm. Serious damage accrues only later, after they have produced one or more generations of offspring. The pertinent question is therefore not "Have we killed all the insects in the hermetic container?" but "Have we stopped them from reproducing?" and "Is this reproductive arrest permanent or at least long lasting?"

We have long known that insects respiring in a hermetic container cause the level of O<sub>2</sub> to fall and the CO<sub>2</sub> to rise. Such changes in gas composition evoke changes in the behavior of insects. These changes are poorly described, particularly for larvae feeding hidden inside the grain.

Insects feeding out of sight in grain are hard to study. Using ultrasonic detection of feeding activity (Shade et al., 1989, 1990) we devised a way to quantitatively monitor insect feeding within seeds. Using this novel feeding monitor device that detects ultrasonic signals generated as the insects feed, Murdock et al. (2012) showed that the rate at which cowpea bruchid larvae feed within cowpea seeds is proportional to the O<sub>2</sub> concentration in the surrounding airspace. As the O<sub>2</sub> level falls in the airspace around the grain, so does the rate at which the insects feed. At a concentration of O<sub>2</sub> in the 2-5% range, the larvae feed very slowly, if at all. In surprising contrast, increasing the levels of CO<sub>2</sub> in the surrounding airspaces had little effect on the rates of larval feeding. Remarkably, as long as the O<sub>2</sub> levels were high, the insects continued feeding even when CO<sub>2</sub> levels approached 20%.

We don't know if the responses of cowpea bruchids to varying ambient gas concentrations is representative of stored products insects in general, or if are they special cases. It would be interesting to explore the effects of varying O<sub>2</sub> and CO<sub>2</sub> levels on the feeding behavior of other major insect pests of stored grain. It would be particularly desirable to carry out such studies over long periods of time of exposure to lowered O<sub>2</sub> and/or increased O<sub>2</sub>. In short, the effects on feeding and other behaviors of chronic exposure of most stored grain insects to such gas changes remains largely unexplored country.

### *10.2. Insect survival times in modified atmospheres*

There is value in knowing how long insects survive when exposed to varying levels of O<sub>2</sub> and CO<sub>2</sub>. Although a few such studies have been done, little attention has been given to survival times of various immature stages. One would suspect that first instar larvae, with their high surface areas relative to their mass, would be the first to die versus older large instars with more favorable surface-to-mass ratios, but validation of this inference requires additional research.

### *10.3. Do air leaks really compromise performance of hermetic containers?*

Traditional thinking about hermetic containers—and in some cases arguments made by those marketing hermetic storage—is that they should be absolutely perfectly airtight, and that any leaks, however miniscule, can compromise performance and protection of the commodity. This appealing idea seems to stand to reason and be obvious, and as a result appears to have been rarely questioned. Our experience suggests that containers that aren't absolutely hermetic can still give good protection of grain against storage insects.

We came to this view from observations made in Niger by Prof. Ibrahim Baoua and his team. They were testing PICS bags on farms. They had admonished their cooperating farmers to not open the PICS bags they were using for storing their cowpea grain. Most of the farmers complied, but when Prof. Baoua visited his farmers to check on how things were going, he observed, in a few cases, that the bags seemed to contain less grain than they had on his previous visit. Queried, the farmers at first declared they hadn't opened the bags but later admitted that they had. They explained that they had to take out grain for the family to eat. They declared that they had closed the bags tightly again after removing some grain and pressing out all the air. When the Baoua team assessed the level of insect damage in grain from such multiply opened (then reclosed) bags, they found no difference between those that had been opened and those that had not. Damage levels were equally low in all cases.

At first this seemed surprising and unexpected, but analysis reveals that it shouldn't be so. Two facts need to be considered. First, that the O<sub>2</sub> required for growth and development of a single

cowpea bruchid from egg to adult is substantial, about 8.5-9 ml of O<sub>2</sub> (Murdock et al., 2012), equivalent to 42-45 ml of air. Next, it should be remembered that when a farmer opens a 50 or 100 kg bag of cowpea grain, he or she only exposes the topmost surface of the grain to fresh air. The bulk of the grain remains undisturbed. Next, if the farmer seals the bag again, expressing any excess air, how much fresh air has actually been admitted into the bag? Very little, in fact. 100 ml? 500 ml? Even a liter? In the last case that would contain about 200 ml of O<sub>2</sub>, enough for full development of 20-25 adults IF it were all used up and there wasn't any competition for it. This set of observations and reasoning suggests that leakage of small amounts of O<sub>2</sub> into a hermetic container would not have devastating consequences for grain preservation. Insects not only need a supply of O<sub>2</sub> to develop normally, they need a continuing supply of it. Clearly, systematic studies of the effects of leaks or of low level seepage of fresh air into hermetic containers is merited versus unverified claims that they are devastating and destroy the value of hermetic containers.

#### *10.4. Water supplies of stored products insects*

All stored grain pest insects are terrestrial. As mentioned, entomologists have long known that the single most serious danger faced by insects that live on land is water loss. Their greatest physiological challenge is to acquire needed water and to minimize its loss. Insects lose water in part thanks to ventilating the airspaces of their tracheal systems. They inspire unsaturated air and expire air that is practically water-saturated thanks to it having passed through the insects' bodies (cf. Wigglesworth, 1967). Additional water is lost through the excreta, though this is minimized in insects that live in extremely dry environments. The major source of water loss is through the cuticle (Wigglesworth, 1967). Although terrestrial insects have waterproofed cuticle to reduce such loss, the cuticle remains the major route through which water is lost in most insects.

Thanks to the ingenuity of Gottfried Fraenkel (Fraenkel and Blewett, 1944) we know that metabolic water for some insects is the major source of water for some insects that live in dry grain. More recently, we showed that this is true for cowpea bruchid; most of its body water can be accounted for as coming from oxidation of their major food source, namely carbohydrates contained in their food. This led us to recognize that atmospheric oxygen is a vital source of water for these insects—and presumably for other insects that have similar life styles and live in dried materials (Murdock et al., 2012). In short, depriving cowpea bruchid larvae of O<sub>2</sub> deprives them of their major source of water, and we surmised that the principal cause of death, which may occur sooner or later depending upon the age and stage of the insect, is desiccation.

Unfortunately and surprisingly, metabolic water has been little studied in insects, especially in those living in stored grain where it is probably of immense importance. Studies are needed to reveal how much water is produced by metabolism in a variety of species.

#### *10.5. How exposure to varying levels of O<sub>2</sub> and CO<sub>2</sub> affect reproduction*

Key to the effectiveness of hermetic conditions in preventing explosive growth of insect populations in stored grain is interference with growth, development, and reproduction as well as survival. In the real world of subsistence farms, O<sub>2</sub> levels fall markedly and CO<sub>2</sub> levels rise when infested grain is stored in PICS bags (Baoua et al., 2012). The degree of these rises and declines may be much less than is sometimes observed in the laboratory, i.e., O<sub>2</sub> may reach and linger at 5 or 10 percent and CO<sub>2</sub> reach a few percent. Even under these conditions, we have documented that good control of insect populations is attained. What accounts for this control? One possibility is that these conditions—lower but still substantial levels of O<sub>2</sub> and elevated levels of

CO<sub>2</sub>-suppress mating behavior, egg laying, or egg and larval development, even though the insects are not killed but merely exhibit changed behavior. While there have been studies of the effects of elevated CO<sub>2</sub> and lowered O<sub>2</sub> on insect reproduction, they have tended to focus on major changes in these gases that are dissimilar to the conditions seen under real-world hermetic storage. Studies that examine the effects on reproduction of modest changes in these gas concentrations may shed more light on the effectiveness of hermetic storage.

#### *10.6. Is there such a thing as hypoxia-evoked aestivation- or hibernation-like states of insects?*

Some insects can survive long periods of exposure to lowered ambient O<sub>2</sub> and elevated ambient CO<sub>2</sub>. We have observed adult cowpea bruchids that have survived for months under conditions in which the O<sub>2</sub> level had fallen into the single digit level and the CO<sub>2</sub> level had attained several percent. Many different explanations for this survival are possible. The insects may have survived thanks to switching to anaerobic metabolism—and there is evidence that this happens to some degree (cf. Navarro, 2012)—anaerobic production of ATP may suffice to maintain cellular and organismal survival until fresh air becomes available again. One of the problems posed by hypoxia is the danger of oxidative stress, namely the accumulation of toxic peroxides and free radicals (reactive oxygen species – ROS) that attack and disable proteins, lipids and DNA and disrupt cellular signaling. Insects surviving the above-described conditions somehow avoid such toxicity. It isn't clear how they do it. One tactic would be to switch off oxidative metabolism completely such that peroxides and free radicals don't accumulate. This could involve using other metabolites as electron acceptors, e.g., pyruvate, and converting it to lactate, as in classical anaerobic energy production. An alternative response might be to turn down the metabolic flame to an extremely low level, i.e., to throttle down all energy-requiring processes (any kind of muscular activity, growth, reproductive processes), thereby preventing the production and accumulation of ROS. Such a state of hypometabolism has been documented in nature, namely in squid which encounter oxygen minimum zones in the ocean and have developed adaptations to deal with them (Seibel et al., 2014). Does, for example, the adenylate energy charge of cells in insects exposed to hypoxia and hypercarbia remain high, or fall markedly because of the inadequate supply of energy required to produce ATP? Further research is needed.

#### *10.7. How gene expression changes during hypoxia and hypercarbia.*

Exposure of insects to hypoxia and hypercarbia leads to substantial changes in gene expression, as has already been documented by Zhu-Salzman and her colleagues at Texas A&M University (Chi et al., 2011; Cheng et al., 2013). Certain classes of genes are expressed more highly, others suppressed. One suspects that when this research area has been thoroughly explored, understanding of changes in gene expression may lead to better understanding of how insects cope with hypoxia and hypercarbia. One of the few published studies suggests that prolonged hypoxia may lead to proliferation of the tracheal system (Centanin et al., 2010), for which one would expect associated changes in gene expression to occur.

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