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Oxygen demand of bean bruchids (*Acanthoscelides obtectus* Say)

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The common bean (*Phaseolus vulgaris* L) is an important crop in many countries and its safe storage is crucial in maintaining a sufficient and high quality food supply for the community. A non-chemical storage technique, hermetic storage, is being used to control the bean bruchid (*Acanthoscelides obtectus* Say), a beetle which can cause large losses to stored beans. Experiments were carried out using hermetically sealed containers of known gas volume at different temperatures (10 and 27°C) and bean moistures (8 and 16% wet basis) to quantify the oxygen requirement of bruchids. Bruchids use between 0.0074 and 0.1043 cm^3 $bruchid^{-1}day^{-1}$, depending on bean temperature and bean moisture content. Days to 100% adult bruchid mortality in hermetic storage, as a function of infestation level, storage volume, temperature and bean moisture content, can be estimated by using these oxygen requirement results. These estimates can be used to design hermetic storage systems to protect beans from damage by bruchids.

Key words: Beans, bean bruchids, hermetic storage, insects, oxygen demand, storage losses.

INTRODUCTION

The common bean, *Phaseolus vulgaris* L., is one of the most important crops in Kenya, Tanzania, Uganda, Zambia and Zimbabwe in East Africa, and elsewhere. In Uganda, beans provide 45% of total protein, 25% of calories and up to 9% of family income in some areas (Gatsby Charitable Foundation, 2014). They are also a cash crop for large scale farmers. Bean production in Kenya, Uganda and Zimbabwe ranges from 2 to 8 mg per farm for smallholders, while large farmers can produce 14 Mg per farm (CIAT, 2006). Smallholders also utilize the green leaves and pods for food. With beans being this important in East Africa and smallholders seeing a decrease in average farm size due to population

pressure, there is a push for both increased production and decreased post-harvest loss (Katungi et al., 2009).

Silos or granaries are common ways to store multi-ton quantities of beans; these beans typically will go to market once the farmer is ready to sell. Beans that are to be used for food by the farmer or for seed will commonly be stored in containers like small pots, baskets, or sacks. Smallholder farmers have a difficult time obtaining new seed due to cost and availability and stored seed is vulnerable to pests, which cause a reduction in seed quality and quantity (Kimani, 2006).

Storage losses account for a major part of post-harvest losses of beans. FAO estimates post-harvest losses of 35

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Figure 1. Bruchid damage to beans (Myers, 2004).

to 50%, with storage losses accounting for 20 to 30% (Grolleaud, 2002). The main cause of losses is from insect pests, particularly the bruchid. Storage losses of beans during six months of storage have been reported from 23% in Uganda to 73% in Kenya from bruchids (Cardona et al., 1989). Rodents have not been a major post-harvest loss since beans in their raw state are toxic to these animals (Jones, 1999). Farmers prevent losses due to fungal growth by drying to 13% moisture (wet basis) or lower which is recommended (Golob et al., 2002).

The bruchid beetle, *Acanthoscelides obtectus* Say, is found worldwide. The adult bruchid has a very short adult life span of 7 to 14 days (Golob et al., 2002). The adult female typically deposits about 45 eggs in cracks or holes in beans and hatching of larvae takes place anywhere from 3 to 30 days later (Cardona et al., 1989). Once larvae hatch, they channel into beans and continue to eat away at the inside. After a few days, larvae molt and look like grubs. Time that bruchids remain in larva stage depends on temperature and bean moisture content and can range from 14 to 180 or more days. Once timing is right, larvae transition into pupae and then finally to adult state prior to emerging from the bean (Metcalf et al., 1962). New adult bruchids will then repeat the cycle, laying eggs within 24 to 48 h after emerging (Parsons and Credland, 2003). The cycle from hatching to adult takes from two to eight weeks (Metcalf et al., 1962).

Damage to beans caused by bruchids (Figure 1) can be great, depending on storage facilities and conditions. In Figure 1, beans on the left are heavily infested while the beans on the right are slightly infested. Extrusion holes, as well as white eggs, are clearly visible. Schmale et al. (2002) found in Colombia that infestation of bruchids can occur in field prior to harvest, with an average of 32 bruchids per kg of beans and a high of 110 bruchids per kg. As bruchids infest beans in field or storage, they damage individual beans and reduce dry matter. After six months of infestation, about 80% of stored beans are infested and are no longer suitable for human food (Cardona et al., 1989).

There have been many practices implemented for reducing bruchid damage to beans, including insecticides, bean tumbling, traditional methods like ash/lime, and hermetic storage. Reducing storage losses by use of pesticides is a common practice among farmers. In Kenya, use of Actellic Super has proven effective in limiting insect damage and allowing beans to be stored as farmers wait for higher market prices (Jones et al., 2011). Pesticides can kill insects as well as stop colonies from forming. However after time and careless use, bruchids develop a tolerance to the pesticide. This requires use of higher toxicity chemicals to control the insects. Increasing the toxicity of the chemical is a short term solution; insects will continue to develop a genetically altered tolerance to these chemicals as exposure increases (Bellinger, 1996). As this would be an ongoing process and also a hazard to humans, countries specify maximum residue levels, as stated by the United Nations. As of 1992, malathion and piperonyl butoxide had the highest allowable residue level for legumes (8 ppm), while permethrin had the lowest at 0.1 ppm (Proctor, 1994). The chemicals are non-hazardous when properly used and applied with correct dilutions; however, if used carelessly, these chemicals are hazardous and must be closely monitored. With chemical residue levels often near maximum, there has been a drive to look for new storage systems that are effective and do not use chemicals (Golob et al., 2002).

Bean tumbling uses the principle that it takes bruchid larvae many hours to penetrate the bean's seed coat and gain entry. Tumbling of beans repositions the opening that the larva has started, forcing it to start a new opening. This may cause the larva to die from lack of food and the drawn out process of constantly working. Quetin et al. (1991) found that in two weeks, a burlap sack initially containing 1,000 adult bruchids, along with 45 kg of beans, could be reduced to 244 bruchids when periodically tumbled compared to 6,857 bruchids when not tumbled. However, this practice of periodic tumbling requires significant effort.

Traditional methods of reducing bruchid infestation include using ash or lime, or solar radiation. Packing ash or lime around beans prevents bruchids from laying eggs directly on the beans, and also decreases the amount of air around beans. To adequately reduce infestation, it is recommended that three or more volumes of ash or lime be spread on four volumes of beans (Fulton et al., 2009). Solar disinfestation has also been utilized. By exposure to the sun, beans can be heated to temperatures which will kill the bruchids (Fulton et al., 2009).

Hermetic storage seeks to eliminate gas exchange between the storage and external environments. It is commonly used in storage of grains and seeds and has been in use since early ages of grain storage. Hermetic storage of grains or seeds that have been infected with insects, if done properly, can cause the insects to die when oxygen is depleted to levels of 1 to 2 % and carbon

dioxide levels increase due to insect respiration (Grainpro, 2011).

Knowing how long the pests will survive is a crucial part of hermetic storage system design. Yakubu et al. (2011) developed a procedure which allows the time to 100% mortality of maize weevils to be estimated. They conducted research on oxygen use by the maize weevil (*Sitophilus zeamais* Motsch.) in shelled maize. Oxygen levels were measured utilizing oxygen sensors. They determined that 100% mortality of adult weevils can be achieved with hermetic storage and that this happens at an average oxygen level of about 4%. Different temperatures and moisture contents were used to duplicate storage conditions in East Africa in tests to determine the oxygen usage of weevils and the time to 100% mortality. Using results from Yakubu, time to 100% mortality in hermetic storage can be estimated for maize moistures between 8 and 16% and temperatures between 10 and 27°C. The calculations involve maize temperature, moisture content, along with volume of maize and container, and weevil population numbers.

For hermetic storage to work effectively for insect control, air volume in the storage container should be reduced as much as possible. Doing this utilizes as much volume as possible for the grain and reduces the total volume of oxygen that insects have to use up before they die. Several systems capable of hermetic storage of beans are available to smallholders (Postcosecha, 2011; Baributsa et al., 2010; Grainpro, 2011; Yakubu et al., 2016). For advancement of hermetic storage systems for the common bean, quantification of the oxygen requirements of bruchids needs to be carried out over a range of temperatures and moistures.

Objective

The objective of this research was to determine the oxygen demand of bruchids in containers with beans at different bean temperatures and different bean moisture contents.

MATERIALS and METHODS

Test chamber

All tests were conducted in a laboratory chamber where temperatures were maintained at desired levels. The chamber used to maintain 10 or 27°C temperatures was a model 307C Fisher Scientific (Thermo Fisher Scientific Inc., Waltham, MA 02454) with a temperature range from -10 to 60°C.

Beans and jars

Organic dark red kidney beans, a common bean in Eastern Africa, were purchased from Wheatsfield Cooperative, 413 Northwestern Avenue, Ames, IA 50010 at 15.9% moisture. Beans were produced in the United States and were stored at 4°C until used. A laboratory drier produced air at 37°C to dry a portion of beans to 8% moisture. Bean moisture content was determined by the 103°C, 72-h oven

method (ASABE, 2008). Density of a randomly selected sample was measured using an Accupyc model 1330 pycnometer (Micromeritics, Gosford, New South Wales, Australia). The average of three measurements was 1.41 g cm^{-3} at 8% moisture and 1.39 g cm^{-3} at 16% moisture. The experimental containers used to contain beans were Kerr 473-mL (one pint) canning jars (Jaden Home Brands, 14611 W. Commence Road, Daleville, Indiana, USA). Each jar was equipped with an AMI model 65 oxygen sensors (AMI, 18269 Gothard Street, Huntington Beach, CA) which was sealed in its lid. Sensor values were read through a PMD into a computer program every hour.

Bruchids

Bruchids (*A. obtectus* Say*)* were obtained from the USDA-ARS stored grain pest lab in Parlier, California, USA. They were placed in one-quart canning jars, with screen lids, containing 14.5% moisture organic kidney beans and kept in a rearing chamber at 27°C for several months to develop a colony to be used for testing.

Experimental design

The experiment consisted of testing at two different temperatures (10 and 27°C) and two different bean moisture contents (8 and 16%), for a total of four treatments. Bean storage conditions in East Africa are typically between temperatures of 10 and 27°C and between 8 and 16% moisture. Each jar contained 200 g of beans and 300 adult bruchids. Initially, 300 bruchids were placed in each jar for each treatment test, however upon further testing at 10°C and 16% bean moisture, bruchids became very inactive and the number of bruchids per jar, for this treatment, was increased to 900. With the inactivity requiring a prolonged storage period, the cause of mortality could not be definitely attributed to lack of oxygen, due to the possibility of natural death. All other treatments remained at 300 bruchids per jar.atments were replicated three times, and then the data were averaged. Daily observation by sight determined when 100% mortality occurred. The determination of mortality was when bruchids no longer moved and also flowed with the grain.

RESULTS AND DISCUSSION

Oxygen level in each jar was noted daily and the number of days to 100% mortality was calculated. Figure 2 shows an example of a single test. Time to 100% mortality was day four, with 0% oxygen remaining, for 10°C temperature and 16% moisture. Figure 3 shows average time to 100% mortality of three replications at 27°C for both 8 and 16% moisture beans. As seen in Figure 3, both treatments had 100% mortality on the second day. Figure 4 shows average time to 100% mortality for three replications at 10°C for both 8% and 16% moisture. The 10°C and 8% moisture treatment reached 100% mortality at 27 days while the 10°C and 16% moisture treatment reached 100% mortality at 4 days. The lesser number of days to 100% mortality at 10°C and 16% moisture is a result of using three times the number of bruchids compared to 10°C and 8% moisture. Thus these two lines should not be directly compared. Oxygen quantification results are shown in Figure 5. The two lines shown on the graph can be used to estimate oxygen consumption and estimate days to 100% adult bruchid mortality, assuming linear relationships.
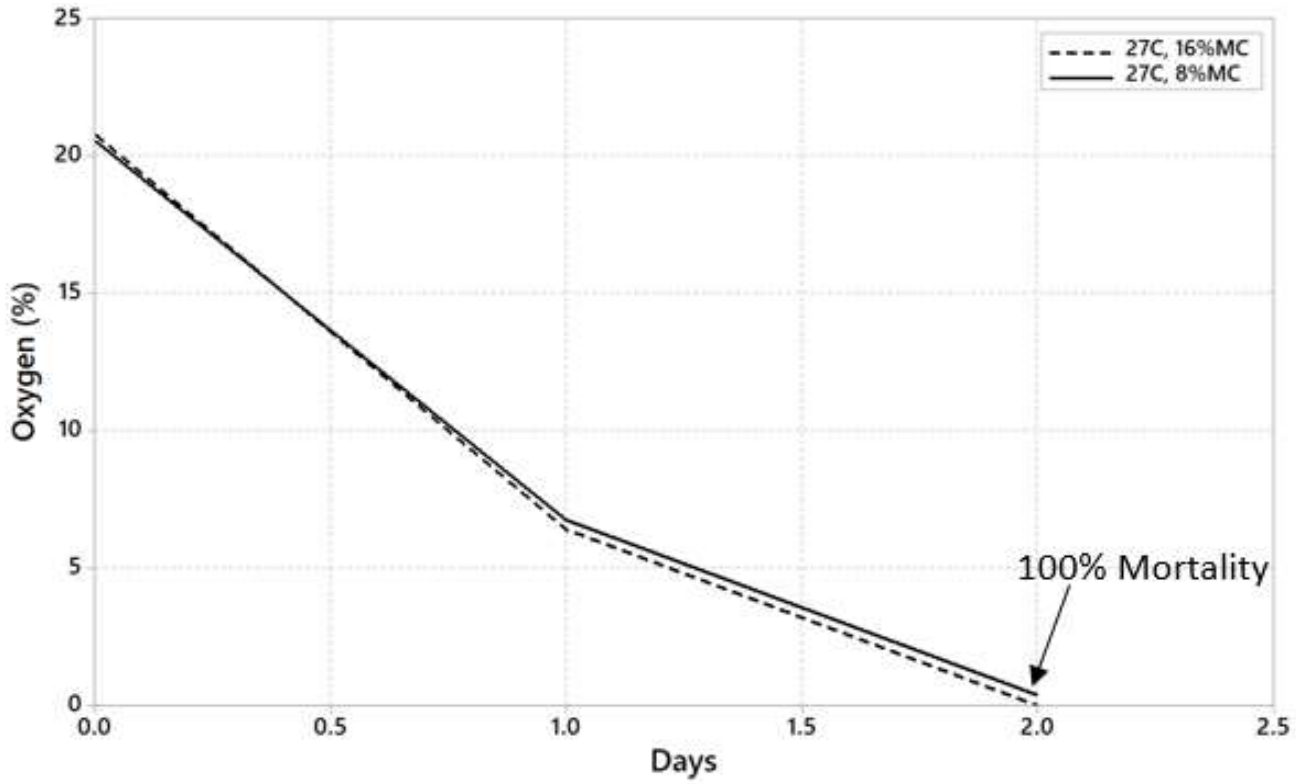
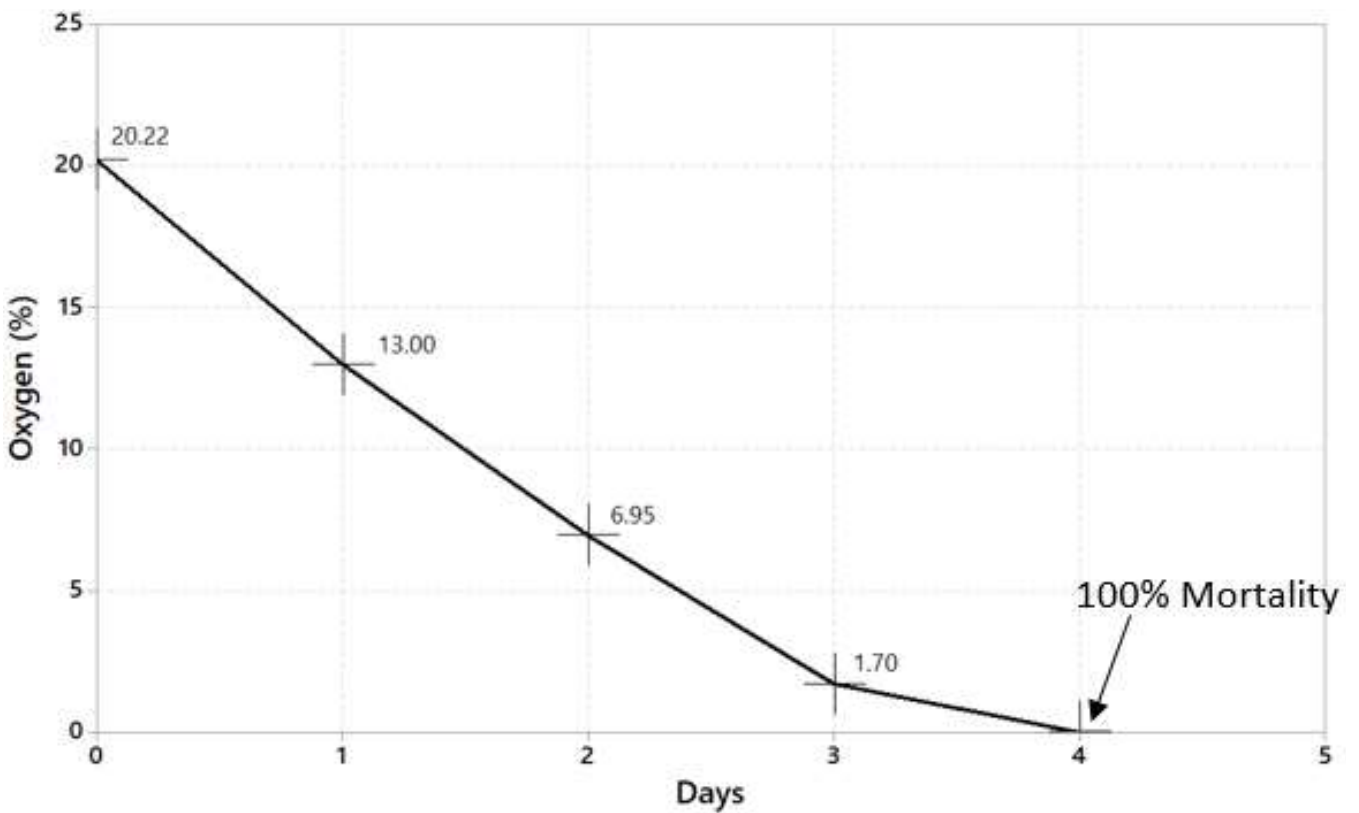**Figure 2.** Days to 100% mortality at 10°C, 16% moisture.

Figure 3. Days to 100% mortality at 27°C, 8% and 16% moisture. Each line is an average of three replications.
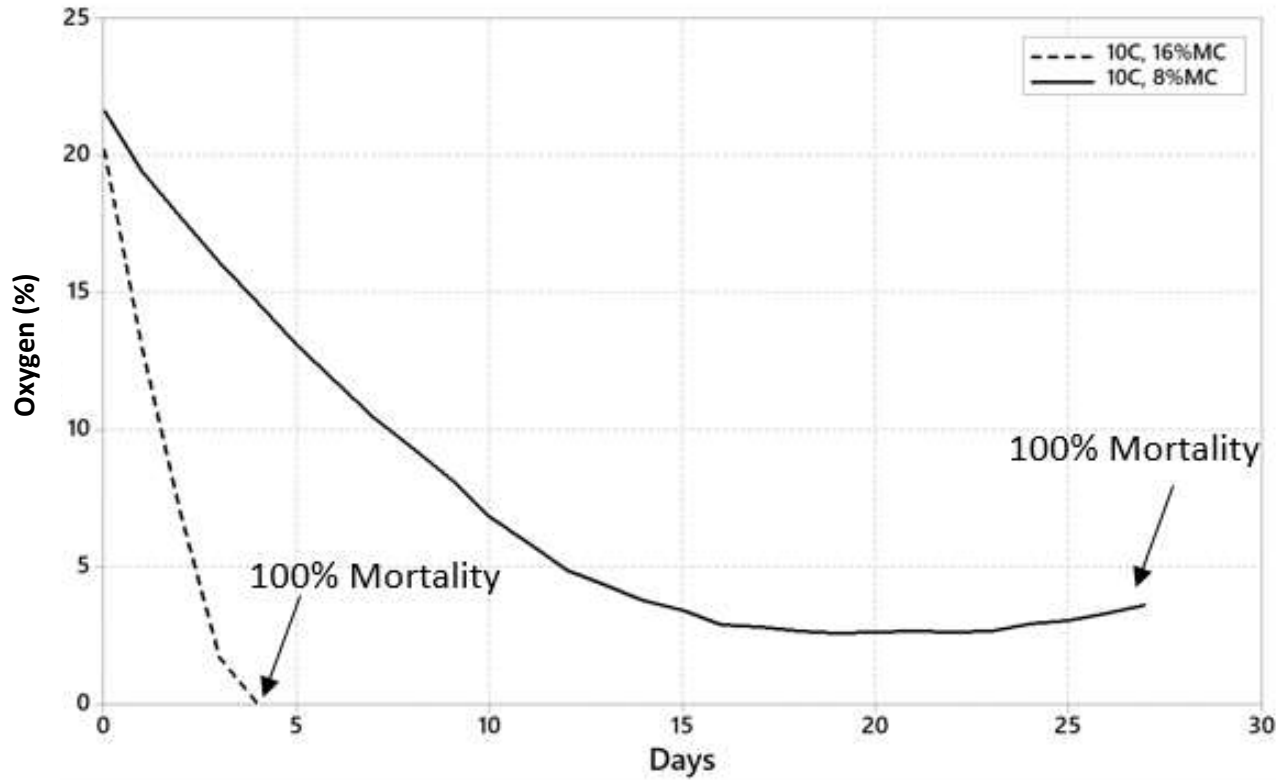
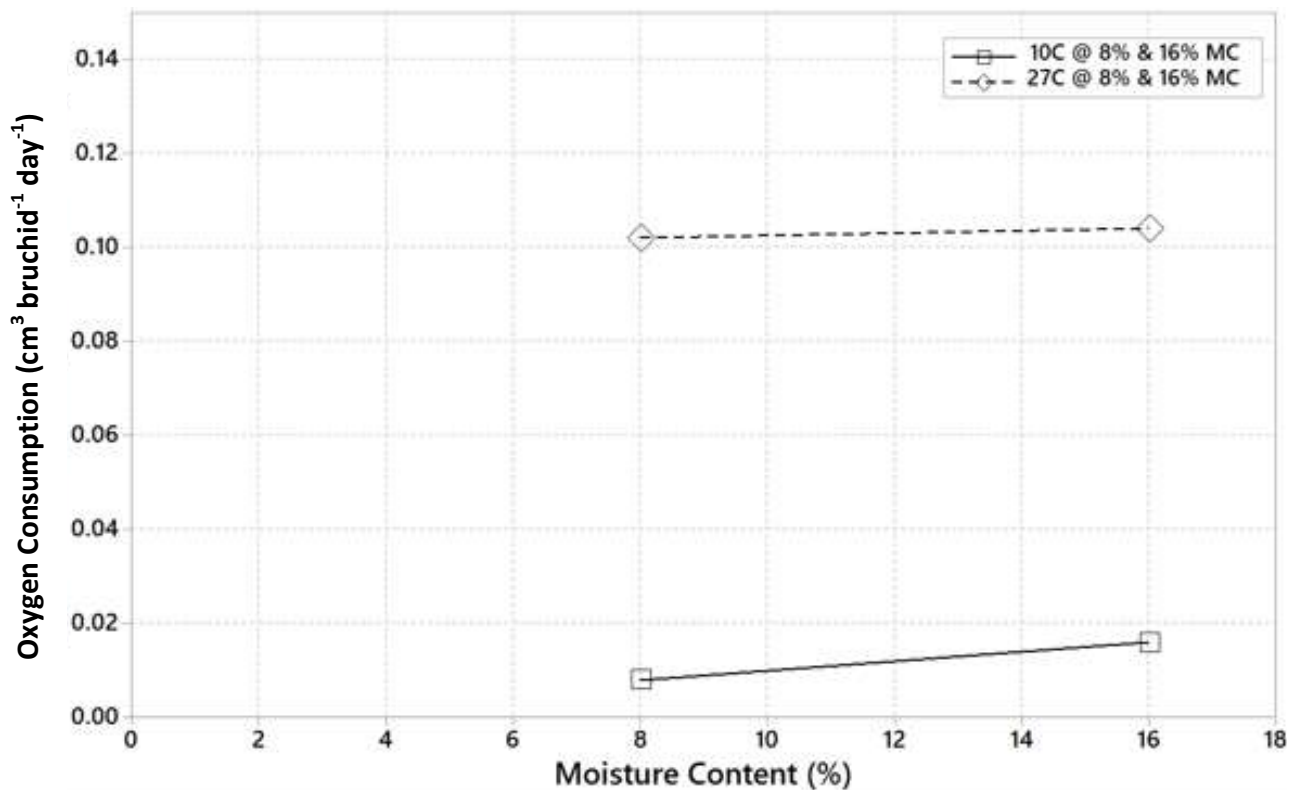**Figure 4.** Days to 100% mortality at 10°C, 8% and 16% moisture. Each line is an average of three replications.

Figure 5. Average oxygen consumption of bruchids in beans.

The line equations are:

$$y = 0.0003x + 0.0995 \text{ (for 27°C and}$$

$$y = 0.001x - 0.0006 \text{ (for 10°C)}$$

where x is moisture content and y is oxygen consumption cm^3 $bruchid^{-1}$ day^{-1}.

Prediction example

Consider a 10 L plastic can stored at 20°C containing 10 kg of beans at 12% moisture which is infested with 100 bruchids per kg of beans. From Figure 5 we can interpolate to get an oxygen utilization value of 0.066 cm^3 $bruchid^{-1}$ day^{-1}. Assuming air is 21% oxygen and that on average bruchids die at around 1% oxygen, and assuming bean particle density is 1.40 g cm^{-3}, we can calculate the predicted time to 100% mortality as about nine days.

DISCUSSION

Freitas et al. (2016) conducted experiments hermetically sealing bruchid-infested beans in silo bags and PET bottles. At the end of 120 days of hermetic storage, they observed no increase in infestation in the hermetic containers, but they reported a significant increase in infestation in non-sealed control containers. They did not quantify the infestation level in bruchid kg^{-1} before or after the hermetic storage. The prediction procedure above will allow estimating how many days of hermetic storage are required for complete mortality of the bruchids in the sealed containers. Yakubu et al. (2011) measured maize weevil oxygen requirements ranging from 0.19 cm^3 $weevil^{-1}$ day^{-1} for weevils at 27°C and 8% moisture to 0.01 cm^3 $weevil^{-1}$ day^{-1} for weevils at 10°C and 16% moisture. The present study measured oxygen requirements for bruchids to range from 0.104 cm^3 $bruchid^{-1}day^{-1}$ to 0.008 cm^3 $bruchid^{-1}day^{-1}$. Comparing maximum values, on a per insect basis, a bruchid requires only about 55% of the oxygen required by a maize weevil. Garcia-Perea et al. (2014) measured oxygen levels in hermetic 275 ml flasks held at 28°C and 75% relative humidity. Each flask was loaded with 150 g of common bean at 10, 12, or 16% moisture and 20 unsexed adult bruchids. After nine days, oxygen levels had decreased to between 0.8 and 2% and all bruchids were dead. Using their data, we calculated the bruchid oxygen use at 0.18 cm^3 $bruchid^{-1}day^{-1}$ for all three bean moisture levels. This is about 40% higher than is predicted using Figure 5.

Conclusions

Bruchids use between 0.0074 and 0.1043 cm^3 $bruchid^{-1}day^{-1}$, of oxygen, depending on bean temperature and bean moisture content:

(i) The equation y=0.0003x + 0.0995 can be used to estimate oxygen consumption for adult bruchids at 27°C, with bean moisture between 8 and 16%*
(ii) The equation y=0.001x - 0.0006 can be used to estimate oxygen consumption for adult bruchids at 10°C, with bean moisture between 8 and 16%*

Where *y is oxygen consumption, $cm^3 bruchid^{-1}day^{-1}$ and x is bean moisture, % wet basis.

These equations can be used to estimate time to 100% adult bruchid mortality in beans stored hermetically at temperatures between 10 and 27°C and at moistures between 8 and 16%.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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