POTENTIAL OF DEVELOPMENT OF MYCOTOXINS IN STORED DURUM WHEAT UNDER NEAR-AMBIENT DRYING CONDITIONS IN WESTERN CANADA

BY

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ABSTRACT

The use of near ambient air drying for the preservation of wheat stored in granaries is common in Western Canada. Guidelines have been developed to assist farmers in selecting appropriate drying methods. During this process the top layer of wheat can remain at moisture contents (m.c.) greater than the safe storage limit, 14.5% wet bulb (wb), for up to 12 weeks. This study tested the effects of this drying procedure on the development of ochratoxin A (OTA) using 1 m^3 bulks of durum wheat at 18% m.c. (wb) contained within steel bins inside a Weather Simulation Lab. In a second study using 20 L volumes of wheat at a m.c. of 20% (wb) within an environmental growth chamber potential development of OTA was also evaluated.

The wheat was exposed to two treatments, *airflow* and n*o airflow*, for a period of 12 weeks under conditions of high relative humidity (greater than 80%) and typical Manitoba fall temperatures. The storage quality parameters of germination, fat acidity value, and presence of OTA were measured weekly.

It was found that high moisture wheat stored under all treatment conditions showed a rapid decrease in germination and increase in fat acidity value over time, with no significant difference between the treatments. Under the tested conditions the development of ochratoxin A was not detected in significant quantities in the 1 m^3 bulks of grain but was detected in the smaller 20 L bulks.

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1 INTRODUCTION

One of the greatest challenges facing the world today is providing a safe food supply for the increasing population. Every year grain that could be used to meet the growing demand for food is lost due to spoilage during storage. Finding economical means of preventing grain spoilage during storage is the subject of research around the world.

It has been estimated that up to 25% of the world's food crops are spoiled by mycotoxins annually (Miller 1996a). By definition, mycotoxins are secondary metabolites of filamentous fungi that are toxic to humans and animals (Smith and Moss 1985; Shapira and Paster 2004). However, some authors agree on a narrower definition that only includes those toxins produced as natural contaminants which show toxicity to humans and animals via a natural route such as ingestion or inhalation (Abramson et al. 2005; Chelkowski 1991). No matter which definition is used there is no doubt that the effects of mycotoxins on animal health are numerous including hepatitis, hemorrhagic disease, reduced feed efficiency, and death (Pitt et al. 2000). As well, the importance of mycotoxins on human health cannot be overstated, according to Miller (1996b) as quoted in Pitt et al. (2000), "Some scientists are of the opinion that the single most effective and beneficial change that could be made in human diets around the world would be the elimination of mycotoxins."

Grain production and export plays a large role in the Canadian economy. In the 2007-2008 crop year Canada exported 14.4 million tonnes (Mt) of wheat (CWB 2008) representing a significant portion of Western Canada's economic activity. To maintain this economic activity Canada must continue to ensure that its wheat quality remains high and meets all foreign trade requirements. Recently, many countries have introduced new

restrictions on the allowable levels of mycotoxins for imported grains in efforts to improve food safety (FAO 2004).

Mycotoxin development during storage is dependent upon many factors which are not completely understood at this time. However, it is known that temperature along with moisture content (m.c.) play a significant role and that grain stored under cool, dry conditions will not develop mycotoxins. Therefore, current grain storage strategies are based on cooling and drying the grain to safe level as soon as possible after harvest.

The current guidelines for drying wheat with near ambient air were developed using mathematical simulations and the input of 33 years of historical weather data (Friesen and Huminicki 1987). However, these guidelines did not consider the development of mycotoxins as a spoilage parameter and therefore may not provide adequate protection against mycotoxin development.

This study was undertaken to determine whether wheat stored in granaries and dried using the current near ambient air guidelines would be at risk of developing mycotoxins in excess of the current international allowable limit for ochratoxin of 5 ppb or 5 μg OTA kg^{-1} (FAO 2004; JECFA 1999).

2 REVIEW OF LITERATURE

2.1 History of Mycotoxins

The presence of visible mould on grain has been used as a spoilage indicator for years. In more recent history mycotoxins were identified as by-products of moulds (Scott 1957; Smith and Moss 1985). However, their importance in contributing to health conditions has been recognized since 1973 (Krogh et al. 1973). The first reported instance of widespread mycotoxicoses was the ergotism seen in Europe during the middle ages. This disease outbreak has been cited as the most influential factor limiting population growth in Europe during this time period (IFST 2006). The effects of mycotoxins on human and animal health are numerous. Aflatoxin B_1 has been proven to be a powerful hepatocarcinogen and ochratoxin A is known to have powerful nephrotoxic effects along with carcinogenic properties (Pitt et al. 2000; Walker 1999).

Over 120 different mycotoxins have been identified in the laboratory, but less than 20% have been found to be naturally occurring (Abramson 1991). Although there are only a few naturally occurring mycotoxins there does not appear to be agreement on which of these few are agriculturally important. Miller (1995) states that there are only five agriculturally important fungal-produced mycotoxins: aflatoxin, deoxynivalenol (DON), fumonisin, ochratoxin A (OTA), and zearalenone. However, Abramson (1991) states that the mycotoxins which pose the greatest risk to consumers of stored cereal products are: aflatoxins, OTA, citrinin, and xanthoquinones.

Even though there is some disagreement as to which mycotoxins are significant with respect to cereal crops as a whole, overall it has generally been accepted that OTA is the

most important mycotoxin with respect to stored wheat (Bayman and Baker 2006; Abramson et al. 1980; Abramson et al. 2005). Citrinin is commonly found in conjunction with OTA and will also be discussed further here.

2.1.1 Ochratoxin

Ochratoxins are white, odourless, crystalline solids with a melting point of 168-173°C (Pohland et al. 1992). "They are composed of an isocoumarin moiety and a phenylalanine moiety linked by an amide bond" (Bayman and Baker 2006). There are three types of known ochratoxins: OTA, ochratoxin B (OTB), and ochratoxin C (OTC). Ochratoxin A, the most prevalent and toxic of the three, is chlorinated (Figure 1) which is uncommon for naturally occurring substances. Ochratoxin B, which is not chlorinated, and OTC, the ethyl ester of OTA, are both less toxic and less common than OTA and therefore have not been studied as frequently.

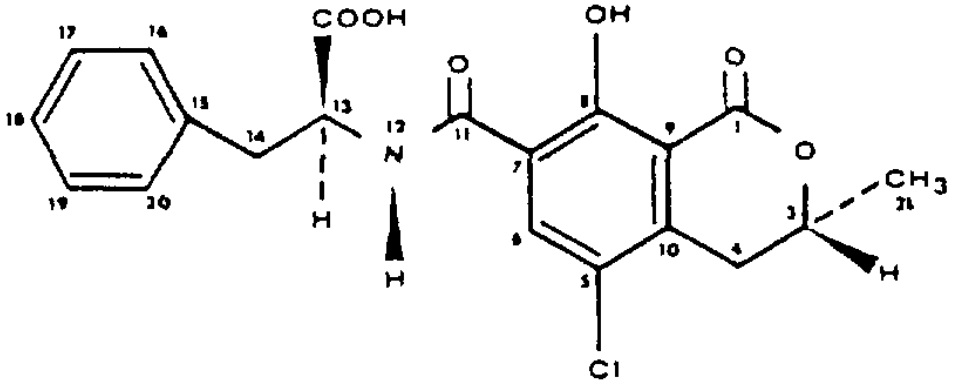

Figure 1. Chemical structure of OTA (FAO 2004).

Ochratoxin A is a powerful nephrotoxin as well as a known liver toxin, an immune suppressant, a potent teratogen, and a carcinogen (Pitt et al. 2000; Walker 1999; Bennet

and Kilch 2003). Although still not considered as significant as Aflatoxin, OTA is quickly becoming one of the most significant and studied mycotoxins in the world today.

2.1.2 Citrinin

The isolation of citrinin from *Penicillium citrinum* Thom was first documented by Hetherington and Raistrick (1931). Since then it has been isolated from over a dozen *Penicillium* species (Bennet and Kilch 2003). Citrinin (Figure 2) is a non water soluble yellow solid with a melting point of 175°C (United States National Library of Medicine 2008).

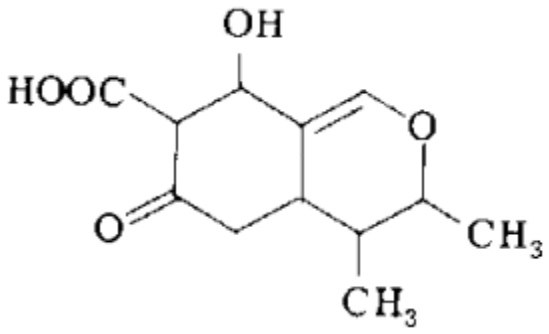

Figure 2. Chemical structure of Citrinin (Micotoxinas 2008).

Citrinin is known to act synergistically with OTA and has proven to be a nephrotoxin in all animal species tested to date (Bennet and Kilch 2003). Citrinin is commonly found in conjunction with OTA and because of the synergistic toxic effects of the two, research into citrinin is increasing.

2.2 Sources of Mycotoxins

It has been well established that mycotoxins are only produced by filamentous fungi. Therefore, an important first step in determining how to prevent mycotoxins development in stored grain is to identify how the spores of the fungi enter into the stored grain bulk. If

entrance of the spores can be prevented then the presence of mycotoxins should be eliminated. Commonly fungi are classified as either field fungi or storage fungi (Chelkowski 1991; Smith and Moss 1985). As the names imply, field fungi flourish under the growth conditions in the field and storage fungi flourish under storage conditions. It has been found that mycotoxins associated with wheat are predominantly storage fungi (Banks et al. 2000; Smith and Moss 1985). Banks et al. (2000) found that pre-harvest fungicide treatment of crops did not decrease overall mycotoxin levels in stored grain. This result corroborates the common belief that the fungal spores are integrated into the seed coat during growth and cannot be eliminated from the harvested crop (Christensen and Kaufmann 1974). Therefore, as proposed by Friesen and Huminicki (1987), the most effective way to prevent grain deterioration due to microflora during storage is through proper drying.

Penicillium and *Aspergillus* are the most important contributors to mycotoxin production in stored wheat (Abramson 1991; Banks et al. 2000; Pitt et al. 2000). Although several species within each genus are capable of producing mycotoxins only a few of them are capable of mycotoxin production in stored wheat, these are summarised below (Table 1).

Table 1. Mycotoxins produced by various fungal species in wheat.

Species	Toxin produced	Reference
P. viridicatum	ochratoxin A , citrinin	Boley and Muller (1986) Smith and Moss (1985) Czerwiecki et al. (2002) Lugauskas (2005)
P. verrucosum	ochratoxin A	Lugauskas (2005) Abramson et al. (1982) Abramson et al. (1990) Banks et al. (2000) Czerwiecki et al. (2002) Pitt and Hocking (1997) IPCS (2001) Lund and Frsivad (2003) Madhyastha et al. (1990)
P. variabile	ochratoxin	Lugauskas (2005)
P. nordinum	ochratoxin A	Lugauskas (2005)
P. cyclopium	ochratoxin A	Czerwiecki et al. (2002)
P. citrinum	citrinin	Smith and Moss (1985)
A. ochraceus	ochratoxin A	Frisvad and Samson (2000) Smith and Moss (1985) Pitt (1995b) IPCS (2001)

Table 1 provides a summary of reported mycotoxins and their sources found in stored wheat. It is important to note that there has been some discrepancy in reported results. Pitt et al. (2000) reported that only *P. verrucosum* was responsible for OTA production in wheat, clearly contradicting other reported findings. This finding was also corroborated by IPCS (2001) which stated that, "It is now clear that ochratoxin A is produced by a single *Penicillium* species, *P. verrucosum*, and a rather remarkable range of *Aspergillus* species." Today, it is widely accepted that *P. verrucosum* is the only *Penicillium* species responsible for OTA production and all earlier reports of OTA production by different *Penicillium* species were due to misidentification (Frisvad 1989; Frisvad and Filtenborg

1989; Frisvad 1995; Pitt and Hocking 1997). Other evidence which supports the conclusion that *P. verrucosum* is the only *Penicillium* species which produces OTA is the claim that the presence of OTA can be correlated to the infestation levels of *P. verrucosum* (Lund and Frisvad 2003; Lindblat et al. 2004). However, this claim implies that all *P. verrucosum* will produce OTA which is contrary to many findings which indicate that it is the presence of *P. verrucosum* as well as specific growing conditions which contribute to OTA production.

It has been reported that *Aspergillius ochraceus* Wilhelm is also capable of producing OTA in stored wheat (Frisvad and Samson 2000; IPCS 2001; Smith and Moss 1985). However, reports of *A. ochraceus* producing OTA in stored cereal products are infrequent and therefore, "its presence (*A. ochreaus*) is not a good indicator of significant mycotoxin production" (Pitt and Hocking 1997).

Citrinin is produced by both *Penicillium* and *Aspergillius* species (Abramson et al. 1995; Madhyastha et al. 1990). It is often found in conjunction with OTA and therefore occurs in many of the same products including wheat and wheat flour (Osborne 1980; Abramson et al. 1990; Boley and Muller 1986).

2.3 Development of Mycotoxins

Moulds undergo chemical reactions to produce biomass and energy. These reactions are commonly termed primary metabolism. Processes that occur within fungi that are not part of primary metabolism are termed secondary metabolism and it is within these processes that mycotoxins are produced (Abramson 1991; Smith and Moss 1985). It has not generally been determined what biological function secondary metabolites serve or even what conditions lead to their formation (Bayman and Baker 2006; Smith and Moss

1985). However, it has been established that mycotoxin formation can only occur under conditions when fungal growth occurs and that production of particular mycotoxins is restricted to a small number of species (Lacey and Magan 1991; Pitt 1995a; Smith and Moss 1985; Lugauskas 2005). These studies indicate that even though fungal spores may not be able to be eliminated from the grain bulk, one method of mycotoxin prevention would be to simply ensure environmental conditions did not allow these spores to germinate and grow.

The link between secondary and primary metabolites occurs in relatively simple intermediate substances (Figure 3).

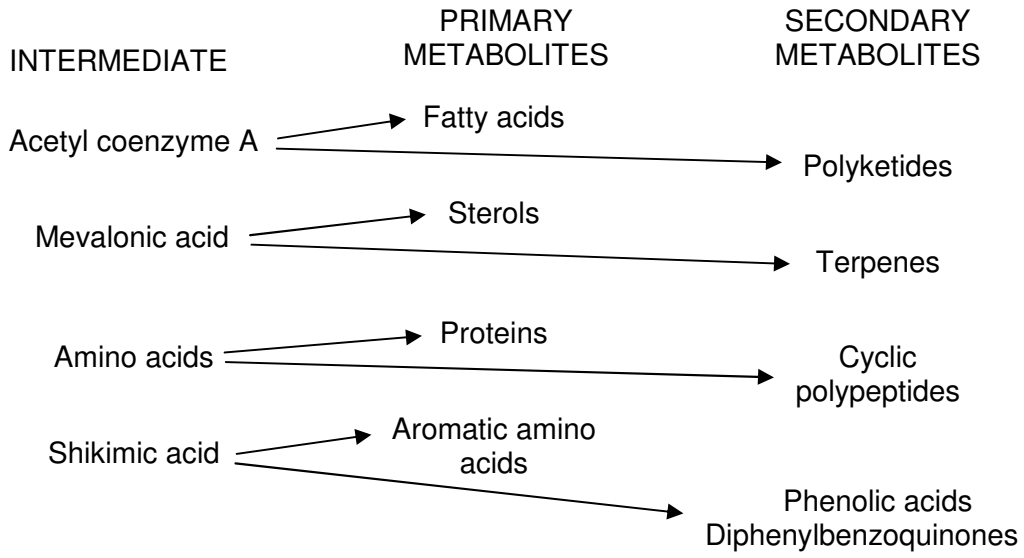

Figure 3. Primary and secondary metabolites produced by fungi from various intermediates (Smith and Moss 1985).

For instance, the intermediate substance, acetyl coenzyme A can lead to fatty acid production if primary metabolism occurs or to polyketides if secondary metabolism occurs (Figure 3). Both OTA and citrinin incorporate five acetyl groups (Figures 1 and 2)

and therefore are termed polyketides (Smith and Moss 1985). These mycotoxins are both secondary metabolites of acetyl coenzyme A (Figure 3).

2.4 Factors Affecting Mycotoxin Development in Storage

Understanding the factors that lead to mycotoxin formation may help researchers develop techniques for preventing mycotoxin formation in stored grain. The interactions of factors such as water activity, temperature, grain condition, substrate type, gas atmosphere, and pH need to be considered to achieve this goal.

2.4.1 Water activity

Grain is a hygroscopic material. Therefore, its m.c. changes to stay in equilibrium with the relative humidity (RH) of the surrounding air. The m.c. value for the grain at a given air RH level is termed the equilibrium moisture content (EMC) and the associated RH to a given EMC is termed the equilibrium relative humidity (ERH). The general relationship between ERH and EMC for cereals and oilseeds is shown in Figure 4. Also, the EMC/ERH relationship is different depending on whether the grain is absorbing or desorbing moisture. This difference is commonly known as hysteresis effect.

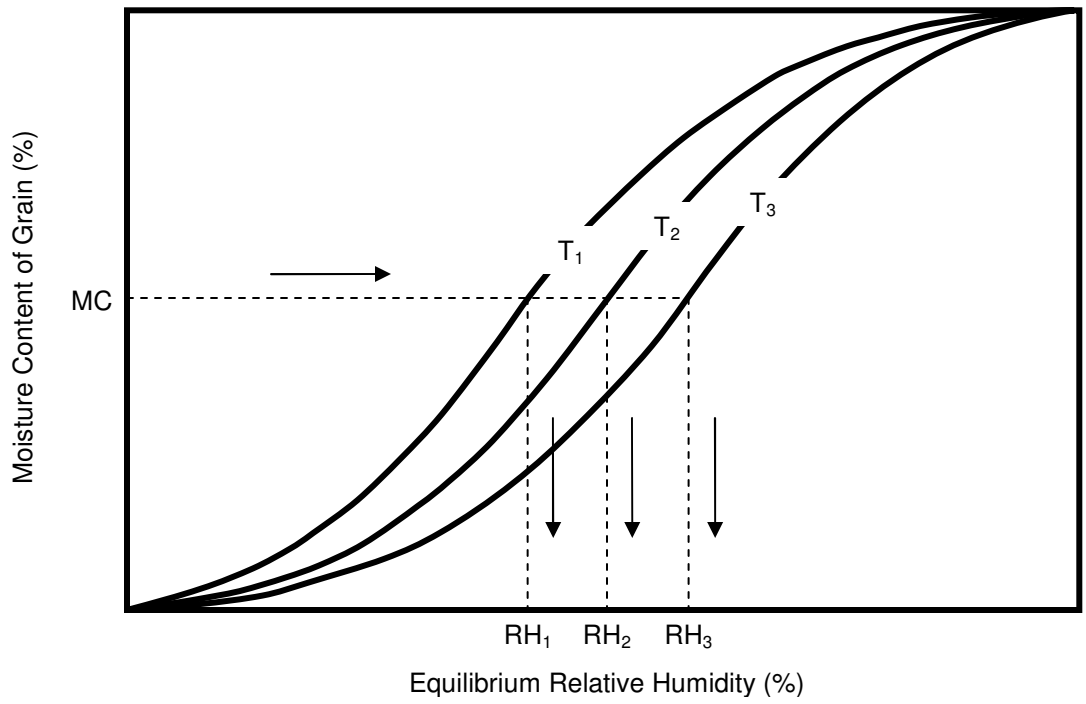

Figure 4. General relationship between grain moisture content and the equilibrium relative humidity (ERH) of the intergranular air at different temperatures (Muir 2001).

Moisture content is a parameter that is commonly used to describe a grain bulk. However, moisture content gives no direct indication of water availability, and it is the latter that is important for microbial growth (Lacey and Magan 1991). Scott (1957) introduced the term water activity, a_w, to quantify the relationship between moisture content of the grain and the amount of water available for microorganism growth. The a_w of an environment is equal to the ERH, expressed as a decimal. It has been found that a_w is one of the most influential factors in the process of stored grain spoilage (Navarro et al. 2002; Lacey and Magan 1991; Pitt 1995a; Abramson et al. 1992; Wicklow 1995).

Relatively high levels of a_w are required for most moulds to grow. However, the ranges of a_w required for mycotoxin development vary between fungal species (Table 2).

Table 2. Water activity ranges at which various fungal species produce mycotoxins.

Species	Mycotoxin	Minimum a_w required for mycotoxin development	Reference
A. ochraceus	ochratoxin A	0.79	Pitt (1995b)
		0.85	Lacey and Magan (1991)
P. verrucosum	ochratoxin A	0.80	IPCS (2001)
		0.88	Lacey and Magan (1991)
		0.86	Pitt (1995c)
		0.90	Cairns-Fuller et al. (2005)
P. citrinum	citrinin	0.90	Lacey and Magan (1991)
P. viridicatum	ochratoxin A	0.83	Abramson (1991)
			Lacey and Magan (1991)

Although there is overlap between the reported results of different researchers, there are also some differences (Table 2). All reviewed literature agrees that if mould growth cannot occur, then mycotoxin production cannot happen. Therefore, the only clear cut boundary that seems to exist is that no mould growth occurs below an a_w of 0.65 (Lacey and Magan 1991; Lacey et al. 1980) and no mycotoxin development can occur at or below this a_w.

It is also important to note that microorganism development is dependent upon local a_w and not the average a_w of the grain bulk. Therefore, it is quite possible for mycotoxins to form in bins with an average m.c. at or below what are considered "safe", due to pockets of high moisture grain.

2.4.2 Temperature

It is widely accepted that temperature is one of the most important factors in the development of OTA and citrinin in stored wheat (Abramson 1991; Cairns-Fuller et al.

2005; Pitt 1995a). However, the ranges of temperature at which mycotoxins can be produced under by different fungal species differ (Table 3).

Table 3. Temperature ranges at which various fungal species produce mycotoxins.

Species	Mycotoxin	Temperature range (°C)	Reference
A. ochraceus	ochratoxin A	15-37	Pitt (1995b)
P. verrucosum	ochratoxin A	0-31	Pitt (1995c)
	ochratoxin A	< 30	IPCS (2001)
	citrinin	10-25	Cairns-Fuller et al. (2005)

It is known that temperature alone cannot be used to predict the formation of mould and mycotoxins (Abramson et al. 1990; Cairns-Fuller et al. 2005; Lacey and Magan 1991). It is generally accepted that OTA production in wheat is limited to *P. verrucosum* in temperate climates and *A. ochraceus* in more tropical climates due to temperature differences (Lund and Frisvad 2003; Lindblat et al. 2004). Recent unpublished studies on durum wheat indicate that in high moisture wheat, 19-20% wb, a temperature of 20ºC is more favourable for OTA production by *P. verrucosum* than a temperature of 10ºC, 30ºC, or 40ºC (Udayakumar 2008). It should also be noted that Wallace et al. (1983) reported *Penicillium* growth in a granary at a temperature range of -5ºC to 8ºC which would indicate that mycotoxin production at these low temperatures may be possible. However, no studies to date have reported mycotoxin production at temperatures below 0ºC, but this may be due to lack of testing at sub zero temperatures. It is clear that more research is needed to relate the effect of temperature on mycotoxin production in stored wheat.

2.4.3 Grain condition

The general term, grain condition, is actually a representation of several individual factors including: soundness, presence of field fungal infection, state of covering tissue and presence of damage either mechanical or from insects. The impact of these factors on mycotoxin production has not been accurately quantified, however there is evidence to support that they do affect mycotoxin development (Lacey and Magan 1991; Wicklow 1995; Lillehoj et al. 1975). Lillehoj et al. (1975) clearly demonstrated that mechanical damage of corn increased the incidence of mycotoxin production when compared with non damaged corn. Pitt (1995a) indicated that kernel damage has an effect on mycotoxin development in stored grain.

2.4.4 Other factors contributing to mycotoxin development

It is suspected that substrate, gas atmosphere, pH, and amount of foreign material all have an effect on the development of mycotoxins in stored grain (Pitt 1995a). However, there is only a small amount of research published pertaining to the effect of these factors. Abramson et al. (1980, 1990, 2005) have performed several experiments on several different grain types and reported that "substrate plays a significant role in mycotoxin development", but did little to further elucidate on this topic. Pitt (1995a) reported that changing the gas atmosphere by increasing CO_2 or N_2 levels does have an effect on mycotoxin development but "more research is needed" in this area. As well, Cairns-Fuller et al. (2005) have determined that a gas atmosphere of 50% CO_2 is required to inhibit growth and OTA production by *P. verrucosum* in moist grain.

Another factor in mycotoxin development that has been identified but not researched is the effect of microbial interactions. If in fact mycotoxins are produced as a mechanism

to increase mould species competitiveness then these effects may prove to be very important. However, based on current literature it is apparent that in order to fully understand the interactions of all the variables that contribute to mycotoxin development more research is needed.

2.5 Post Harvest Operations

2.5.1 Storage

The importance of grain storage in the stability of the world's food supply cannot be overstated. At any given time, approximately half of the world's grain production is in storage (Jayas et al. 1995). Often the time between harvest of the grain and consumption is considerable and without effective storage techniques the quality of the grain would quickly deteriorate. There are many abiotic and biotic factors affecting grain storage. When all of the interrelations of these factors are considered the study of grain storage becomes a study of a complex ecological system or in other words a stored grain ecosystem.

The goal in grain storage is to control the variables within the stored grain ecosystem in such a way as to preserve the life of the grain. Moisture content and temperature have been found to be two of the most critical factors when it comes to grain storage. In general, cool, dry grain spoils more slowly than moist, warm grain. Therefore, drying and cooling are two post harvest operations that are critical to effective grain storage.

2.5.2 Drying

Drying is one of the most important techniques used in the preservation of grains. The goal of drying is to lower the m.c. of the grain to a level that is suitable for safe storage

thus preventing spoilage from occurring. Safe storage guidelines have been developed for many cereal grains (Friesen and Huminicki 1987; Udayakumar 2008). However in practice, the choice of drying method is highly dependent upon economic variables. There are many methods available for grain drying ranging from low to high technology methods such as solar drying to microwave drying, respectively (Mujumdar and Beke 2003). Heated air drying and near ambient air drying are the two most prevalent grain drying methods utilized in Western Canada.

Heated air drying is the process of heating ambient air to high temperatures, usually "in the range of 40-120ºC but in some cases as high as 275ºC" and passing through the moist grain (Nellist and Bruce 1995). Due to the increase in temperature, the RH of the air decreases to a level that is well below saturation and as the air passes through the intergranular space within the grain it draws the moisture off the grain. On small farms heated air drying is achieved by having a supplemental heat source connected to the bin aeration/drying system and is a deep bed drying process. On larger farms or commercial elevators heated air drying is achieved using dedicated dryers and a thin layer drying process. Heated air drying is an effective method of decreasing the m.c. of wet grain but has the following potential negative consequences if not managed properly: loss of grade through over heating and shrinkage, loss of profit due to high input costs, and potential of fire.

Near ambient air drying is the process of passing ambient air through a wet grain bulk when the RH of the incoming air is less than the ERH at the given m.c. As the air passes over the moist grain it draws moisture off the grain until equilibrium is reached. This process is much slower than heated air drying but requires less capital cost to implement

and has lower operating costs. Near ambient air drying is normally a deep bed drying process.

2.5.3 Deep-bed drying

Deep-bed drying is generally used for on farm drying. The drying system consists of a bin with a perforated floor attached to a fan with or without a supplemental heat source. Underneath the perforated floor is a void space or plenum which acts to produce a fairly uniform pressure front over the entire area of the perforated floor. The grain bulk to be dried rests on top of the perforated floor and air is either forced through the grain bulk from the bottom to the top or drawn from the top to the bottom. Vertical airflow up through the grain bulk is the more common method.

Deep-bed drying occurs through the following process. Air enters into the bottom of the grain bulk and absorbs moisture from the grain until the ERH is reached. This moisture transfer occurs in a finite zone termed the moisture front. Once the air has left the moisture front it continues up through the grain bulk generally at the ERH. If there are drier sections of the grain above the moisture front then the air will lose moisture to these sections bringing the grain bulk above the moisture front to very uniform m.c.

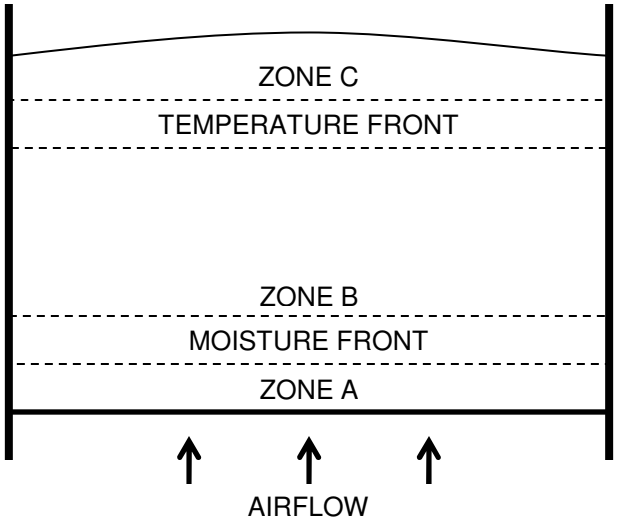

Figure 5. A grain bin dried with air at a uniform flow rate, and constant temperature and relative humidity (Sanderson 1986).

Once the moisture front is established it moves through the grain bulk in the direction of the air movement. It is the goal of deep bed drying to move the moisture front through the top of the grain before spoilage occurs. The speed at which the moisture front moves through the grain is dependent upon the volumetric airflow through the bulk and the RH of the air. Due to the energy requirements, and thereby increased costs, the rate of drying is usually kept at a minimum level to prevent spoilage. Hence, accurate safe storage guidelines are required by deep bed drying operators to ensure that the drying rate selected is appropriate for the initial grain conditions.

2.5.4 Temperature control

Once in storage, a grain bulk represents a significant thermal mass. It would take a great deal of energy to significantly change the temperature of that mass from that of the ambient air temperature. However, due to the large amount of intergranular air spaces within the stored grain bulk, simply passing ambient air through the grain bulk at low

volumes, ~1 (L/s)/m^3, has proven to be an effective method of maintaining uniform temperatures throughout. In climates, where there are cooler temperatures during harvest season, e.g., Western Canada, temperature control is usually not a problem. However, in warmer climates, e.g., Southern USA, temperature control becomes a difficult problem to mitigate and moisture control becomes much more critical.

2.5.5 Safe storage guidelines

The term "safe storage guideline" is not absolute for two main reasons. The first reason is that it is a guideline based on numerous experiments. But as stated earlier the stored grain ecosystem is complex and it is difficult to determine the combined effect of all the variables. Therefore, the guidelines are effective in the majority of cases but not all. The second reason is that the end use of the grain needs to be known for a "safe" level of spoilage to be determined. For instance, grain that is no longer useful for human consumption may be perfectly adequate for animal consumption, or grain destined for foreign markets may have different requirements than grain used in domestic markets. In both of these cases the term "safe" is relative to the intended use. However, most of the studies that have been conducted have used the metric of grain intended for human consumption as the "safe" level. Since the requirements on this grain are the most stringent "safe storage guidelines" usually encompass all of the required categories.

Current safe storage guidelines typically are based on the following grain quality parameters: CO_2 levels, Fat Acidity Value (FAV), germinability, presence of visible mould, and the presence of invisible mould (Friesen and Huminicki 1987; Udayakumar 2008). Mycotoxin development was never considered in the development of the current

guidelines and therefore they need to be evaluated to determine if they prevent mycotoxin development.

2.6 Quality Assessment Parameters

Determining if stored grain is spoiling has been the subject of intensive research over the past 50 years. However, the term quality grain does not have an absolute meaning, for example grain that has low protein levels and high starch levels may be good for malting while not so good for baking. In general the following quality assessment parameters have been accepted as reliable indicators of spoilage: germination, fat acidity values (FAV), CO_2 levels, odour, presence of visible moulds, and more recently the presence of mycotoxins. The quality assessment parameters used in this study included: germination, FAV, and the presence of mycotoxins.

2.6.1 Germination

Germination is a measure of the capability of a grain seed to develop into a plant and in the context of grain storage it is a measure of the viability of the stored product. It has been found that the ability of a seed to germinate is very sensitive to spoilage and therefore, germination can be used as a reliable indicator of spoilage (Nellist and Bruce 1995). In general, stored products that have germination values greater than 90% are considered sound (Metzger 1981). Germination values decrease as the amount of spoilage increases.

2.6.2 Fat acidity value

Deteriorative changes in grain may be produced by either an oxidative process or a hydrolytic process. A sound grain kernel is not readily susceptible to the negative effects

of oxygen within the air and therefore oxidative deterioration is not a significant problem in stored grain. However, during storage the fats in grains are readily broken down by lipases into free fatty acids (FFA) and glycerols. Microorganisms in the grain increase the rate of lypolytic activity thereby increasing the rate of FFA production. Wallace et al. (1983) reported that there is a positive correlation between the presence of microorganisms and FFA and that FFA can be used as a reliable indicator of deterioration.

The amount of FFA present in a grain sample is determined by measuring how much base, usually KOH, is required to neutralize all the acid present. The amount of KOH required to neutralize the FFA in a 100 g of dried sample is referred to as the FAV. The relative change in the FAV can be correlated to spoilage in grain, with higher FAV being positively correlated with greater degree of spoilage (Sinha 1983).

2.6.3 Mycotoxins

In the past, the presence of mycotoxins was not used as grain quality parameter. The presence of mycotoxins has become a grain quality parameter with the introduction of maximum limits on the allowable levels of mycotoxin being imposed by international agencies. From a measurement perspective the definition of quality in this area is very simple; the grain is within the allowable limit or it is not. The difficulty in measuring this parameter is more one of obtaining a sample which is representative enough of the grain bulk for the testing to accurately represent the required detection limits which are in the 5 ppb range (FAO 2004).

2.7 Objectives

As a grain exporting nation it is incumbent upon Canada to ensure that its grain exports meet all foreign trade requirements. It can be seen from the variety of literature on the subject that the development of mycotoxins is dependent on many factors that are not completely understood at this time. On farm grain storage and drying is a critical component of the Canadian grain distribution system and near ambient air drying is one of the principal methods used by farmers to dry their grain. Under the current near ambient air drying guidelines the top section of a grain bulk can remain at the original moisture content for up to 12 wk and it is unknown at this time if this top section of grain is in jeopardy of developing mycotoxins in excess of the current international allowable limit for ochratoxin of 5 ppb or 5 μg OTA kg^{-1} (FAO 2004; JECFA 1999). This study was undertaken to determine if the upper section of a bulk of durum wheat that is dried using the near ambient air drying guidelines is at risk of developing OTA.

3 MATERIALS AND METHODS

3.1 Materials

The CWB provided 120 t of dry, ~12% m.c. (wb), durum wheat. This 120 t was comprised of 3 loads of 40 t. One each from Kindersley, Weyburn, and Moosejaw (Saskatchewan).

3.2 Equipment

The Canadian Wheat Board Centre for Grain Storage Research (CWBCGSR) is a world class facility that provides researchers the equipment to study many aspects of grain storage. The facility can perform the following functions: drying, cleaning, conditioning, weather simulation, and storage.

3.2.1 Storage

Storage is achieved within the research centre using seven bins designated BN01 to BN05, SB01, and SB02 (Figure 6). Each type of bin performs a unique role within the research centre.

BN01 and BN02 – These bins, located within the main room of the research lab, are round, smooth walled, steel, hopper bottom bins each with 90 m^3 of capacity. Both of these bins have aeration fans and are connected to the dust collection system. BN01 is mounted on load sensors and is normally used as the primary receiving bin. The primary function of these bins is to provide storage of material to be used in experiments, however BN01 has been retrofitted with several samples ports which allows for it to be used for storage experiments.

BN03, BN04, and BN05 - These bins are round, smooth walled, steel, hopper bottom bins each with 30 m^3 of capacity. They are all located within the Simulated Weather Lab of the CWBCGSR, share a common aeration fan, and are connected to the dust collection system. A unique feature of these bins is that they can have an aeration floor inserted into them at the transition to the hopper bottom which allows them to be used either as flat bottom or hopper bottom bins. These bins are the primary research bins within the research centre.

SB01 and SB02 – These bins are constructed as rectangular bins with corrugated steel side walls and smooth wall hopper bottoms. They have a capacity of 10 m^3 each and are located on the roof with the hoppers extending through the roof into the main room of the CWBCGSR. These bins have no aeration or dust collection and their primary function is to provide surge capability when material is being fed to either the grain conditioner or the grain dryer.

3.2.2 Environmental control

The Simulated Weather Lab of the CWBCGSR was designed to provide researchers with the ability to simulate the climate of any location and any year. The initial design called for temperature control from -50ºC to +50ºC with relative humidity control from 20% to 95%. Temperature is controlled using two refrigeration systems and one heating system. The RH is controlled using steam to increase the RH and a steam operated wheel style dehumidifier to decrease RH. During the course of this study the dehumidification system was inoperable.

The CWBCGSR also houses 4 stand alone environmental chambers, (Conviron, Controlled Environments Limited, Winnipeg, MB). These chambers allow researchers to

conduct small scale experiments with a greater degree of temperature and RH control than the larger Weather Simulation Lab. These chambers do not have a mechanism for reducing RH other than by increasing the temperature and therefore are not useful for low RH experiments during the summer months.

3.2.3 Distribution system

The distribution system within the CWBCGSR is comprised of two 60 tph bucket elevators with dual 8 spout swing flow distributors capable of delivering grain to any one of the 7 bins and the grain cleaning system. Reclaim out of the bins is achieved using belt conveyors (Figure 6) feeding into the bucket elevators.

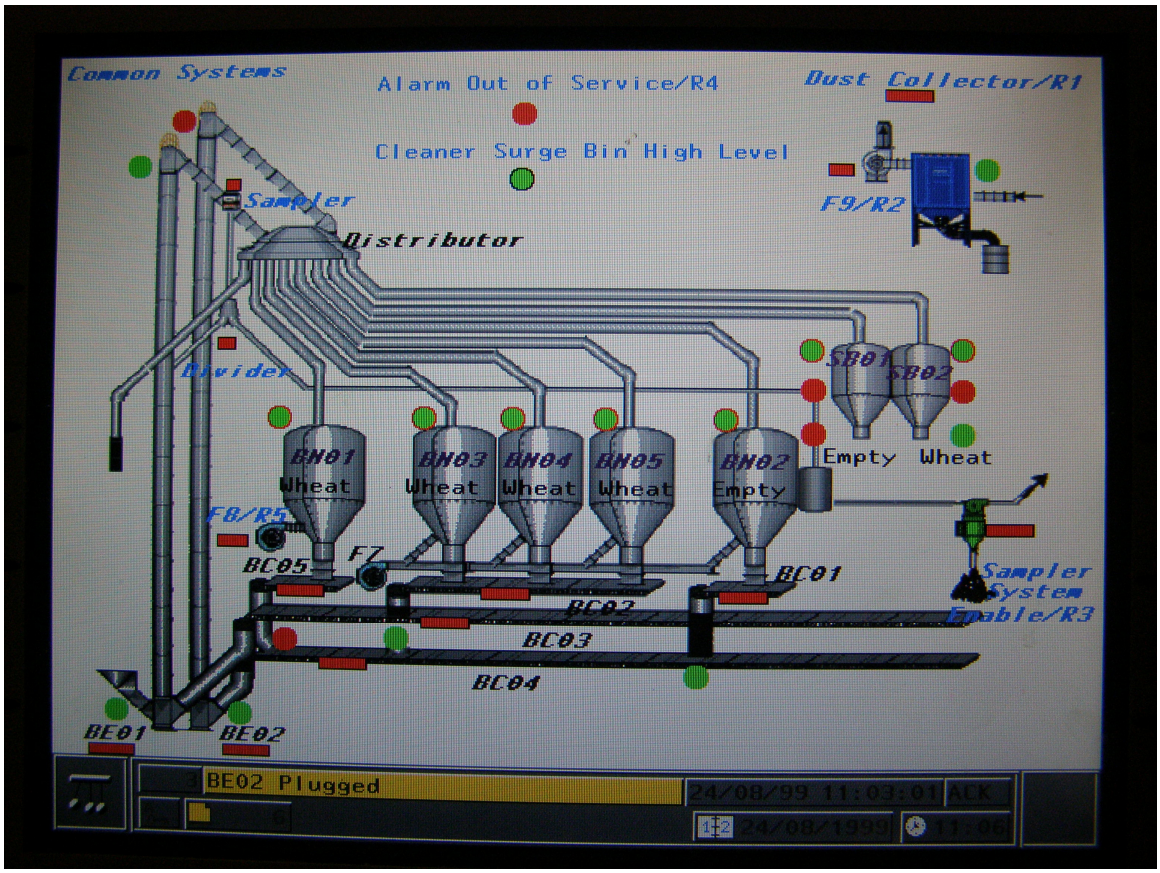

Figure 6. Flow diagram of CWBCGSR distribution system.

3.2.4 Grain conditioner

The grain conditioner is composed of a screw conveyor with mixing paddles (Figure 7). As material passes thorough this conveyor heated water at approximately 65°C is sprayed onto the grain. The amount of water added can be controlled automatically by setting the desired m.c. of the outgoing grain into the control system or by manually setting the opening of the water valve. It has been found that the grain conditioner can raise the moisture content of wheat in a single pass by a maximum amount of 4% (wb).

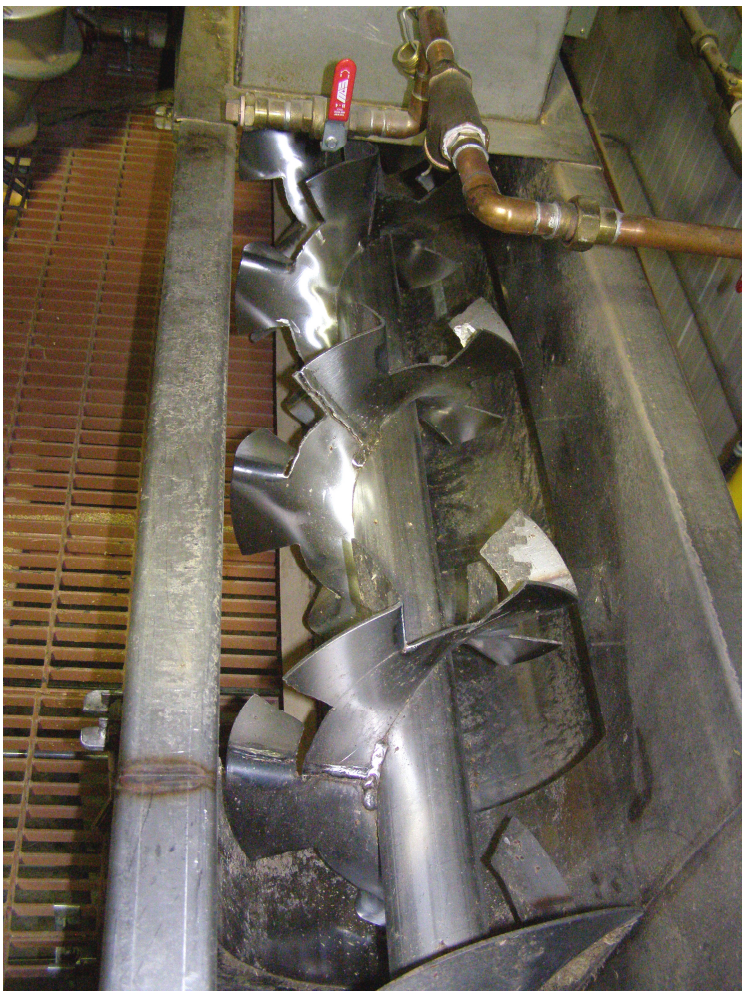

Figure 7. Grain conditioner screw conveyer with mixing paddles.

3.3 Bin Study Experimental Design and Procedure

3.3.1 Experimental design

The purpose of the bin study was to simulate what occurs in the uppermost layer of a stored grain bulk when dried using near ambient air during a wet drying season to determine the following:

1) Is grain dried using the current near ambient air guidelines at risk of developing mycotoxins in excess of the current international allowable limit of 5 ppb; and
2) Does airflow have an effect on the spoilage rate of grain when the air is not providing drying.

During a wet drying season the uppermost layer of grain would stay moist and be exposed to high humidity airflow for up to 12 weeks.

The Bin Study was to be conducted over three separate trials designated Bin Study 1, Bin Study 2, and Bin Study 3. The experimental design was a completely randomized block design comprised of 3 treatments, *Airflow*, *Airflow Inoculated*, and *No Airflow* each with 3 replicates run concurrently over a 12 week period. The Airflow Inoculated treatment was included to guarantee that mycotoxin producing fungal strains were present in the grain bulk during the storage period. All quadrants were sampled according to the testing parameters detailed in Table 4.

Table 4. Parameters, frequency, and testing protocols used for Bin Studies.

Parameter	Frequency	Testing Protocol
Seed germination	Weekly	Wallace and Sinha (1962)
Moisture content	Weekly	ASAE (2003)
FAV	Weekly	AACC (1962)
OTA*	Weekly	HPLC (Appendix 4)

*OTA detection limit of 10 ppb.

The experimental design called for statistical analysis to be conducted on seed germination, FAV, moisture content and OTA levels. However, OTA test results indicated that statistical analysis would provide no additional value and therefore it was not conducted on OTA test results.

3.3.2 Experimental procedure

3.3.2.1 Construction of experimental apparatus

The experimental apparatus consisted of BN03, BN04, and BN05 within the Weather Simulation Lab. These bins were divided into 4 equal quadrants each with an approximate volume of 1 m^3 (Figure 8).

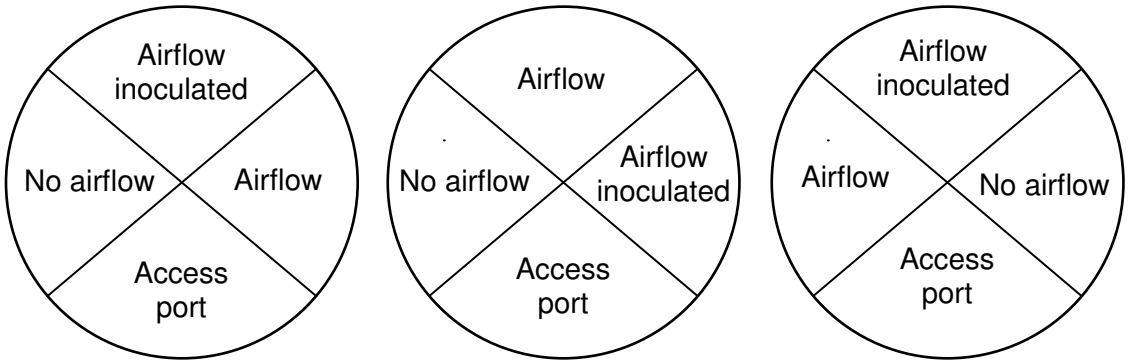

Figure 8. Schematic diagram of experimental setup within the bins for Bin Study 1.

The quadrants were divided by a wooden structure and two of the quadrants, *no airflow* and access port, were lined with 6 mil poly along the floor to prevent airflow from passing through (Figure 9). The access port quadrant was used as an area for collecting samples from the other three quadrants.

Figure 9. Wooden divider within experimental bins.

Once the experimental apparatus was constructed a disinfectant solution comprised of water and a 5% sodium hypochlorite bleach mixed at a 4:1 ratio was sprayed on the following equipment: inside of BN03, BN04, BN05, bucket elevators, and the belt conveyors. It should be noted that the inside of the bin walls were only sprayed to a height of approximately 3 m. This was as high as a person could easily reach with the spraying equipment and it was believed that since the airflow was travelling up and out of the bin there was no real danger of mycotoxin contamination moving down onto the grain. The perforated bin floor was also sprayed but the hopper bottom was not accessible and therefore was not sprayed.

Air was provided to the bins using a common 30 hp fan controlled by a variable frequency drive (VFD). The VFD allowed the operator to control the speed of the fan. The fan is located within the Weather Simulation Lab and draws air from within the lab and forces it up through the perforated floors of the bins. Once through the grain bulk 90% of the air is recirculated back into the chamber. To avoid cross contamination between the bins by the recirculated air a two stage filter system was constructed and fitted to the inlet of the fan. The two stage filter system was comprised of a large first stage particle filter with a MERV 8 rating (3.0-10 μm), followed by a second stage cartridge style HEPA filter with a filtration size of 0.3 μm.

3.3.2.2 Environmental control

Temperature and RH were controlled using the control system of the Weather Simulation Lab. Based on historical data, 1969 was one of the wettest drying years on record. Therefore, to simulate drying conditions during a wet year the weather simulation lab was programmed to provide temperatures and RH similar to those of 1969. Environment Canada data were obtained and the temperature and RH at 6 h intervals were tabulated (Appendix 1) and programmed into the Weather Simulation Lab control system on a weekly basis.

To monitor the effectiveness of the temperature and RH control, remote sensors using HOBOware U10-003 data logger (Onset Computer Corporation, Bourne, MA) were placed in the following locations: 2 at fan inlet (1 primary and a backup), 1 in each grain quadrant buried approximately 15 cm below the grain surface. These remote sensors were programmed to take a data reading every 30 min for the length of the trial.

3.3.2.3 Sample preparation

Durum wheat was received into BN01 and BN02. First 40 t into BN01, the second 40 t into BN02, and the third 40 t split 20 t each into BN01 and BN02. As the grain was received into the bins, 100 g samples were obtained every 5 min by passing a small cup through the grain flow. These samples were then mixed together and the initial m.c. of the grain was measured to be approximately 12.5% (wb) using the hot air oven method by drying 10 g of unground sample, in triplicate, at 130ºC for 19 h (ASAE 2003). The grain was then circulated, 50% flow from each of BN01 and BN02 into filling BN03, BN04, and BN05. Then using ¼ flow from BN03, BN04, and BN05 along with ¼ from BN01 back into BN02 until full then back into BN01 until full. This process was carried out 2 times to completely mix the grain. Once the mixing of the grain was complete, 90 t was transported out to the Agriculture and Agri-food Canada Glenlea Research Farm for storage. The remaining 30 t was stored in BN01 at approximately 12.5% m.c. (wb) until the experimental apparatus was set up. Based on the volume of each quadrant it was determined that 7 t of 12.5% m.c. (wb) grain would need to be conditioned to 20% m.c. (wb) for each trial.

3.3.2.3.1 Grain conditioning

The grain conditioning equipment of the CWBCGSR can add up to four percentage points moisture to wheat in a single pass. Therefore, two passes through the equipment were required to condition the experimental grain up to 20% m.c. (wb). The following procedure was used to condition the grain and start the experiment:

Step 1) Grain moved from BN01 to SB01

Step 2) Grain moved from SB01, through conditioner with water valve 75% open, to SB02

Step 3) Grain tempered in SB02 for 24 h

Step 4) Grain moved from SB02 to SB01

Step 5) Grain tempered in SB01 for 24 h

Step 6) Grain moved from SB01, through conditioner with water valve 75% open, to SB02

Step 7) Grain tempered in SB02 for 24 h

Step 8) Grain moved from SB02 into experimental bins

Step9) Grain allowed to sit for 24 h in experimental bins at 5ºC, prior to starting fan for experiment

Step 10) Started fan and measured airflow through quadrants. Adjusted airflow using VFD until an airflow of approximately 10 (L/s)/m^3 was reached in each *airflow* and *airflow inoculated* quadrant

3.3.2.3.2 Preparation and application of inoculant

Penicillium sp. was isolated from a mould culture taken from a sample of experimental grain that had been allowed to spoil, and sent to the Canadian Grain Commission (CGC) for positive identification as a toxic strain of *P. verrucosum*. The CGC provided a pure inoculum which was multiplied on agar in Petri plates. Fungal spores were removed from Petri plates by washing into a beaker with a squeeze bottle containing sterile distilled water and a few drops of the surfactant Tween 80; the surface of the agar was gently scraped with a small flat spatula to remove embedded fungi. The

slurry of water/fungi was made up to about 1 L with additional water and 0.3 L of the mixture was sprayed onto the grain in each *airflow inoculated* treatment quadrant.

3.3.2.4 Sampling procedure

On a weekly basis grain samples of approximately 400 g each were collected from each quadrant using a torpedo type sampler. The order of bin sampling, treatment sampling, and sample location within the quadrant were randomized by the person conducting the sampling. After being drawn, the samples were put into plastic freezer bags. On the day of sampling, germination and moisture content tests were initiated. The remainder of the sample was stored in a freezer for FAV testing and mycotoxin testing which were done at a later date.

To minimize the possibility of cross contamination during sampling the torpedo probe was sanitised with isopropyl alcohol on both the inside and outside surfaces between all samples. The probe was air dried for 1 min after sanitation to allow the isopropyl alcohol to evaporate prior to taking the next sample.

3.3.2.4.1 Mycotoxin rapid test kit

To provide preliminary mycotoxin testing results, ochratoxin detection in wheat samples was carried out by ELISA test using RIDASCREEN FAST OTA testing kits. The basic principle of this test is the antigen-antibody reaction. The microtiter wells in this testing kit are coated with capture antibodies directed against anti-OTA antibodies. The ochratoxin standard solutions (0, 5, 10, 20 and 40 ppb) and sample solutions were added with OTA conjugate and anti-OTA antibodies in the wells. The free OTA and OTA conjugate competed for OTA antibody binding sites and unbound enzyme

conjugate was removed by the washing step. Then chromogen was added to the wells, the bound enzyme conjugate converted the chromogen into a blue color and addition of stop solution changed the color from blue to yellow. The absorbance of the material was measured by spectrophotometer at 450 nm. The absorbance is inversely proportional to the ochratoxin A concentration in the sample.

3.4 Chamber Study Experimental Design

The Chamber Study was proposed to provide a convenient method for testing the same hypothesis as the Bin Study. The testing parameters used in the Chamber Study are outlined in Table 4. The Chamber Study consisted of only two treatments, *airflow* and *no airflow*, with 3 replicates run concurrently. Statistical analysis using the t-test was conducted on seed germination and FAV results.

3.4.1 Construction of experimental apparatus

This experiment was conducted in three wooden structures, each with its own fan. Each structure had two compartments, each with a volume of 20.25 L (Figure 10). One of these compartments had the bottom sealed off with a piece of wood so that one compartment received no airflow from the fan and the other did not. All three wooden structures were placed in a single Conviron environmental chamber (Conviron, Winnipeg, MB) for the duration of the trial.

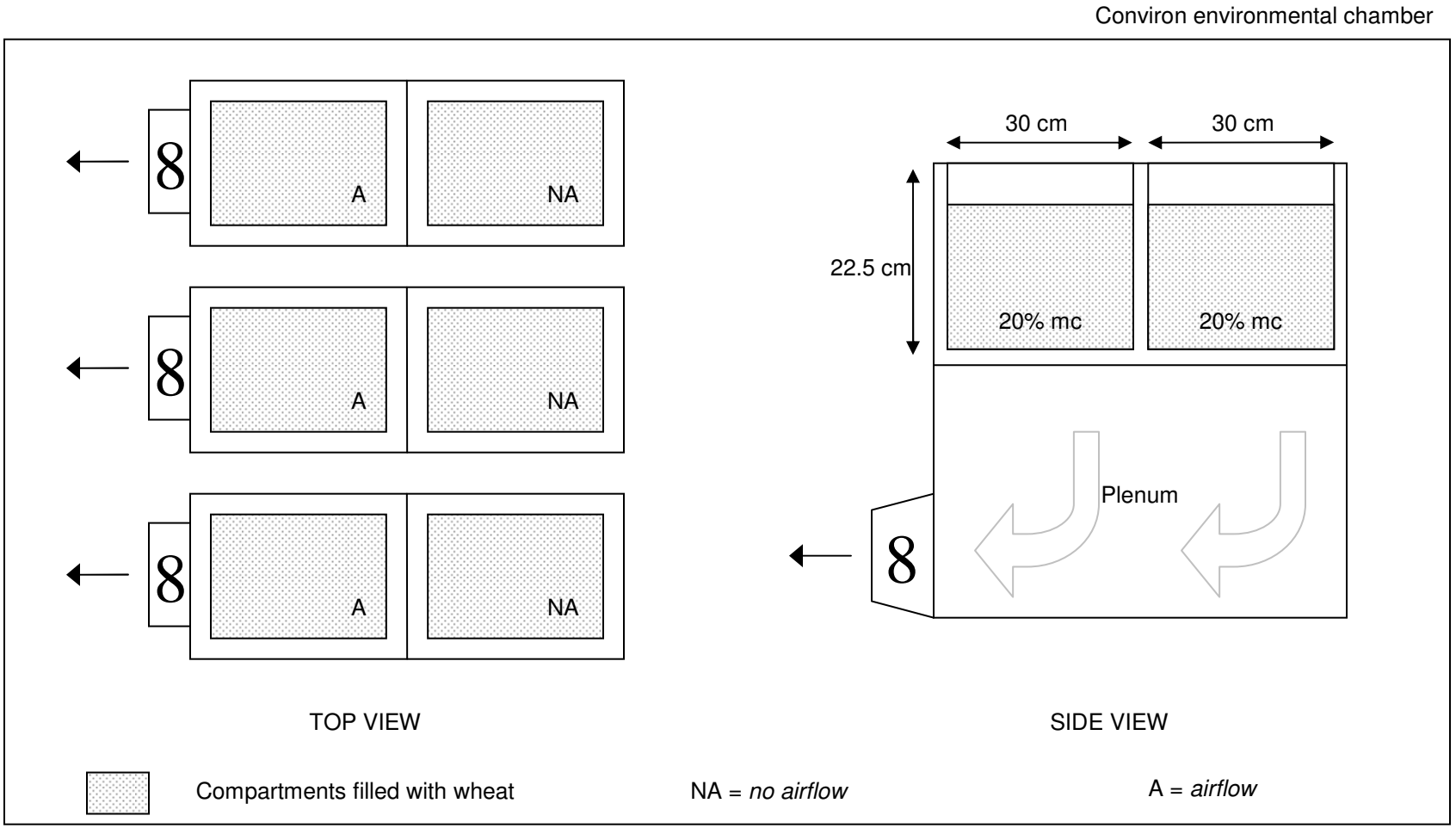

Figure 10. Experimental setup for Chamber Study.

3.4.2 Sample preparation

Ten kilograms of durum wheat were taken from the grain stored in BN01. The initial m.c. of this wheat was determined using the hot air oven method (ASAE 2003). The grain was then conditioned to 20% ± 0.2% m.c. (wb) by adding and mixing a calculated quantity of distilled water; the sample was stored in a plastic bag in a freezer at -5ºC ± 2ºC for 72 h. Prior to use the grain sample was mixed thoroughly and the final moisture was determined by the hot air oven method.

3.4.3 Sampling procedure

On a weekly basis grain samples of approximately 150 g each were collected from each treatment using a 45 cm nickel plated trier. As with the Bin Study, the order of treatment sampling, and sample location within the treatment were randomized by the person conducting the sampling. After being drawn the samples were put into plastic freezer bags. On the day of sampling, germination and moisture content tests were initiated. The remainder of the sample was stored in a freezer for FAV testing and mycotoxin testing which were done at a later date.

To minimize the possibility of cross contamination during sampling the trier was sanitised with isopropyl alcohol on the both the inside and outside surfaces between all samples. The probe was air dried for 1 min after sanitation to allow the isopropyl alcohol to evaporate prior to taking the next sample.

Each treatment box was filled with a total grain volume of 18 L. The temperature settings of the Conviron chamber were as shown in Table 5.

Table 5. Temperature settings of Conviron environmental chamber.

Time (24 hour clock notation)	Temperature (ºC)
0:00	0
4:00	10
8:00	15
12:00	20
16:00	15
20:00	10

The RH of the chamber was held constant at 85% and the experiment was run for 12 wk. The airflow was measured to be approximately 25 (L/s)/m^3 using a hot wire anemometer (Model TA 35, TOPAC, Cohasset MA, USA) through the grain. This value is an approximation because it was difficult to obtain a steady reading.

4 RESULTS

4.1 Bin Study 1

4.1.1 Environmental data

The weather data from Environment Canada (Appendix 1) were used to program the temperature and RH set points for the Weather Simulation Lab (Figure 11).

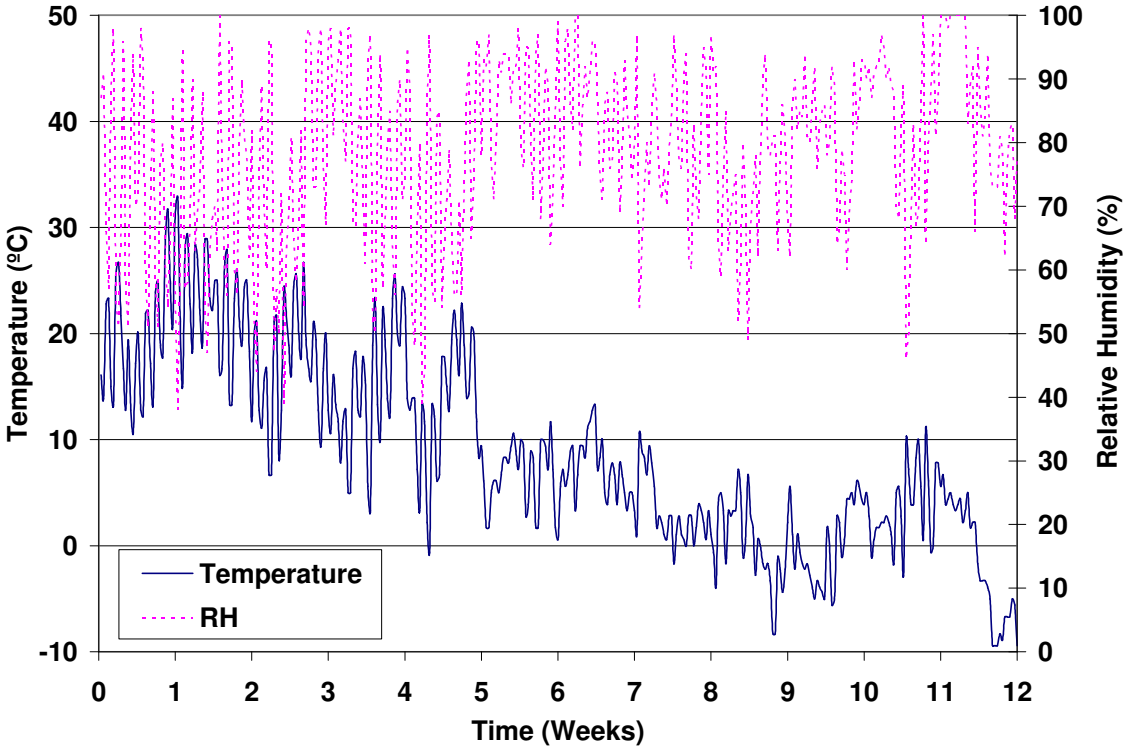

Figure 11. Environment Canada weather data from 1969 Aug 15 to 1969 Nov 15 used to program Weather Simulation Lab.

Figure 12 shows the temperature and RH of the air in the environmental chamber as it entered the fan, measured with HOBO data collectors placed at the fan inlet.

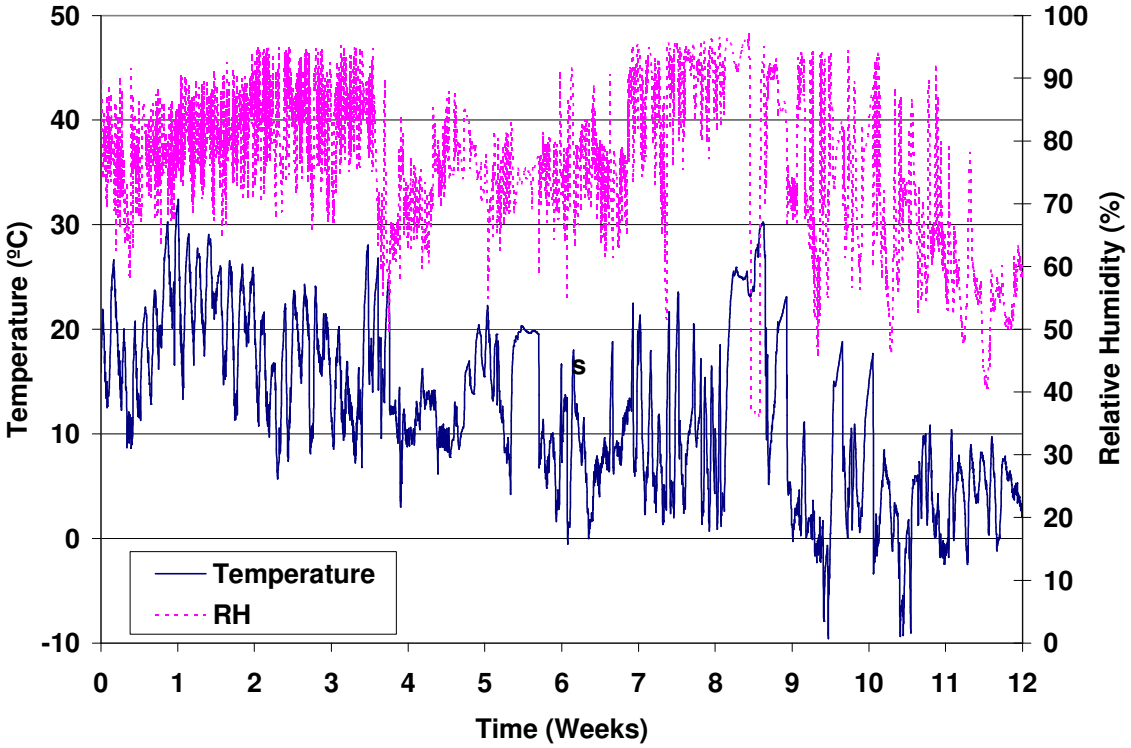

Figure 12. Temperature and RH at fan inlet to BN03 recorded at 30 min intervals.

HOBO data collectors were also placed within each of the grain bulks (*no airflow*, *airflow*, and *airflow inoculated* for each of BN03, BN04, and BN05) to measure the temperature and RH of the air within the grain bulk. The data collected for the three sections of BN03 are displayed in Figures 13, 14, and 15, respectively.

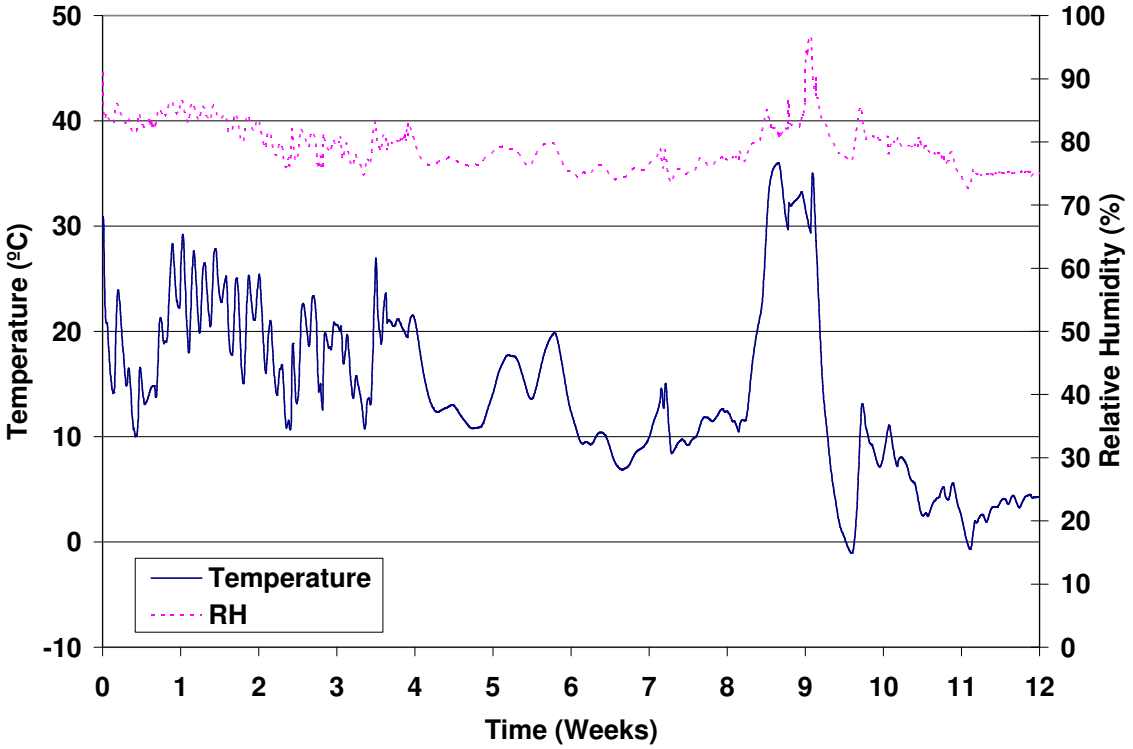

Figure 13. Temperature and RH for *no airflow* treatment in BN03 measured at 30 min intervals.

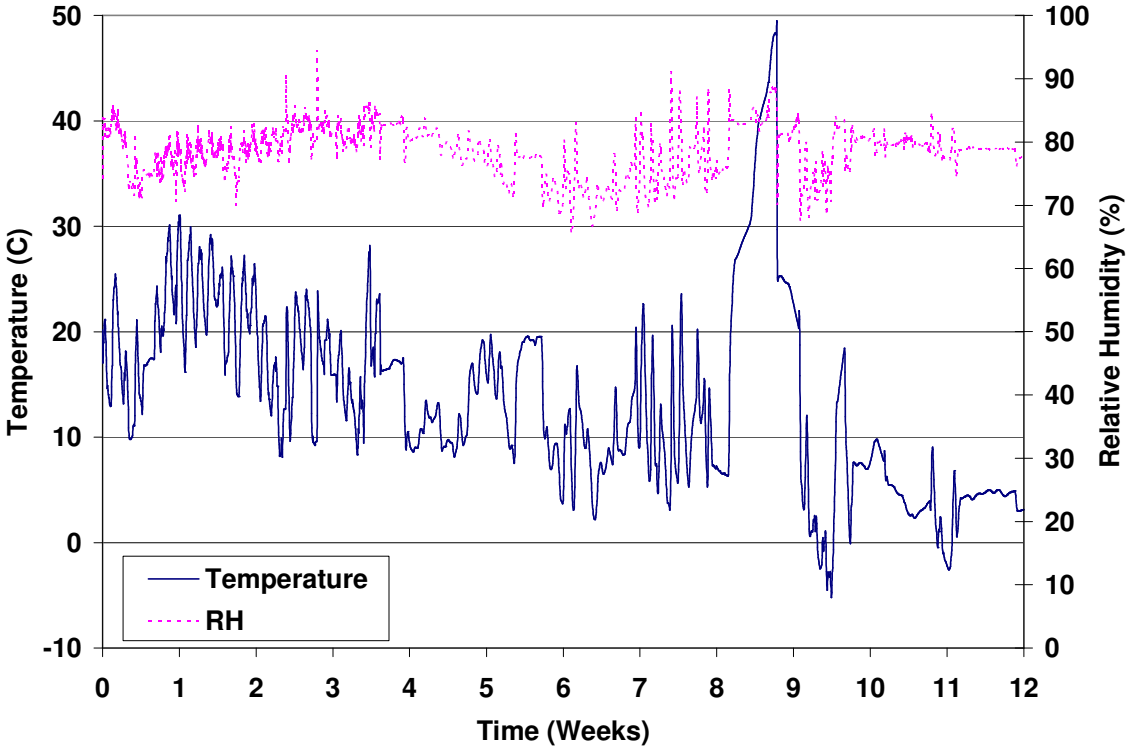

Figure 14. Temperature and RH for *airflow* treatment in BN03 measured at 30 min intervals.

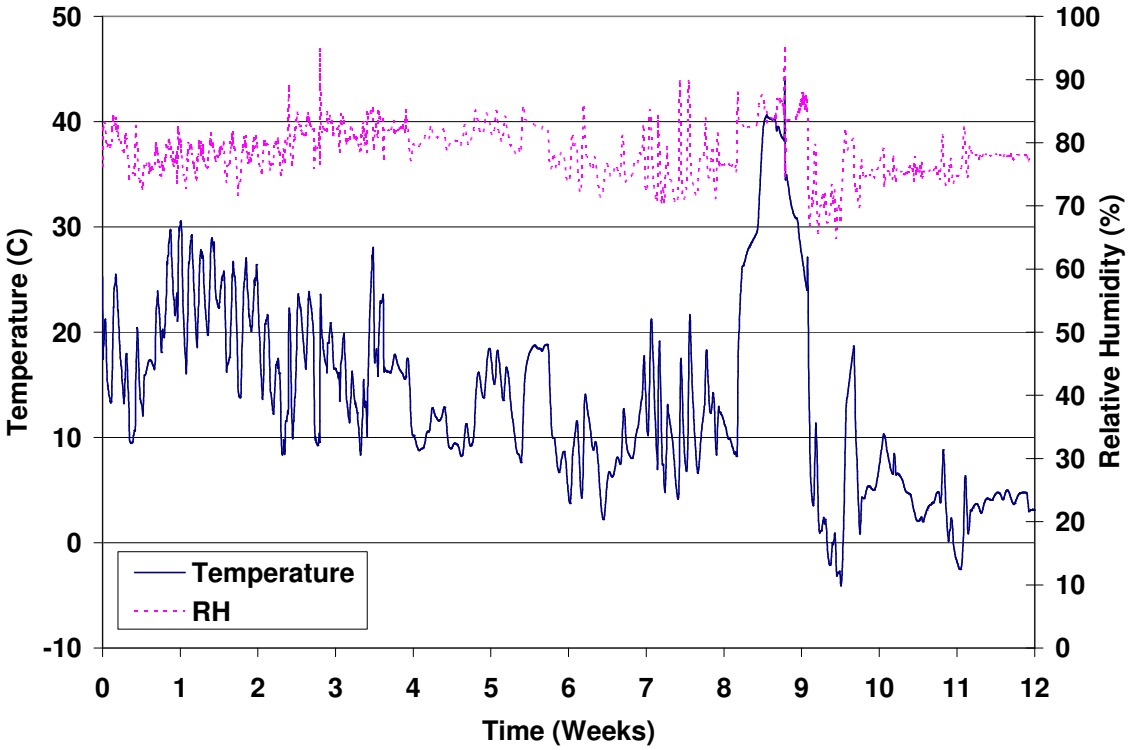

Figure 15. Temperature and RH for *airflow inoculated* treatment in BN03 measured at 30 min intervals.

The temperature within the Weather Simulation Lab closely followed the temperature pattern of the year 1969 except for a temperature spike which occurred during week 8 (Figure 12). This temperature spike was the result of the refrigeration coils icing up and not being detected right away. Once detected, a defrost cycle which was run to prevent the cooling coils from icing up. The Weather Simulation Lab was unable to maintain RH values in line with those of the year 1969, when the RH was regularly above 90% (Figure 12) but only above 90% infrequently in the Weather Simulation Lab (Figure 15).

4.1.2 Germination

Germination of wheat kernels at different times for different treatments is shown in Figure 16. Germination was approximately 90% for all three treatments in the start of the

experiment and reduced to less than 15% for *no airflow* treatment samples from the second week onwards.

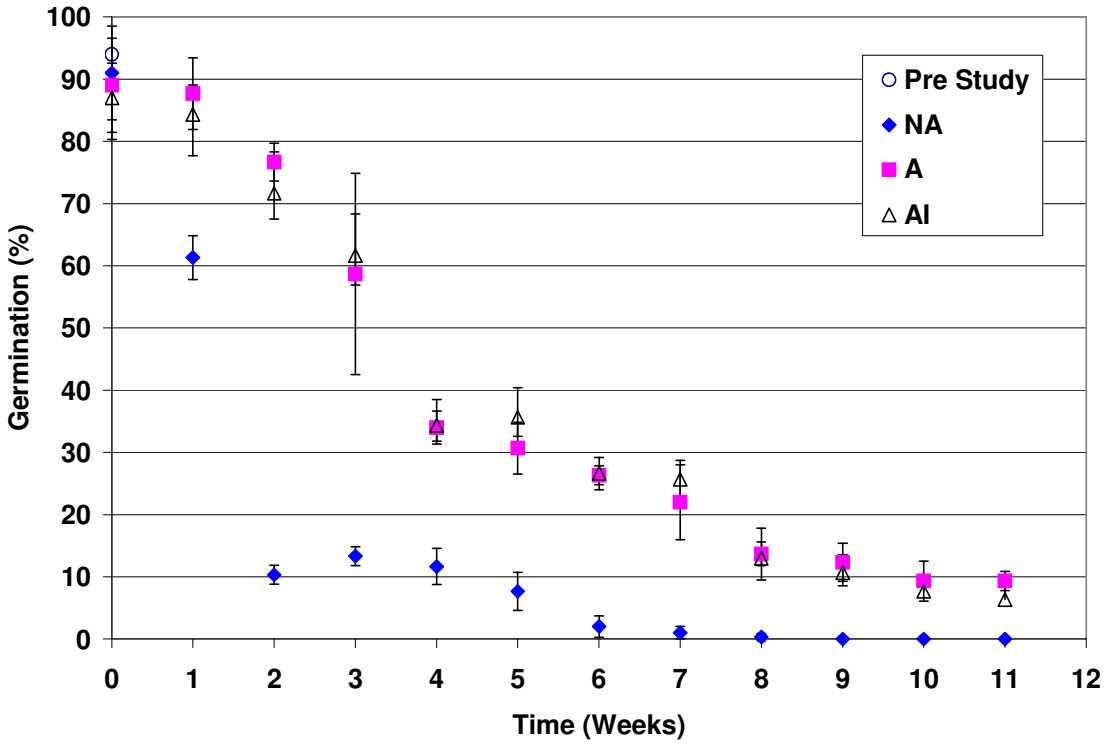

Figure 16. Germination of wheat kernels before the beginning of the study and for *no airflow* (NA), *airflow* (A), and *airflow inoculated* (AI) treatments for Bin Study 1.

Table 6. Comparison of germination for *no airflow (NA)* vs. *airflow* (A) treatments for Bin Study 1.

	Mean (Standard Deviation)		
Week	NA	A	P value (two tail)
0	91.0 (7.5)[a]	89.0 (7.5)[a]	0.7619
1	61.3 (13.5)[a]	87.7 (5.8)[b]	0.0066
2	10.3 (1.5)[a]	76.7 (5.8)[b]	<0.0001
3	13.3 (1.5)[a]	58.7 (16.2)[b]	0.0402
4	11.7 (2.9)[a]	34.0 (2.6)[b]	0.0006
5	7.7 (3.0)[a]	30.7 (14.2)[b]	0.0015
6	2.0 (1.7)[a]	26.3 (1.5)[b]	<0.0001
7	1.0 (1.0)[a]	22.0 (6.0)[b]	0.0268
8	0.3 (0.5)[a]	13.7 (4.2)[b]	0.0316
9	0.0 (0.0)[a]	12.3 (3.0)[b]	0.0198
10	0.0 (0.0)[a]	9.3 (3.2)[b]	0.0373
11	0.0 (0.0)a	9.3 (1.5)[b]	0.0088

[a]In a row numbers followed by the same character are statistically the same (P<0.05).

Table 7. Comparison of germination for *airflow* (A) vs. *airflow inoculated* (AI) treatments for Bin Study 1.

	Mean (Standard Deviation)		
Week	A	AI	P value (two tail)
0	89.0 (7.5)[a]	87.0 (5.6)[a]	0.7306
1	87.7 (5.8)[a]	84.3 (4.7)[a]	0.4822
2	76.7 (3.0)[a]	71.7 (6.7)[a]	0.4822
3	58.7 (16.2)[a]	61.7 (6.7)[a]	0.7857
4	34.0 (2.6)[a]	34.3 (4.2)[a]	0.9142
5	30.7 (4.2)[a]	35.7 (4.7)[a]	0.2411
6	26.3 (1.5)[a]	26.7 (2.5)[a]	0.8570
7	22.0 (6.0)[a]	25.7 (3.0)[a]	0.4151
8	13.7 (4.2)[a]	13.0 (2.6)[a]	0.8300
9	12.3 (3.0)[a]	10.7 (2.9)[a]	0.5300
10	9.3 (3.2)[a]	7.7 (1.1)[a]	0.4600
11	9.3 (1.5)[a]	6.3 (2.1)[a]	0.1145

[a]In a row numbers followed by the same character are statistically the same (P<0.05).

Germination of the *airflow* and *airflow inoculated* samples was always higher than that of *no airflow* treatment. The data were analyzed using the t-test procedure (Microsoft Office Excel v2003). There were no significant differences in germination between *airflow* and the *airflow inoculated* treatments ($P < 0.05$), but there were significant differences between the *no airflow* and *airflow* treatment ($P < 0.05$) (Tables 6 and 7).

4.1.3 FAV

Free fatty acid tests conducted in accordance with AACC 1962 were carried out for all samples (Figure 17). The initial FAV for the *no airflow* treatment sample was 5.4 and it increased to 45.8 at the end of the study (Table 8). FAV values for the *airflow* and *airflow inoculated* samples also increased, from 5.4 to 34.8 and from 4.8 to 34.6, respectively (Tables 8 and 9). There were significant differences between *airflow* and *no airflow* treatments in FAV values from the second week onward, but there were no significant differences between *airflow* and *airflow inoculated* samples throughout the study (Tables 8 and 9).

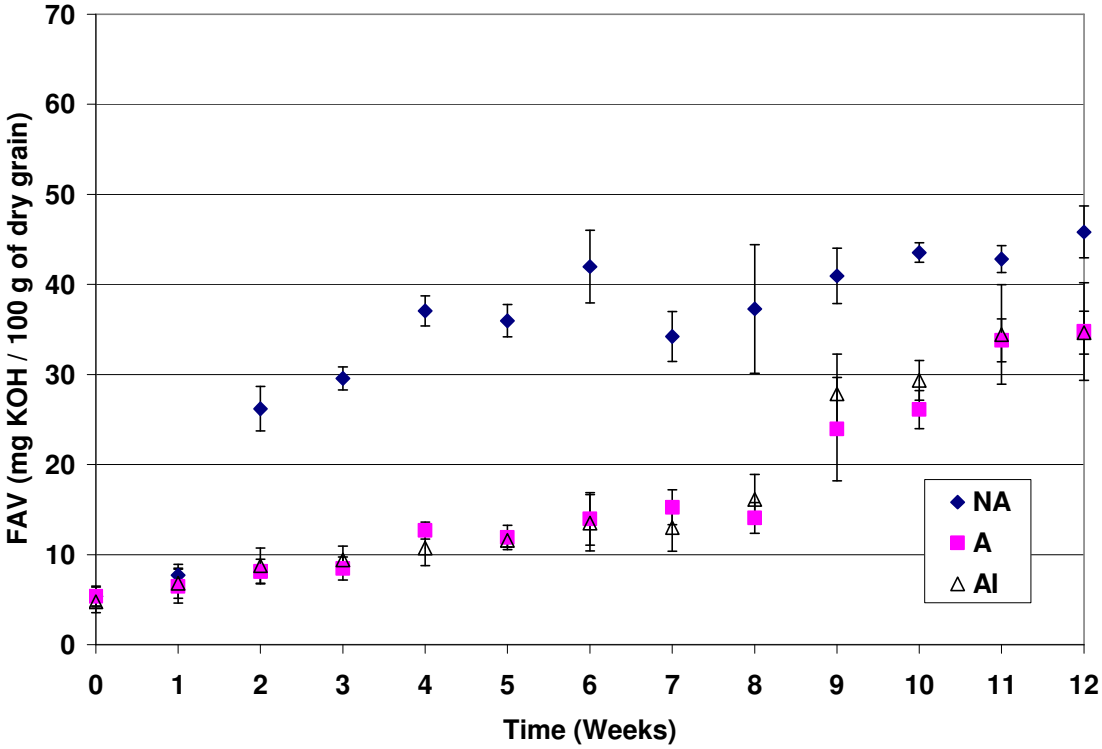

Figure 17. FAV of wheat kernels for *no airflow* (NA), *airflow* (A), and *airflow inoculated* (AI) treatments for Bin Study 1.

Table 8. Comparison of FAV for *no airflow* (NA) vs. *airflow* (A) treatments for Bin Study 1.

Week	Mean (Standard Deviation)		P value (two tail)
	NA	A	
0	5.4 (1.0)[a]	5.4 (1.1)[a]	1.0000
1	7.7 (1.2)[a]	6.5 (1.9)[a]	0.1347
2	26.2 (2.4)[a]	8.1 (1.4)[b]	<0.0001
3	29.6 (1.3)[a]	8.4 (1.3)[b]	<0.0001
4	36.0 (2.0)[a]	10.9 (1.7)[b]	<0.0001
5	36.0 (1.8)[a]	11.9 (1.3)[b]	<0.0001
6	42.0 (4.0)[a]	14.0 (2.9)[b]	<0.0001
7	34.2 (2.8)[a]	15.2 (1.9)[b]	<0.0001
8	37.3 (7.1)[a]	14.1 (1.7)[b]	<0.0001
9	41.0 (3.9)[a]	23.9 (2.2)[b]	<0.0001
10	43.5 (1.1)[a]	26.1 (2.1)[b]	<0.0001
11	42.8 (1.5)[a]	33.8 (2.4)[b]	<0.0001
12	45.8 (2.9)[a]	34.8 (5.4)[b]	0.0002

[a]In a row numbers followed by the same character are statistically the same (P<0.05).

Table 9. Comparison of FAV for *airflow* (A) vs. *airflow inoculated* (AI) treatments for Bin Study 1.

Week	Mean (Standard Deviation)		P value (two tail)
	A	AI	
0	5.4 (1.2)[a]	4.8 (1.2)[a]	0.3240
1	6.5 (1.9)[a]	6.8 (1.7)[a]	0.7056
2	8.1 (1.4)[a]	8.8 (1.9)[a]	0.4275
3	8.4 (1.3)[a]	9.4 (1.5)[a]	0.1666
4	10.9 (1.7)[a]	10.5 (1.2)[a]	0.5371
5	11.9 (1.3)[a]	11.6 (0.8)[a]	0.5446
6	14.0 (2.9)[a]	13.5 (3.1)[a]	0.7634
7	15.2 (1.9)[a]	13.0 (2.6)[a]	0.0543
8	14.1 (1.7)[a]	16.1 (2.8)[a]	0.0774
9	23.9 (2.2)[a]	27.8 (1.9)[b]	0.0011
10	26.1 (2.1)[a]	29.4 (2.2)[b]	0.0057
11	33.8 (2.4)[a]	34.4 (5.5)[a]	0.7524
12	34.8 (5.4)[a]	34.6 (2.4)[a]	0.9511

[a]In a row numbers followed by the same character are statistically the same ($P<0.05$).

4.1.4 Moisture content

Moisture contents of wheat samples at different times for all three treatments are shown in Figure 18. The initial m.c. was approximately 18.5% (wb). Due to problems with the mechanical systems in the Weather Simulation Lab of the CWBCGSR, the m.c. of some sections dropped below 17% (wb) (Figure 18). Moisture content of the *no airflow* treatment samples was approximately 16.8% (wb) at the end of study. The final m.c. of the *airflow* and *airflow inoculated* treatments was approximately 16.2% (wb).

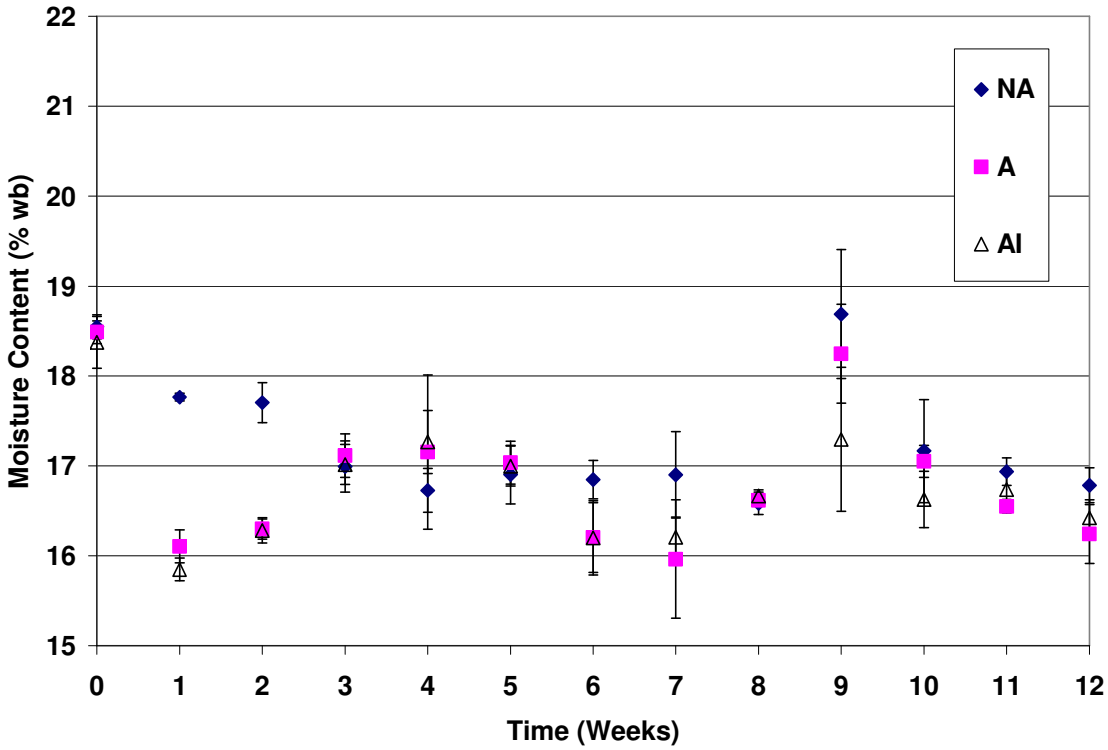

Figure 18. Moisture contents (wb) for *no airflow* (NA), *airflow* (A), and *airflow inoculated* (AI) treatments for Bin Study 1.

4.1.5 Mycotoxins

Rapid ochratoxin testing indicated that at week 12 only one treatment (BN03, *no airflow*) had ochratoxin present (Table 10).

Table 10. Rapid OTA test results for week 12 of Bin Study 1.

Sample	Ochratoxin level (ppb)
B3 Airflow	< 5
B3 Airflow Inoculated	< 5
B3 No Airflow	**> 40**
B4 Airflow	< 5
B4 Airflow Inoculated	< 5
B4 No Airflow	< 5
B5 Airflow	< 5
B5 Airflow Inoculated	< 5
B5 No Airflow	< 5

Samples from week 7 and week 12 were sent to CGC for mycotoxin analysis (Table 11). The levels of OTA and OTB were below 10 ppb for all samples. The level of zearalenone was below 100 ppb for all samples.

Table 11. Ochratoxin analysis of Bin Study 1 (AI- air flow inoculated, NA- no airflow) samples at weeks 7 and 12.

Sample ID	Ochratoxin A(ppb)	Ochratoxin B(ppb)	Zearalenone(ppb)
B3 AI Wk7	<10	<10	<100
B4 AI Wk7	<10	<10	<100
B5 AI Wk7	<10	<10	<100
B3 NA Wk7	<10	<10	<100
B4 NA Wk7	<10	<10	<100
B5 NA wk 7	<10	<10	<100
B3 AI Wk12	<10	<10	<100
B4 AI Wk12	<10	<10	<100
B5 AI Wk12	<10	<10	<100
B3 NA Wk12	<10	<10	<100
B4 NA Wk12	<10	<10	<100
B5 NA wk 12	<10	<10	<100

4.2 Bin Study 2

Bin Study 2 had initial germination values greater than 90% and relatively low FAVs. However, by the end of week 2 the germination had dropped to near zero and FAV had increased significantly. This trial was discontinued after week two because it was believed that the grain had been negatively affected by storage within the CWBCGSR lab and fresh grain was moved in for Bin Study 3.

4.3 Bin Study 3

The results of Bin Study 1 and the Chamber Study indicated that the *airflow inoculated* treatment did not provide any additional information to the experiment. Therefore, this treatment was not included in Bin Study 3.

4.3.1 Germination

Germination of wheat kernels at different times for different treatments are shown in Figure 19. Germination was approximately 85% for both treatments in the first week of experiment and reduced to less than 10% for both treatments at week 2. There were no significant differences ($P < 0.05$) between the treatments (Table 12).

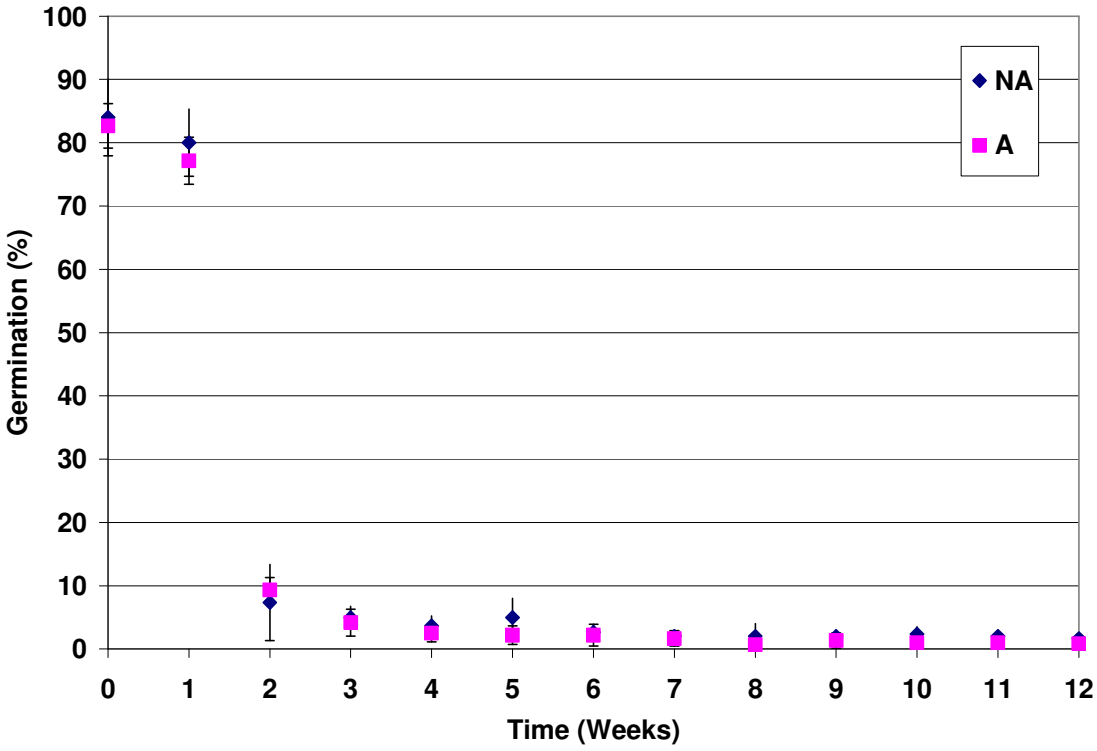

Figure 19. Germination of wheat kernels for *no airflow* (NA) and *airflow* (A) treatments for Bin Study 3.

Table 12. Comparison of germination for *no airflow* (NA) vs. *airflow* (A) treatments for Bin Study 3.

Week	Mean (Standard Deviation)		P value (two tail)
	NA	A	
0	84.0 (6.1)[a]	82.7 (3.5)[a]	0.7483
1	80.0 (5.3)[a]	77.2 (3.7)[a]	0.4670
2	7.3 (6.0)[a]	9.3 (2.0)[a]	0.6318
3	5.0 (1.7)[a]	4.2 (2.1)[a]	0.5576
4	3.7 (1.5)[a]	2.5 (1.4)[a]	0.3272
5	5.0 (3.0)[a]	2.2 (1.5)[a]	0.2623
6	2.7 (1.1)[a]	2.2 (1.7)[a]	0.6243
7	2.0 (1.0)[a]	1.7 (1.2)[a]	0.6793
8	2.0 (2.0)[a]	0.7 (1.0)[a]	0.3574
9	2.0 (1.0)[a]	1.3 (1.2)[a]	0.4206
10	2.3 (1.1)[a]	1.0 (0.6)[a]	0.1590
11	2.0 (1.0)[a]	1.0 (0.9)[a]	0.2171
12	1.7 (0.5)[a]	0.8 (0.8)[a]	0.1255

[a]In a row numbers followed by the same character are statistically the same ($P<0.05$).

4.3.2 FAV

Free fatty acid value (FAV) tests were carried out for all samples (Figure 20). The initial FAV for the *no airflow* and *airflow* treatment samples were 8.9 and 7.5; these increased to 62.4 and 58.7 respectively by the end of the study (Table 13). There were significant differences between *no airflow* and *airflow* treatments in FAV values at week 6, 7, 10 and 12 (Table 13).

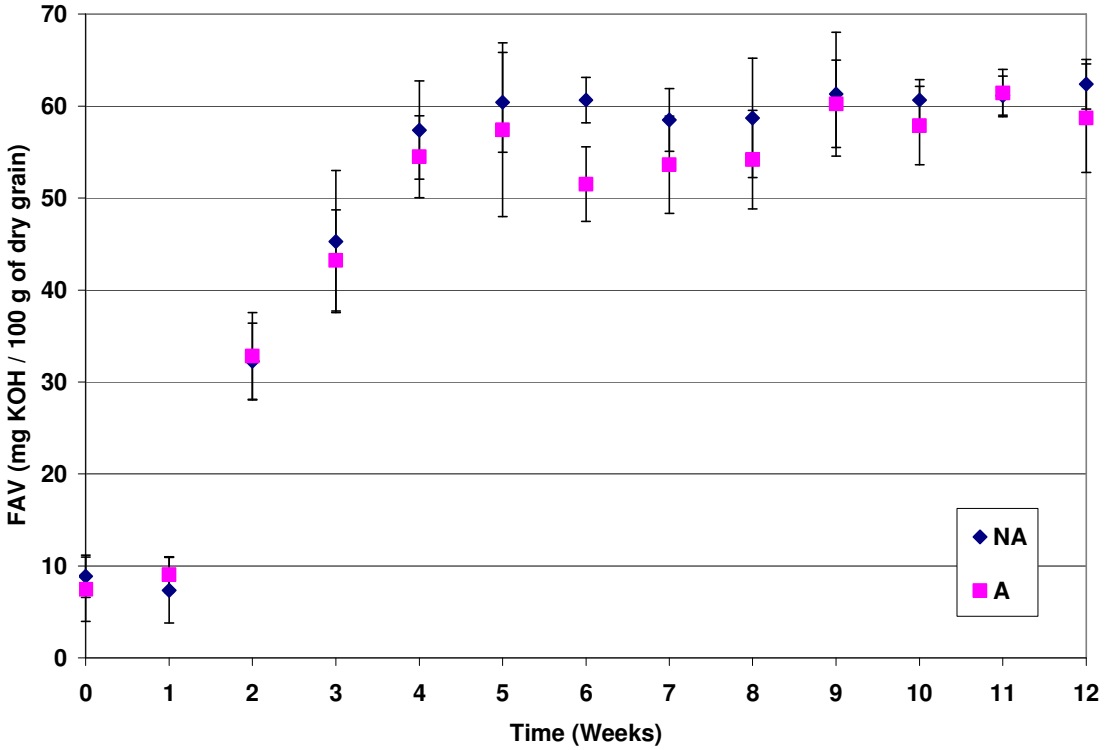

Figure 20. FAV results for *no airflow* (NA) and *airflow* (A) treatments for Bin Study 3.

Table 13. Comparison of FAV for *no airflow* (NA) vs. *airflow* (A) treatments for Bin Study 3.

	Mean (Standard Deviation)		
Week	NA	A	P value (two tail)
0	8.9 (2.3)[a]	7.5 (3.5)[a]	0.2246
1	7.4 (3.6)[a]	9.0 (1.9)[a]	0.2142
2	32.3 (4.1)[a]	32.8 (4.8)[a]	0.7634
3	45.3 (7.7)[a]	43.2 (5.5)[a]	0.4883
4	57.4 (5.3)[a]	54.5 (4.5)[a]	0.1818
5	60.4 (5.4)[a]	57.4 (9.4)[a]	0.3070
6	60.6 (2.5)[a]	55.4 (5.9)[b]	0.0030
7	58.7 (13.6)[a]	53.6 (5.3)[b]	0.0075
8	58.7 (6.5)[a]	54.2 (5.4)[a]	0.0928
9	61.3 (6.7)[a]	60.2 (4.8)[a]	0.6852
10	60.6 (2.2)[a]	57.9 (4.3)[b]	0.0341
11	61.1 (2.1)[a]	61.4 (2.6)[a]	0.7776
12	62.4 (2.7)[a]	58.7 (5.9)[b]	0.0359

[a]In a row numbers followed by the same character are statistically the same (P<0.05).

4.3.3 Moisture content

The initial m.c. of the *no airflow* and *airflow* treatments in this Bin Study 3 was approximately 17.5% (wb). The m.c. of the *airflow* treatment was generally higher than the *no airflow* treatment; however, throughout most of the study the moisture contents of both treatments remained within 1% (wb) of each other.

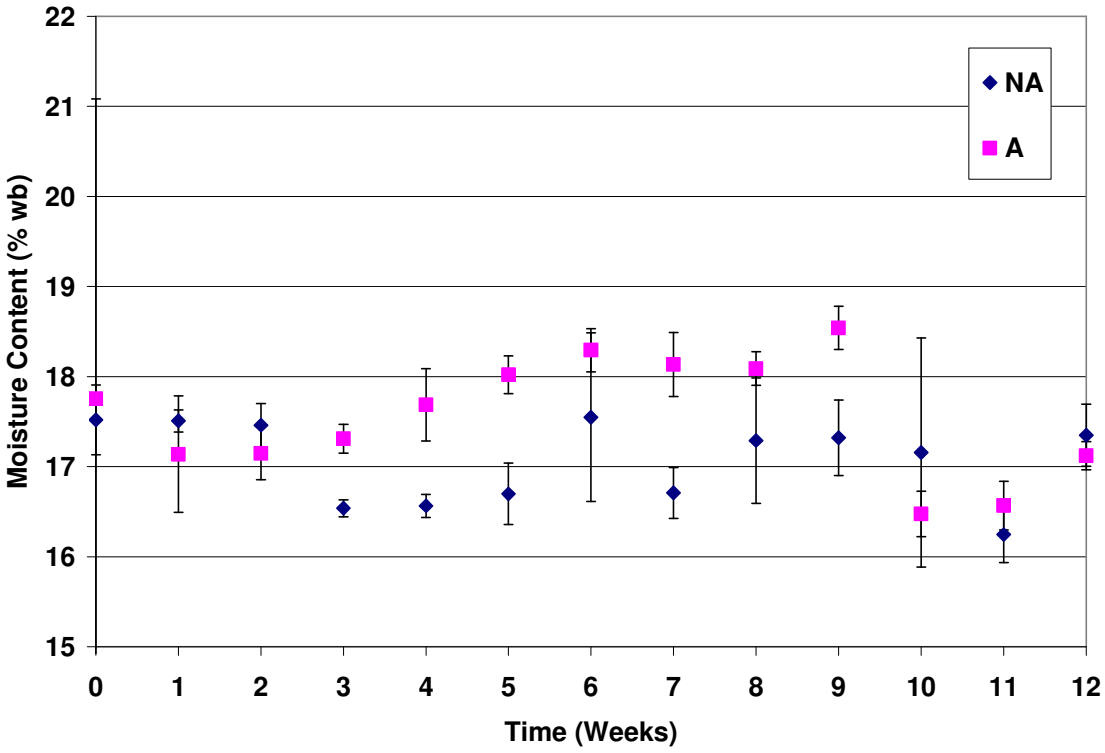

Figure 21. Moisture contents for *no airflow* (NA) and *airflow* (A) treatments for Bin Study 3.

4.3.4 Mycotoxins

Samples from Bin Study 3 were sent out for testing to Central Testing Labs Ltd. (Winnipeg, MB). Two of the samples (week 10 *no airflow* and week 11 *no airflow*) showed the presence of mycotoxins in excess of 5 ppb (Table 14).

Table 14. Ochratoxin analysis for *no airflow* (NA) and *airflow* (A) treatments for various weeks from Bin Study3.

Week	Treatment	OTA (ppb)
0	NA	<5
0	A	<5
4	NA	<5
4	A	<5
8	NA	<5
8	A	<5
10	NA	34
		<5
		<5
10	A	<5
11	NA	30
		<5
		<5
12	NA	<5
		<5
		<5
12	A	<5

4.4 Chamber Study

4.4.1 Germination

Germination of wheat kernels at different times for the *no airflow* and *airflow* treatments are given (Figure 22). The initial germination was approximately 91% for both treatments and reduced to less than 20% from the fifth week onwards. There were no significant differences ($P < 0.05$) between the *no airflow* and *airflow* treatments throughout the study (Table 15).

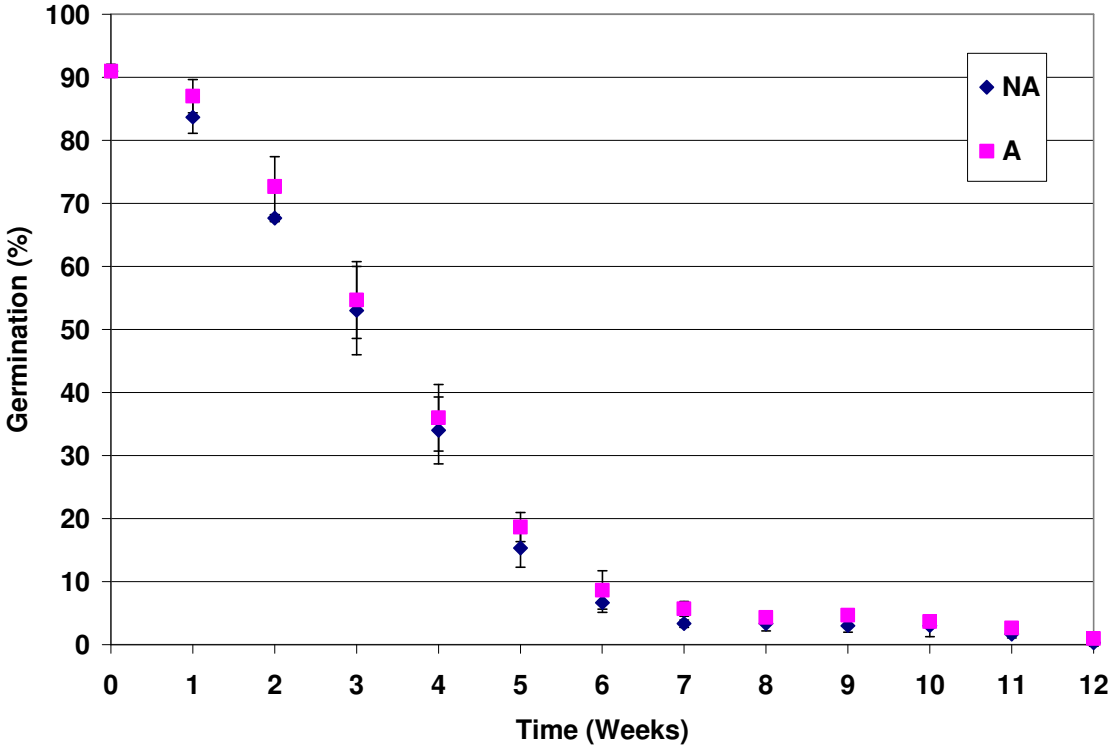

Figure 22. Germination values for *no airflow* (NA) and *airflow* (A) treatments for the Chamber Study.

Table 15. Comparison of germination for *no airflow* (NA) and *airflow* (A) treatments for the Chamber Study.

	Mean (Standard Deviation)		
Week	NA	A	P value (two tail)
0	91.0 (1.0)[a]	91.0 (1.0)[a]	1.0000
1	83.7 (2.5)[a]	87.0 (2.6)[a]	0.1890
2	67.7 (0.5)[a]	72.7 (4.8)[a]	0.2105
3	53.0 (7.0)[a]	54.7 (6.1)[a]	0.7716
4	34.0 (5.3)[a]	36.0 (5.3)[a]	0.6675
5	15.3 (3.0)[a]	18.7 (2.3)[a]	0.2062
6	6.7 (1.5)[a]	8.7 (3.0)[a]	0.3852
7	3.3 (0.5)[a]	5.7 (1.1)[a]	0.0520
8	3.3 (1.1)[a]	4.3 (0.5)[a]	0.2822
9	3.0 (1.0)[a]	4.7 (0.5)[a]	0.0877
10	3.0 (1.7)[a]	3.7 (0.5)[a]	0.5918
11	1.7 (0.5)[a]	2.7 (0.5)[a]	0.1012
12	0.3 (0.5)[a]	1.0 (1.0)[a]	0.3910

[a]In a row numbers followed by the same character are statistically the same (P<0.05).

4.4.2 FAV

Fat acidity values increased nearly five times at the end of the study (week 12) in both treatments (Figure 23). There was no significance difference in FAV values between *no airflow* and *airflow* samples throughout the study ($P<0.05$) (Table 16).

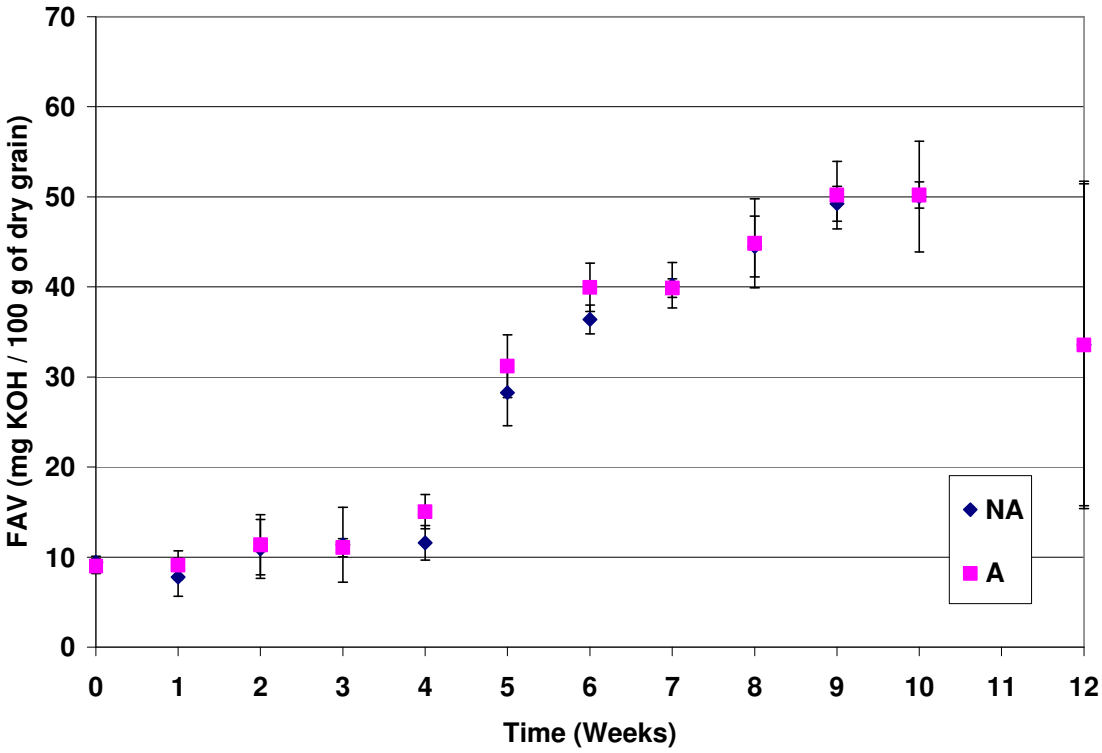

Figure 23. FAV results for *no airflow* (NA) and *airflow* (A) treatments for Chamber Study.

Table 16. Comparison of FAV for *no airflow* (NA) and *airflow* (A) treatments for the Chamber Study.

Week	Mean (Standard Deviation)		P value (two tail)
	NA	A	
0	9.4 (0.7)[a]	9.0 (0.8)[a]	0.2443
1	7.8 (2.1)[a]	9.1 (1.6)[a]	0.2623
2	10.9 (3.3)[a]	11.4 (3.4)[a]	0.8267
3	11.4 (4.2)[a]	11.0 (1.0)[a]	0.8583
4	11.6 (1.9)[a]	15.0 (1.9)[b]	0.0014
5	28.3 (3.7)[a]	31.2 (3.5)[a]	0.1861
6	36.4 (1.6)[a]	40.0 (2.7)[b]	0.0233
7	40.2 (2.5)[a]	39.9 (1.0)[a]	0.7308
8	44.5 (3.4)[a]	44.8 (4.9)[a]	0.8926
9	49.2 (1.9)[a]	50.2 (3.7)[a]	0.5880
10	50.0 (6.3)[a]	50.2 (1.5)[a]	0.9510
11	*	*	
12	33.6 (17.9)[a]	33.6 (18.2)[a]	1.0000

[a]In a row numbers followed by the same character are statistically the same ($P<0.05$).
*Sample lost.

4.4.3 Moisture content

Moisture content of the wheat samples at different times for the *no airflow* and *airflow* treatments are given in Figure 24. The m.c. was approximately 20% (wb) throughout the study.

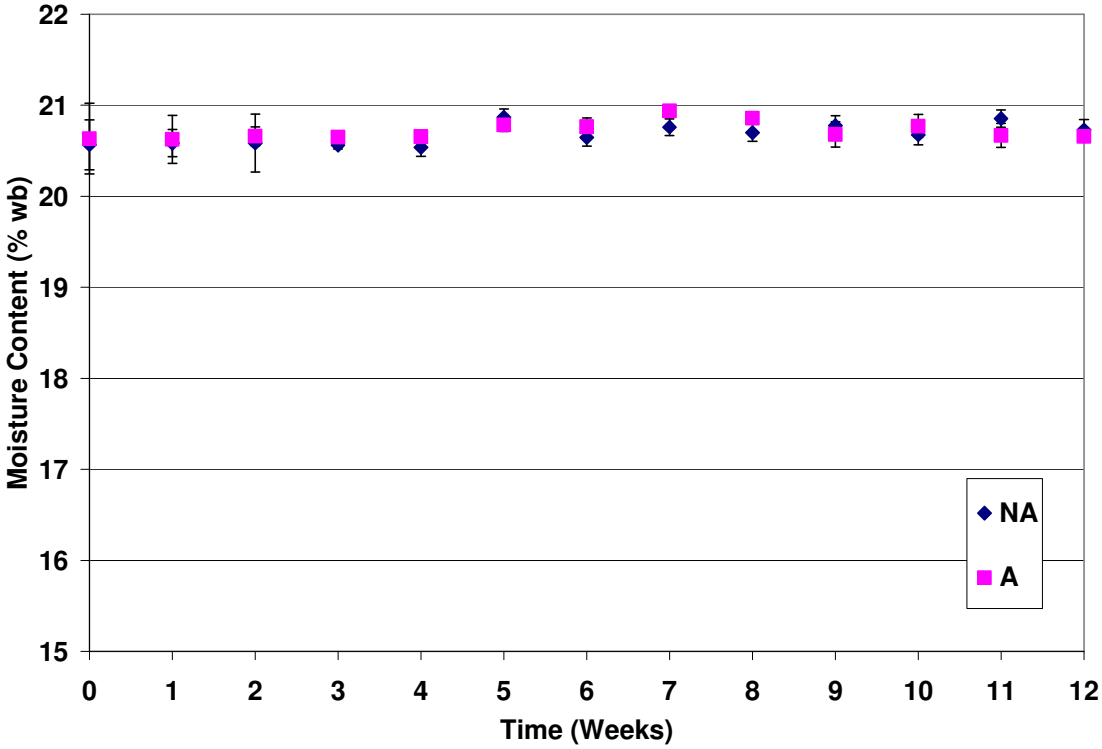

Figure 24. Moisture contents for *no airflow* (NA) and *airflow* (A) treatments of the Chamber Study.

4.4.4 Mycotoxins

Representative samples from the chamber study were sent to Canadian Grain Commission for mycotoxin analysis and results obtained from the analysis are given in Table 17. Presence of OTA was identified in both the *no airflow* (NA) and *airflow* (A) samples from week 7 onwards.

Table 17. Results of Ochratoxin analysis for Chamber Study.

Sample ID	Ochratoxin A(ppb)	Ochratoxin B(ppb)	Zearalenone(ppb)
B2 A Wk7	67	(tr)	<100
B2NA Wk7	63	(tr)	<100
B2 A Wk8	52	(tr)	<100
B2A Wk10	110	(tr)	<100
B1 A Wk12	170	(tr)	(tr)

5 DISCUSSION

5.1 Germination

It is well accepted that germination is a reliable and sensitive indicator of spoilage. In general, high moisture grain is more susceptible to spoilage than low moisture grain and one would expect that germination rates of stored high moisture grain would decrease with time. The germination results of this study support the accepted theory.

In all the studies and treatments the germination decreased over time as expected. Germination results for Bin Study 1 indicate that airflow, even if it has a high RH, significantly decreases the rate of spoilage in stored grain. However, both Bin Study 3 and the Chamber Study, contradict this finding. As well, the m.c. (wb) of the grain in the *airflow* treatments of Bin Study 1 decreased a couple of percentage points during the first two weeks (Figure 18). This fact combined with the results of Bin Study 3 and the Chamber Study indicates that airflow does not have a significant effect on the rate of spoilage unless drying is occurring.

A comparison of the germination results of Bin Study 1 (Figure 16) and the Chamber Study (Figure 23) shows that the germination values of Bin Study 1 decreased more rapidly than in the Chamber Study. This result is unexpected because these experiments started with the same wheat and the m.c. of the Chamber Study wheat was approximately 2% (wb) higher than the m.c. of the wheat in Bin Study 1. This result indicates that the spoilage process may have already been underway in the Bin Study 1 grain when the experiment started. This spoilage could have been due to the differences in the grain

conditioning processes used in the two experiments or potentially some other source as discussed in the sources of error section of this thesis.

5.2 FAV

The increase of free fatty acids and thereby increased FAV has proven to be a reliable indicator of spoilage in wheat. In general, unspoiled wheat should have FAV in the range of 5 to 10 mg KOH / 100 g of dry grain. The FAV increases as spoilage increases. The results of this study are consistent with expected results.

The FAV results of all the studies follow the reverse trends to the germination results, with FAV increasing over time in all trials and treatments. Bin Study 1 showed a significant difference in FAV between the *no airflow* and *airflow* treatments with the *no airflow* treatment having higher values (Table 8). Bin Study 3 and the Chamber study showed no significant differences in FAV between the treatments (Tables 13 and 16). The significant differences found in Bin Study 1 were probably due to the drying that occurred and not merely the presence of airflow.

5.3 Moisture Content

A m.c. of 14.5% for wheat is considered safe according to Canadian storage guidelines for wheat. However, in Western Canada farmers will typically put their wheat into storage at higher moisture contents and use a drying regime to dry the grain. This study used durum wheat that was at moisture contents near the upper range of what a farmer would generally store.

In Bin Study 1 the *airflow* treatment had a significantly lower m.c. than the *no airflow* treatment during the first 2 weeks of the trial. This difference was likely caused by the

fact that some of the moisture from the conditioning process was still on the surface of the grain and therefore easily removed by the airflow. As well, the Weather Simulation Lab was not able to maintain the RH of the lab at the ERH required for a m.c. of 18.5% (wb) in the grain. This problem was rectified for Bin Study 3 and the m.c. of the *airflow* treatment was consistently higher than the *non airflow* treatment. In general the m.c. for all the studies was significantly higher than the safe storage limit of 14.5% (wb) for wheat.

5.4 Mycotoxins

Ochratoxin A was not detected with any regularity in either of the Bin Studies. Even with the rapid loss in germinability of the wheat in Bin Study 3 ochratoxin was not detected. This result is unexpected because the durum wheat in Bin Study 3 represented a worst case storage scenario, high RH air and high moisture grain combined with low viability grain should support microorganism growth, and thereby support mycotoxin production more readily than higher viability wheat. However, except for a couple of tests in week 10 no OTA was detected.

The Chamber Study showed significant amounts of OTA production as early as the 7^{th} week of the study. This result confirms two things:

1) That the grain used for all the studies had the capacity to grow mycotoxin producing strains of *P. verrocosum* if the growing conditions were right; and
2) That even under extremely negative storage conditions OTA development occurs long after germination and FAV indicate major spoilage.

The Chamber Study represents one of the worst possible storage situations that could occur in Western Canada and is actually highly unlikely. However, it is worth noting that

the current grain drying guidelines, are such that if a farmer put 20% (wb) grain in a bin and followed the guidelines the grain would not be at 20% m.c. (wb) for 12 weeks.

5.5 Sources of Error

5.5.1 Conditioning

An accepted procedure for conditioning grain is to add the required amount of water to achieve the desired mc and then let the grain temper in an airtight container for 72 h at approximately 5ºC (Udayakumar 2008). Due to the volume of grain required for the bin study experiment and the available equipment, achieving the desired moisture levels and cool storage temperatures was impossible. Therefore, the amount of time the grain was allowed to temper was significantly reduced in an attempt to decrease the amount of spoilage that would occur prior to the experiment start. The researchers knew that some spoilage could possibly occur but since the aim of the study was to test for mycotoxin development in an approximate time period of 6-12 weeks, the few days of pre-experiment spoilage was considered acceptable. As well, all the experimental grain was exposed to the same treatment so it was believed that any differences that occurred would still be relevant.

Unfortunately, because of the short tempering time of the conditioning process some of the moisture was still loosely bound on the surface of the grain. Due to the mechanical problems with the humidity control system, the RH of the room was not consistently at the required ERH to maintain the high m.c. of the grain exposed to airflow once the fans were started. This led to the grain exposed to the airflow treatments having lower average m.c. than the non-airflow treatments, confounding the results.

5.5.2 Equipment sanitation

Prior to all the bin studies, the bins and material handling equipment were sanitized in accordance with the procedure described in the procedure section of this thesis. The results from Bin study 1 were consistent with the expected results and at the end of this study significant amounts of grain were spoiled in all quadrants. To empty the bins, manual slide gates were opened in the perforated bin floor and the grain flowed out through the hopper bottoms and through the material handling equipment to a truck. After Bin Study 1, a second trial, Bin study 2 was initiated. In this trial the grain spoiled within the first 2 weeks and it was believed that this spoilage was caused because the experimental grain had been in storage BN01 under varying temperature conditions for over 6 months and that some loss of viability had already occurred. Therefore, 10 t of the original durum wheat was shipped into the CWBCGSR to be used in Bin Study 3. Germination of this grain prior to the experiment start was greater than 95%. However, as shown in Figure 20 the grain in Bin Study 3 also spoiled within the first two weeks of the study.

It is now believed that the sanitization process used did not adequately clean the bins and equipment, especially the hopper bottoms, which are unreachable when the perforated floor is in the bins. This allowed the air that was blown through the bins to potentially pass over spoiled grain trapped in the hoppers prior to entering the experimental treatment thereby introducing spoilage factors from an outside source.

5.5.3 Cross contamination in the Chamber Study

In the Conviron chamber there is no mechanism for adding fresh air other than opening the chamber door. Sampling was conducted on a weekly basis so the door was

not opened frequently enough to provide a significant amount of fresh air to the experiment. Therefore, the air within the chamber for the chamber study was continuously recycled through all three treatments. Since there were no filters on the experimental apparatus it was possible that fungal spores from one treatment could pass to another.

5.5.4 Mechanical breakdowns

Prior to the start of the experiment the Weather Simulation Lab equipment was run for 30 days to ensure that the required environmental parameters could be adequately controlled. However, early in Bin Study 1 the system which controlled the RH of the room malfunctioned causing the RH in the room to drop. This mechanical problem was not easily rectified. To allow the experiment to continue, portable humidifiers were placed in the room in an attempt to maintain a high RH. This solution was not able to keep the RH of the room high enough to maintain the m.c. of the airflow grain near the 18% wb range and therefore the airflow treatments experienced some drying.

5.5.5 Storage of grain prior to experiment

Prior to the beginning of the trials, the grain that was to be used for the experiment was stored in BN01. This bin is within the CWBCGSR and therefore is maintained at a temperature of approximately 23ºC. This storage situation was considered acceptable because 23ºC combined with the low m.c. of the grain, 12.5% (wb), should not have caused any significant spoilage in the grain. However, based on the spoilage which occurred in Bin Study 2 and 3 a more thorough examination of the storage conditions that

existed in BN01 revealed two potential causes of spoilage: condensation due to convection currents and contamination from the dust collection system.

Due to the location of BN01 the grain within this bin can easily be at a different temperature than the air within the distributor and spouting from the bucket elevators, which are outside the building. During the summer months this situation would not be an issue. However, in the winter months the warm air within the bin would naturally rise up the distribution spout. Since this bin is connected to a fan at the hopper bottom which is open to the air within the CWBCGSR a natural convection current of warm air from the fan, through the grain bulk and up through the spout would occur. Depending on several factors condensation could occur within the spout and moisture could drip down into BN01.

A second source of spoilage within BN01 during this experiment was the presence of a common dust collection system within the lab. Having a common dust collection system is standard practice within grain storage facilities and does not normally lead to problems. However, in the situation where high temperature and high moisture air is being blown up through the bins in the Weather Simulation Lab the common dust collection system, becomes an issue. During the winter months cold air can travel down the spouts of the distribution system into the head space of BN01 and BN02. When the warm moist air is forced through the common dust collection system into either BN01 or BN02 condensation can occur.

5.5.6 Sampling probability

The FAO allowable mycotoxin limit is currently 5 ppb (FAO 2004). Over the 12 week period the sampling procedure for each quadrant would have included 36 probe

insertions from random locations and a total mass of approximately 6 kg of grain. Each quadrant of grain contain approximately 800 kg of grain. To achieve a strong representative sample much larger samples would need to have been drawn and split with the excess put back into the experiment. In light of the drawbacks of disturbing the experimental material and the amount of labour required to draw that many samples it was decided that the potential for error from sampling would be acceptable.

6 CONCLUSIONS

Based on the results of this study grain that is stored and dried in accordance with the current safe storage guidelines is not at risk of developing OTA levels in excess of 5 ppb. Also, that airflow within a grain bulk has no significant affect on spoilage unless it is drying the grain.

6.1 Recommendations

This study revealed the following areas that merit further investigation, including:

1) Research correlating sampling probability and detected mycotoxin levels to actual mycotoxin levels within a grain bulk.
2) Research into the effect a single or multiple truckloads of wheat with high mycotoxin levels would have on the grain distribution system in Western Canada.
3) Research into current grading standards and procedures to determine if they are indirectly detecting mycotoxin infested grain by monitoring other physical characteristics of the grain?
4) Conduct similar experiments on a smaller scale.
5) Design experiments on mycotoxin formation in such a way that larger or more representative samples can be drawn.

Further recommendations include examining the existing grain distribution equipment at the CWB Centre for Grain Storage Research to determine if there are possible ways to decrease the potential for cross contamination between experiments and increase the reliability of the existing equipment.

7 REFERENCES

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8 APPENDIX 1 – Bin Study 1 Raw Data

8.1 Environmental Control

8.1.1 Environment Canada Weather Data

Point	Year Month	Date	Time (24 hour clock notation)	Temperature (ºC)	RH (%)
1	1969 Aug	15	0	16.1	87
2	1969 Aug	15	600	13.9	91
3	1969 Aug	15	1200	22.8	66
4	1969 Aug	15	1800	23.3	57
5	1969 Aug	16	0	15.0	88
6	1969 Aug	16	600	13.3	98
7	1969 Aug	16	1200	25.5	55
8	1969 Aug	16	1800	26.7	51
9	1969 Aug	17	0	21.7	66
10	1969 Aug	17	600	16.7	96
11	1969 Aug	17	1200	12.8	57
12	1969 Aug	17	1800	19.4	51
13	1969 Aug	18	0	12.8	84
14	1969 Aug	18	600	10.6	94
15	1969 Aug	18	1200	17.2	70
16	1969 Aug	18	1800	20.0	72
17	1969 Aug	19	0	12.8	98
18	1969 Aug	19	600	12.2	93
19	1969 Aug	19	1200	21.9	59
20	1969 Aug	19	1800	22.2	51
21	1969 Aug	20	0	17.2	70
22	1969 Aug	20	600	13.3	88
23	1969 Aug	20	1200	23.9	57
24	1969 Aug	20	1800	25.0	51
25	1969 Aug	21	0	18.9	77
26	1969 Aug	21	600	17.8	80
27	1969 Aug	21	1200	28.9	60
28	1969 Aug	21	1800	31.7	54
29	1969 Aug	22	0	24.7	65
30	1969 Aug	22	600	20.6	87
31	1969 Aug	22	1200	31.1	57
32	1969 Aug	22	1800	32.8	38
33	1969 Aug	23	0	21.1	63
34	1969 Aug	23	600	15.0	95
35	1969 Aug	23	1200	27.8	57
36	1969 Aug	23	1800	29.4	58

37	1969 Aug	24	0	22.8	67
38	1969 Aug	24	600	18.3	90
39	1969 Aug	24	1200	28.3	63
40	1969 Aug	24	1800	27.2	69
41	1969 Aug	25	0	22.2	73
42	1969 Aug	25	600	18.9	88
43	1969 Aug	25	1200	28.9	54
44	1969 Aug	25	1800	28.9	47
45	1969 Aug	26	0	23.3	63
46	1969 Aug	26	600	22.2	68
47	1969 Aug	26	1200	25.0	70
48	1969 Aug	26	1800	25.0	63
49	1969 Aug	27	0	16.1	100
50	1969 Aug	27	600	16.7	75
51	1969 Aug	27	1200	26.7	60
52	1969 Aug	27	1800	27.8	56
53	1969 Aug	28	0	13.3	96
54	1969 Aug	28	600	13.3	95
55	1969 Aug	28	1200	23.3	60
56	1969 Aug	28	1800	26.1	56
57	1969 Aug	29	0	21.1	80
58	1969 Aug	29	600	18.9	90
59	1969 Aug	29	1200	24.4	81
60	1969 Aug	29	1800	25.0	78
61	1969 Aug	30	0	18.3	62
62	1969 Aug	30	600	11.7	82
63	1969 Aug	30	1200	20.0	52
64	1969 Aug	30	1800	21.1	44
65	1969 Aug	31	0	12.8	75
66	1969 Aug	31	600	11.1	89
67	1969 Aug	31	1200	15.6	70
68	1969 Aug	31	1800	16.7	60
69	1969 Sep	1	0	6.7	96
70	1969 Sep	1	600	6.7	96
71	1969 Sep	1	1200	21.1	47
72	1969 Sep	1	1800	21.7	57
73	1969 Sep	2	0	8.3	50
74	1969 Sep	2	600	12.8	72
75	1969 Sep	2	1200	24.2	39
76	1969 Sep	2	1800	21.7	63
77	1969 Sep	3	0	18.9	69
78	1969 Sep	3	600	16.1	81
79	1969 Sep	3	1200	24.4	65
80	1969 Sep	3	1800	25.6	58
81	1969 Sep	4	0	20.0	68
82	1969 Sep	4	600	17.8	82
83	1969 Sep	4	1200	26.7	54
84	1969 Sep	4	1800	19.4	96
85	1969 Sep	5	0	16.7	98
86	1969 Sep	5	600	15.6	95

87	1969 Sep	5	1200	21.1	73
88	1969 Sep	5	1800	19.4	73
89	1969 Sep	6	0	12.8	95
90	1969 Sep	6	600	9.4	98
91	1969 Sep	6	1200	16.7	75
92	1969 Sep	6	1800	20.0	67
93	1969 Sep	7	0	12.8	96
94	1969 Sep	7	600	10.6	98
95	1969 Sep	7	1200	16.1	81
96	1969 Sep	7	1800	13.3	89
97	1969 Sep	8	0	11.7	93
98	1969 Sep	8	600	7.8	98
99	1969 Sep	8	1200	11.7	83
100	1969 Sep	8	1800	12.8	77
101	1969 Sep	9	0	5.0	98
102	1969 Sep	9	600	5.0	98
103	1969 Sep	9	1200	16.7	67
104	1969 Sep	9	1800	18.3	67
105	1969 Sep	10	0	12.8	81
106	1969 Sep	10	600	12.2	87
107	1969 Sep	10	1200	17.8	72
108	1969 Sep	10	1800	15.6	61
109	1969 Sep	11	0	5.6	93
110	1969 Sep	11	600	3.3	97
111	1969 Sep	11	1200	18.9	52
112	1969 Sep	11	1800	23.3	50
113	1969 Sep	12	0	13.3	81
114	1969 Sep	12	600	10.0	94
115	1969 Sep	12	1200	22.2	57
116	1969 Sep	12	1800	19.4	68
117	1969 Sep	13	0	13.9	80
118	1969 Sep	13	600	12.2	85
119	1969 Sep	13	1200	23.3	63
120	1969 Sep	13	1800	25.6	56
121	1969 Sep	14	0	20.0	86
122	1969 Sep	14	600	18.9	90
123	1969 Sep	14	1200	24.4	71
124	1969 Sep	14	1800	23.3	74
125	1969 Sep	15	0	13.9	95
126	1969 Sep	15	600	12.8	89
127	1969 Sep	15	1200	13.9	53
128	1969 Sep	15	1800	13.9	48
129	1969 Sep	16	0	7.2	66
130	1969 Sep	16	600	3.3	80
131	1969 Sep	16	1200	13.3	39
132	1969 Sep	16	1800	11.7	44
133	1969 Sep	17	0	2.2	87
134	1969 Sep	17	600	-0.6	97
135	1969 Sep	17	1200	13.3	57
136	1969 Sep	17	1800	12.2	55

137	1969 Sep	18	0	6.1	84
138	1969 Sep	18	600	6.7	85
139	1969 Sep	18	1200	17.8	54
140	1969 Sep	18	1800	17.8	65
141	1969 Sep	19	0	15.0	71
142	1969 Sep	19	600	12.8	79
143	1969 Sep	19	1200	19.4	63
144	1969 Sep	19	1800	22.2	56
145	1969 Sep	20	0	19.4	56
146	1969 Sep	20	600	16.1	71
147	1969 Sep	20	1200	22.8	56
148	1969 Sep	20	1800	18.9	73
149	1969 Sep	21	0	13.9	88
150	1969 Sep	21	600	14.4	93
151	1969 Sep	21	1200	20.6	65
152	1969 Sep	21	1800	20.0	75
153	1969 Sep	22	0	11.7	96
154	1969 Sep	22	600	8.3	96
155	1969 Sep	22	1200	9.4	78
156	1969 Sep	22	1800	6.1	93
157	1969 Sep	23	0	1.7	92
158	1969 Sep	23	600	1.7	97
159	1969 Sep	23	1200	5.0	77
160	1969 Sep	23	1800	6.1	71
161	1969 Sep	24	0	6.1	80
162	1969 Sep	24	600	5.0	93
163	1969 Sep	24	1200	6.7	91
164	1969 Sep	24	1800	8.3	94
165	1969 Sep	25	0	8.3	94
166	1969 Sep	25	600	7.8	94
167	1969 Sep	25	1200	9.4	86
168	1969 Sep	25	1800	10.6	92
169	1969 Sep	26	0	8.9	92
170	1969 Sep	26	600	7.2	98
171	1969 Sep	26	1200	10.0	79
172	1969 Sep	26	1800	9.4	76
173	1969 Sep	27	0	2.8	95
174	1969 Sep	27	600	3.9	95
175	1969 Sep	27	1200	8.9	80
176	1969 Sep	27	1800	8.3	71
177	1969 Sep	28	0	1.7	94
178	1969 Sep	28	600	1.7	97
179	1969 Sep	28	1200	10.0	68
180	1969 Sep	28	1800	10.0	77
181	1969 Sep	29	0	9.4	92
182	1969 Sep	29	600	7.2	89
183	1969 Sep	29	1200	11.7	64
184	1969 Sep	29	1800	7.2	72
185	1969 Sep	30	0	1.7	86
186	1969 Sep	30	600	0.6	99

187	1969 Sep	30	1200	5.6	77
188	1969 Sep	30	1800	7.2	70
189	1969 Oct	1	0	5.6	96
190	1969 Oct	1	600	6.7	98
191	1969 Oct	1	1200	8.9	86
192	1969 Oct	1	1800	9.4	82
193	1969 Oct	2	0	3.3	100
194	1969 Oct	2	600	6.7	100
195	1969 Oct	2	1200	9.4	76
196	1969 Oct	2	1800	9.4	86
197	1969 Oct	3	0	8.3	92
198	1969 Oct	3	600	11.1	87
199	1969 Oct	3	1200	11.7	91
200	1969 Oct	3	1800	12.8	96
201	1969 Oct	4	0	13.3	96
202	1969 Oct	4	600	7.2	87
203	1969 Oct	4	1200	8.3	75
204	1969 Oct	4	1800	10.0	71
205	1969 Oct	5	0	5.0	86
206	1969 Oct	5	600	3.9	88
207	1969 Oct	5	1200	6.7	76
208	1969 Oct	5	1800	7.8	77
209	1969 Oct	6	0	6.7	91
210	1969 Oct	6	600	3.9	90
211	1969 Oct	6	1200	7.8	69
212	1969 Oct	6	1800	6.7	76
213	1969 Oct	7	0	5.0	93
214	1969 Oct	7	600	3.3	85
215	1969 Oct	7	1200	5.0	81
216	1969 Oct	7	1800	5.0	74
217	1969 Oct	8	0	3.3	80
218	1969 Oct	8	600	1.1	97
219	1969 Oct	8	1200	10.6	54
220	1969 Oct	8	1800	8.9	64
221	1969 Oct	9	0	8.3	73
222	1969 Oct	9	600	6.7	80
223	1969 Oct	9	1200	9.4	73
224	1969 Oct	9	1800	7.8	85
225	1969 Oct	10	0	5.6	91
226	1969 Oct	10	600	1.7	86
227	1969 Oct	10	1200	2.8	74
228	1969 Oct	10	1800	2.2	71
229	1969 Oct	11	0	1.1	76
230	1969 Oct	11	600	0.6	70
231	1969 Oct	11	1200	2.8	79
232	1969 Oct	11	1800	2.8	84
233	1969 Oct	12	0	-1.7	97
234	1969 Oct	12	600	1.1	89
235	1969 Oct	12	1200	2.8	77
236	1969 Oct	12	1800	1.1	76

237	1969 Oct	13	0	0.6	91
238	1969 Oct	13	600	0.0	94
239	1969 Oct	13	1200	2.8	62
240	1969 Oct	13	1800	2.8	60
241	1969 Oct	14	0	0.0	83
242	1969 Oct	14	600	1.7	72
243	1969 Oct	14	1200	3.9	68
244	1969 Oct	14	1800	3.3	85
245	1969 Oct	15	0	2.2	95
246	1969 Oct	15	600	0.6	91
247	1969 Oct	15	1200	3.3	75
248	1969 Oct	15	1800	1.1	97
249	1969 Oct	16	0	-0.6	91
250	1969 Oct	16	600	-3.9	92
251	1969 Oct	16	1200	4.4	64
252	1969 Oct	16	1800	5.0	59
253	1969 Oct	17	0	3.3	75
254	1969 Oct	17	600	-1.7	85
255	1969 Oct	17	1200	3.3	63
256	1969 Oct	17	1800	2.8	69
257	1969 Oct	18	0	3.3	72
258	1969 Oct	18	600	3.3	75
259	1969 Oct	18	1200	7.2	52
260	1969 Oct	18	1800	5.0	61
261	1969 Oct	19	0	-1.1	80
262	1969 Oct	19	600	0.6	70
263	1969 Oct	19	1200	6.7	49
264	1969 Oct	19	1800	2.8	67
265	1969 Oct	20	0	1.7	72
266	1969 Oct	20	600	-2.8	78
267	1969 Oct	20	1200	0.6	62
268	1969 Oct	20	1800	0.0	77
269	1969 Oct	21	0	-1.7	90
270	1969 Oct	21	600	-2.2	94
271	1969 Oct	21	1200	-1.7	79
272	1969 Oct	21	1800	-3.3	78
273	1969 Oct	22	0	-8.3	81
274	1969 Oct	22	600	-8.3	81
275	1969 Oct	22	1200	-1.1	63
276	1969 Oct	22	1800	-2.2	77
277	1969 Oct	23	0	-4.4	86
278	1969 Oct	23	600	-2.2	81
279	1969 Oct	23	1200	2.2	66
280	1969 Oct	23	1800	5.6	62
281	1969 Oct	24	0	1.1	86
282	1969 Oct	24	600	-2.2	90
283	1969 Oct	24	1200	1.1	82
284	1969 Oct	24	1800	-0.6	85
285	1969 Oct	25	0	-1.7	84
286	1969 Oct	25	600	-2.2	94

287	1969 Oct	25	1200	-1.7	81
288	1969 Oct	25	1800	-2.8	80
289	1969 Oct	26	0	-3.9	87
290	1969 Oct	26	600	-5.0	92
291	1969 Oct	26	1200	-3.3	76
292	1969 Oct	26	1800	-3.9	83
293	1969 Oct	27	0	-4.4	85
294	1969 Oct	27	600	-5.0	86
295	1969 Oct	27	1200	1.7	78
296	1969 Oct	27	1800	0.0	80
297	1969 Oct	28	0	-5.6	92
298	1969 Oct	28	600	-5.0	89
299	1969 Oct	28	1200	2.8	64
300	1969 Oct	28	1800	2.2	66
301	1969 Oct	29	0	-1.1	79
302	1969 Oct	29	600	0.6	75
303	1969 Oct	29	1200	4.4	60
304	1969 Oct	29	1800	4.4	69
305	1969 Oct	30	0	5.0	74
306	1969 Oct	30	600	3.9	93
307	1969 Oct	30	1200	6.1	82
308	1969 Oct	30	1800	5.6	86
309	1969 Oct	31	0	4.4	93
310	1969 Oct	31	600	3.9	93
311	1969 Oct	31	1200	5.0	90
312	1969 Oct	31	1800	2.8	92
313	1969 Nov	1	0	-1.1	87
314	1969 Nov	1	600	0.6	91
315	1969 Nov	1	1200	1.7	92
316	1969 Nov	1	1800	1.7	92
317	1969 Nov	2	0	2.2	97
318	1969 Nov	2	600	2.2	95
319	1969 Nov	2	1200	2.8	90
320	1969 Nov	2	1800	2.2	92
321	1969 Nov	3	0	1.1	89
322	1969 Nov	3	600	-1.7	88
323	1969 Nov	3	1200	5.0	70
324	1969 Nov	3	1800	5.6	68
325	1969 Nov	4	0	2.8	82
326	1969 Nov	4	600	-2.8	89
327	1969 Nov	4	1200	10.0	46
328	1969 Nov	4	1800	7.2	50
329	1969 Nov	5	0	3.9	68
330	1969 Nov	5	600	3.9	83
331	1969 Nov	5	1200	8.3	67
332	1969 Nov	5	1800	10.0	69
333	1969 Nov	6	0	5.6	86
334	1969 Nov	6	600	0.6	100
335	1969 Nov	6	1200	11.1	64
336	1969 Nov	6	1800	6.1	80

337	1969 Nov	7	0	-0.6	88
338	1969 Nov	7	600	0.0	97
339	1969 Nov	7	1200	7.8	81
340	1969 Nov	7	1800	7.8	87
341	1969 Nov	8	0	5.6	100
342	1969 Nov	8	600	6.7	100
343	1969 Nov	8	1200	3.9	100
344	1969 Nov	8	1800	4.4	98
345	1969 Nov	9	0	5.0	100
346	1969 Nov	9	600	3.9	100
347	1969 Nov	9	1200	3.3	100
348	1969 Nov	9	1800	3.9	100
349	1969 Nov	10	0	4.4	100
350	1969 Nov	10	600	2.2	100
351	1969 Nov	10	1200	3.3	100
352	1969 Nov	10	1800	5.0	83
353	1969 Nov	11	0	1.7	89
354	1969 Nov	11	600	2.2	92
355	1969 Nov	11	1200	2.2	66
356	1969 Nov	11	1800	-1.7	95
357	1969 Nov	12	0	-3.3	86
358	1969 Nov	12	600	-3.3	81
359	1969 Nov	12	1200	-3.3	84
360	1969 Nov	12	1800	-3.9	94
361	1969 Nov	13	0	-5.0	76
362	1969 Nov	13	600	-9.4	73
363	1969 Nov	13	1200	-9.4	73
364	1969 Nov	13	1800	-9.4	73
365	1969 Nov	14	0	-8.3	81
366	1969 Nov	14	600	-8.9	79
367	1969 Nov	14	1200	-6.7	62
368	1969 Nov	14	1800	-6.7	77
369	1969 Nov	15	0	-6.7	82
370	1969 Nov	15	600	-5.0	83
371	1969 Nov	15	1200	-5.6	68
372	1969 Nov	15	1800	-9.4	78

8.2 Germination

			Germination (%)		
Date	**Week**	**Bin**	**NA**	**A**	**AI**
2008 Jul 24	0		94		
2008-07-29	0	3	98	96	93
2008-07-29	0	4	83	81	86
2008-07-29	0	5	92	90	82
2008-08-05	1	3	61	91	88
2008-08-05	1	4	58	81	86
2008-08-05	1	5	65	91	79
2008-08-12	2	3	10	74	70
2008-08-12	2	4	9	80	79
2008-08-12	2	5	12	76	66
2008-08-19	3	3	15	76	65
2008-08-19	3	4	13	44	66
2008-08-19	3	5	12	56	54
2008-08-26	4	3	15	33	39
2008-08-26	4	4	10	32	33
2008-08-26	4	5	10	37	31
2008-09-02	5	3	7	34	41
2008-09-02	5	4	11	26	34
2008-09-02	5	5	5	32	32
2008-09-10	6	3	1	25	27
2008-09-10	6	4	4	28	29
2008-09-10	6	5	1	26	24
2008-09-17	7	3	2	16	29
2008-09-17	7	4	1	28	25
2008-09-17	7	5	0	22	23
2008-09-24	8	3	0	17	15
2008-09-24	8	4	1	9	10
2008-09-24	8	5	0	15	14
2008-10-01	9	3	0	15	14
2008-10-01	9	4	0	9	9
2008-10-01	9	5	0	13	9
2008-10-08	10	3	0	8	9
2008-10-08	10	4	0	7	7
2008-10-08	10	5	0	13	7
2008-10-15	11	3	0	11	4
2008-10-15	11	4	0	9	7
2008-10-15	11	5	0	8	8

NA = *no airflow*; A = *airflow*; AI = *airflow inoculated*

8.3 FAV

			FAV Values								
			B3			B4			B5		
Date	Week	Rep	B3 NA	B3 A	B3 AI	B4 NA	B4 A	B4 AI	B5 NA	B5 A	B5 AI
2008 Jul 29	0	1	4.87	5.84	3.90	3.89	2.92	4.87	6.82	5.84	4.87
2008 Jul 29	0	2	4.86	6.82	3.90	5.84	5.84	6.82	4.88	4.88	5.84
2008 Jul 29	0	3	4.87	5.84	2.92				6.83	4.88	4.88
2008 Aug 06	1	1	8.76	9.76	5.84	7.80	5.85	8.78	5.85	5.84	4.88
2008 Aug 06	1	2	8.78	5.85	6.83	7.80	8.76	7.79	6.82	3.91	4.87
2008 Aug 06	1	3	8.77	5.85	6.83	8.79	7.79	9.74	5.86	4.87	5.85
2008 Aug 12	2	1	26.29	7.80	7.78	25.36	6.83	7.80	27.30	7.80	9.76
2008 Aug 12	2	2	24.39	9.74	10.72	21.44	6.82	6.83	28.26	7.80	10.73
2008 Aug 12	2	3	26.30	10.73	6.82	30.22	6.82	6.82	26.29	8.77	11.71
2008 Aug 19	3	1	30.20	7.81	8.77	27.31	8.78	9.75	28.26	8.77	9.75
2008 Aug 19	3	2	29.25	6.83	8.77	31.20	9.75	6.83	30.23	8.78	11.69
2008 Aug 19	3	3	31.19	6.83	8.77	29.25	7.80	8.78	29.23	10.73	11.71
2008 Aug 26	4	1	38.04	13.63	10.72	34.08	11.68	9.75	34.15	8.77	11.71
2008 Aug 26	4	2	38.02	11.71	12.66	34.12	10.72	10.73	37.06	8.76	9.74
2008 Aug 26	4	3	35.12	12.68	8.78	34.12	10.72	9.75	38.94	9.75	10.72
2008 Sep 02	5	1	39.01	14.61	11.70	33.13	10.71	11.70	36.09	10.71	12.68
2008 Sep 02	5	2	37.05	12.68	12.66	34.14	10.72	10.73	37.01	11.72	11.70
2008 Sep 02	5	3	35.12	12.68	10.73	35.15	12.67	10.72	37.08	10.72	11.70
2008 Sep 10	6	1	38.91	9.72	9.74	37.00	10.73	8.77	42.81	14.61	15.58
2008 Sep 10	6	2	42.82	11.66	11.67	45.83	13.66	12.64	49.72	18.49	17.54
2008 Sep 10	6	3	38.96	14.62	14.60	38.96	14.60	13.63	42.88	17.55	17.53
2008 Sep 17	7	1	31.16	17.52	10.71	32.18	15.57	8.77	31.18	17.53	11.69
2008 Sep 17	7	2	35.09	13.64	14.61	38.02	16.55	12.66	37.05	15.59	13.63
2008 Sep 17	7	3	31.20	11.69	16.56	36.08	15.60	11.70	36.04	13.64	16.56
2008 Sep 24	8	1	36.09	14.61	15.60	28.29	14.61	11.71	42.90	17.53	17.54
2008 Sep 24	8	2	34.14	13.63	19.48	27.31	12.66	12.67	44.86	12.66	19.49
2008 Sep 24	8	3	40.00	14.61	17.54	34.14	11.68	16.58	47.81	14.61	14.62
2008 Oct 01	9	1	38.98	27.31	29.25	42.94	21.45	27.29	42.88	24.37	27.29
2008 Oct 01	9	2	39.03	26.33	31.18	35.10	22.43	26.31	44.83	22.42	26.33
2008 Oct 01	9	3	38.03	26.33	30.22	39.02	23.42	26.32	47.80	21.44	26.33
2008 Oct 08	10	1	43.90	27.31	28.26	43.85	27.29	29.23	43.84	30.22	29.26
2008 Oct 08	10	2	42.91	25.35	31.20	41.89	25.34	26.31	44.85	22.40	29.25
2008 Oct 08	10	3	43.85	25.35	34.12	41.91	26.32	28.26	44.86	25.35	28.27
2008 Oct 15	11	1	41.95	34.13	32.20	44.83	37.08	29.24	42.90	31.22	34.13
2008 Oct 15	11	2	43.87	33.09	43.91	41.91	34.12	31.18	39.99	31.21	33.15
2008 Oct 15	11	3	41.96	38.01	43.89	43.89	33.18	31.16	43.91	32.14	31.17
2008 Oct 22	12	1	45.84	28.30	31.17	48.76	39.94	35.07	41.91	34.14	37.01
2008 Oct 22	12	2	41.89	28.26	33.11	45.84	40.99	36.08	45.86	34.09	31.22
2008 Oct 22	12	3	49.72	29.24	35.08	48.78	41.90	35.07	43.89	36.10	38.03

NA = *no airflow*; A = *airflow*; AI = *airflow inoculated*

8.4 Moisture content

			MC (% WB)								
			B3			B4			B5		
Date	Week	Rep	B3 NA	B3 A	B3 AI	B4 NA	B4 A	B4 AI	B5 NA	B5 A	B5 AI
2008 Jul 29	0	1	18.63	18.57	18.67	18.68	18.43	18.08	18.33	18.71	18.60
2008 Jul 29	0	2	18.54	18.61	18.65	18.65	18.45	18.15	18.31	18.32	18.45
2008 Jul 29	0	3	18.64	18.54	18.60	18.60	18.45	17.95	18.55	18.40	18.40
2008 Jul 29	0	4	18.67	18.66	18.69	18.60	18.42	17.90	18.37	18.32	18.35
2008 Aug 06	1	1	17.69	16.24	15.89	17.82	16.34	16.03	17.78	16.07	15.76
2008 Aug 06	1	2	17.76	15.86	15.84	17.77		16.02	17.81	16.11	15.74
2008 Aug 06	1	3	17.81	15.89	15.85	17.71	16.33	16.00	17.78	16.03	15.70
2008 Aug 06	1	4	17.74	15.88	15.85	17.71	16.33	15.89	17.81	16.09	15.63
2008 Aug 12	2	1	17.87	16.35	16.30	17.74	16.44	16.27	17.59	16.19	16.19
2009 Aug 12	2	2	17.47	16.24	16.31	17.85	16.31	16.27	18.04	16.22	16.25
2008 Aug 12	2	3	17.42	16.39	16.71	17.90	16.36	16.23	17.61	16.13	16.24
2008 Aug 12	2	4	17.38	16.42	16.19	17.96	16.41	16.20	17.63	16.13	16.26
2008 Aug 19	3	1	16.88	17.21	16.94	17.32	17.09	17.30	17.08	17.08	17.12
2009 Aug 19	3	2	16.81	17.18	16.88	17.40	17.10	17.48	17.05	17.12	16.98
2008 Aug 19	3	3	16.53	16.41	16.84	17.33	17.27	17.18	16.93	17.11	16.99
2008 Aug 19	3	4	16.53	17.39	16.78	17.13	17.26	16.69	16.96	17.15	17.03
2008 Aug 26	4	1	16.85	17.50	17.40	16.71	16.95	17.32	17.08	17.81	17.44
2009 Aug 26	4	2	16.96	17.42	17.40	16.81	17.31	17.27	16.68	17.34	17.39
2008 Aug 26	4	3	16.86	17.60	17.81	16.24	17.35	17.27	16.63	17.33	17.39
2008 Aug 26	4	4	16.90	17.57	16.53	16.34	17.15	16.64	16.66	14.53	17.35
2008 Sep 02	5	1	17.25	17.19	17.13	16.67	17.16	17.20	16.73	16.69	16.72
2008 Sep 02	5	2	17.32	17.38	17.17	16.67	17.10	17.17	16.71	16.77	16.68
2008 Sep 02	5	3	17.40	17.18	17.08	16.57	17.09	17.11	16.81	16.75	16.72
2008 Sep 10	6	1	16.51	16.19	16.78	17.00	16.52	16.00	16.91	15.83	15.76
2008 Sep 10	6	2	16.57	16.16	16.72	16.96	16.98	16.05	17.04	15.87	15.88
2008 Sep 10	6	3	16.63	16.07	16.70	16.99	16.44	16.05	17.01	15.78	15.86
2008 Sep 17	7	1	16.36	16.04	16.36	16.79	16.09	16.12	17.46	14.57	15.88
2008 Sep 17	7	2	16.38	15.93	16.38	16.84	16.10	16.13	17.49	17.14	15.96
2008 Sep 17	7	3	16.42	15.96	16.42	16.87	16.14	16.07	17.50	15.73	16.54
2008 Sep 24	8	1	16.68	16.59	16.71	16.64	16.72	16.74	16.39	16.52	16.69
2008 Sep 24	8	2	16.71	16.61	16.63	16.61	16.65	16.71	16.43	16.64	16.66
2008 Sep 24	8	3	16.70	16.56	16.52	16.63	16.67	16.72	16.45	16.62	16.63
2008 Oct 01	9	1	18.78	17.32	17.19	17.85	18.86	17.58	19.42	18.25	18.00
2008 Oct 01	9	2	18.65	17.60	17.45	17.82	18.69	17.32	19.52	18.29	18.16
2008 Oct 01	9	3	18.72	18.03	17.36	17.87	18.97	17.21	19.56	18.22	15.36
2008 Oct 08	10	1	17.57	16.83	16.25	16.67	17.12	16.88	17.58	17.21	16.78
2008 Oct 08	10	2	17.42	16.86	16.26	16.34	17.07	16.86	17.73	17.31	16.79
2008 Oct 08	10	3	16.69	16.80	16.14	16.63	17.12	16.90	17.86	17.15	16.78
2008 Oct 15	11	1	17.02	16.60	16.53	16.86	16.42	16.68	17.07	16.57	17.03
2008 Oct 15	11	2	16.95	16.61	16.52	16.60	16.53	16.64	17.04	16.64	17.03
2008 Oct 15	11	3	16.85	16.56	16.44	16.95	16.45	16.77	17.10	16.59	16.99
2008 Oct 22	12	1	16.82	16.56	16.16	16.68	15.91	16.55	16.74	16.43	16.63
2008 Oct 22	12	2	17.01	16.62	16.15	16.55	15.88	16.57	16.73	16.06	16.58
2008 Oct 22	12	3	17.12	16.67	16.19	16.54	15.90	16.51	16.88	16.16	16.46

NA = *no airflow*; A = *airflow*; AI = *airflow inoculated*

9 APPENDIX 2 – Bin Study 3 Raw Data

9.1 Germination

			Germination (%)		
Date	**Week**	**Bin**	**NA**	**A1**	**A2**
2009 Jul 09	0	3	91	79	85
2009 Jul 09	0	4	80	83	78
2009 Jul 09	0	5	81	87	84
2009 Jul 15	1	3	84	78	82
2009 Jul 15	1	4	82	74	81
2009 Jul 15	1	5	74	74	74
2009 Jul 22	2	3	8	8	10
2009 Jul 22	2	4	1	8	7
2009 Jul 22	2	5	13	12	11
2009 Jul 29	3	3	3	4	2
2009 Jul 29	3	4	6	3	5
2009 Jul 29	3	5	6	3	8
2009 Aug 04	4	3	4	3	2
2009 Aug 04	4	4	5	4	0
2009 Aug 04	4	5	2	3	3
2009 Aug 12	5	3	8	2	3
2009 Aug 12	5	4	2	3	4
2009 Aug 12	5	5	5	1	0
2009 Aug 19	6	3	2	0	5
2009 Aug 19	6	4	2	2	1
2009 Aug 19	6	5	4	3	2
2009 Aug 26	7	3	3	1	1
2009 Aug 26	7	4	2	4	1
2009 Aug 26	7	5	1	2	1
2009 Sep 02	8	3	4	0	0
2009 Sep 02	8	4	0	0	2
2009 Sep 02	8	5	2	2	0
2009 Sep09	9	3	2	0	1
2009 Sep09	9	4	3	2	0
2009 Sep09	9	5	1	3	2
2009 Sep 17	10	3	1	1	1
2009 Sep 17	10	4	3	1	0
2009 Sep 17	10	5	3	2	1
2009 Sep 24	11	3	2	0	1
2009 Sep 24	11	4	3	2	0
2009 Sep 24	11	5	1	1	2
2009 Sep 29	12	3	1	1	0
2009 Sep 29	12	4	2	1	0
2009 Sep 29	12	5	2	2	1

NA = *no airflow*; A1 = *airflow 1*; A2 = *airflow 2*

9.2 FAV

			FAV Values								
			B3			B4			B5		
Date	Week	Rep	B3 NA	B3 A1	B3 A2	B4 NA	B4 A1	B4 A2	B5 NA	B5 A1	B5 A2
2009 Jul 09	0	1	6.83	3.90	6.82	11.70	6.83	6.82	9.74	9.76	3.90
2009 Jul 09	0	2	3.90	2.92	7.80	8.77	3.90	10.73	9.75	9.74	15.59
2009 Jul 09	0	3	8.77	1.95	6.82	10.72	8.77	12.67	9.74	7.80	7.80
2009 Jul 15	1	1	4.58	9.76	10.43	8.09	10.72	9.45	12.67	10.72	10.71
2009 Jul 15	1	2	4.58	10.72	9.45	2.83	6.82	6.53	10.72	10.72	9.75
2009 Jul 15	1	3	10.43	9.75	11.41	3.61	4.87	6.53	8.77	6.82	7.79
2009 Jul 22	2	1	35.10	37.98	27.31	30.21	32.15	41.93	30.20	32.15	32.15
2009 Jul 22	2	2	40.91	31.17	27.28	32.17	23.40	39.94	29.22	32.19	35.08
2009 Jul 22	2	3	35.05	35.06	27.29	30.21	33.13	33.15	27.31	31.20	38.01
2009 Jul 29	3	1	50.66	48.76	38.98	42.90	46.76	37.01	44.87	38.97	50.71
2009 Jul 29	3	2	40.92	48.74	37.03	42.88	44.86	42.89	46.74	37.04	48.77
2009 Jul 29	3	3	35.09	44.85	44.81	40.95	42.90	35.07	62.37	37.00	52.59
2009 Aug 04	4	1	54.61	56.04	52.62	44.85	52.15	44.33	58.45	60.38	58.51
2009 Aug 04	4	2	58.48	50.19	56.51	64.36	50.17	54.60	58.44	56.48	58.48
2009 Aug 04	4	3	58.47	56.06	54.54	60.44	54.06	46.77	58.45	58.44	60.41
2009 Aug 12	5	1	62.33	56.02	54.54	46.77	50.18	50.22	62.37	66.24	70.17
2009 Aug 12	5	2	62.32	54.11	60.43	60.41	50.19	44.87	62.43	62.33	74.03
2009 Aug 12	5	3	58.42	44.35	62.42	64.27	44.34	52.65	64.34	66.25	70.12
2009 Aug 19	6	1	58.49	56.05	58.49	56.49	54.11	44.38	62.37	64.34	68.16
2009 Aug 19	6	2	62.38	50.22	52.63	60.44	52.18	54.61	60.47	56.56	50.67
2009 Aug 19	6	3	62.38	48.26	60.38	58.51	50.24	58.47	64.31	60.48	56.49
2009 Aug 26	7	1	54.55	44.38	58.46	56.57	54.09	42.40	52.66	52.66	62.36
2009 Aug 26	7	2	60.48	50.15	58.49	60.42	50.20	52.67	64.33	58.52	58.46
2009 Aug 26	7	3	60.42	50.16	56.50	58.42	48.21	56.56	60.44	56.49	54.58
2009 Sep 02	8	1	54.62	42.38	58.44	52.65	50.21	54.08	64.36	60.44	54.57
2009 Sep 02	8	2	52.60	50.24	58.46	62.41	52.16	56.57	58.53	58.49	56.53
2009 Sep 02	8	3	56.49	42.43	58.53	54.58	59.95	56.52	72.07	50.66	54.62
2009 Sep09	9	1	60.42	54.06	62.39	62.34	56.04	59.98	48.78	64.32	52.67
2009 Sep09	9	2	56.58	59.98	58.53	58.48	56.07	58.52	72.07	64.36	62.36
2009 Sep09	9	3	64.28	58.01	58.46	60.41	56.04	64.38	68.27	68.18	70.17
2009 Sep 17	10	1	61.58	50.03	59.39	59.45	52.28	54.45	61.60	61.60	63.79
2009 Sep 17	10	2	63.87	52.26	61.68	63.80	54.47	59.47	57.26	59.44	59.47
2009 Sep 17	10	3	59.44	54.50	63.83	59.44	54.42	57.17	59.47	61.63	61.65
2009 Sep 24	11	1	57.20	62.92	61.60	59.47	62.95	60.71	61.61	61.68	59.47
2009 Sep 24	11	2	63.80	58.52	61.59	59.43	67.39	59.41	61.63	59.40	59.44
2009 Sep 24	11	3	61.59	58.53	61.61	61.65	67.32	61.65	63.79	59.39	61.56
2009 Sep 29	12	1	61.68	52.83	66.07	61.63	55.03	50.63	66.04	61.64	61.61
2009 Sep 29	12	2	63.87	55.06	63.88	63.81	48.41	63.83	59.40	63.85	61.59
2009 Sep 29	12	3	63.80	50.64	63.85	63.80	50.60	61.61	57.27	61.59	63.83

NA = *no airflow*; A1 = *airflow 1*; A2 = *airflow 2*

9.3 Moisture Content

			MC (% WB)								
			B3			B4			B5		
Date	Week	Rep	B3 NA	B3 A1	B3 A2	B4 NA	B4 A1	B4 A2	B5 NA	B5 A1	B5 A2
2009 Jul 09	0	1	17.76	16.95	16.96	17.62	16.97	17.03	17.06	16.92	16.87
2009 Jul 09	0	2	17.58	17.07	31.08	17.96	16.87	16.99	17.04	17.00	16.94
2009 Jul 09	0	3	17.75	17.10	16.91	17.91	16.91	17.01	16.98	17.01	16.97
2009 Jul 15	1	1	17.60	17.31	17.06	17.56	17.25	17.20	17.47	17.58	17.39
2009 Jul 15	1	2	17.50	14.62	17.01	17.40	17.27	17.13	17.45	17.60	17.39
2009 Jul 15	1	3	17.37	17.35	17.15	17.77	17.27	17.20	17.44	17.42	17.30
2009 Jul 22	2	1	17.88	16.80	16.84	17.35	17.17	17.72	17.29	17.07	17.10
2009 Jul 22	2	2	17.71	16.94	16.87	17.27	17.21	17.73	17.33	17.23	17.09
2009 Jul 22	2	3		16.82	16.95		17.22	17.67	17.39	17.05	17.18
2009 Jul 29	3	1	16.66	17.48	17.00	16.50	17.29	17.43	16.47	17.31	17.31
2009 Jul 29	3	2	16.62	17.46		16.39	17.26	17.54	16.50	17.21	17.41
2009 Jul 29	3	3	16.51	17.49	17.02	16.68	17.38	17.09	16.52	17.27	17.31
2009 Aug 04	4	1	16.57	16.56	17.37	16.75	17.61		16.52	17.83	18.24
2009 Aug 04	4	2	16.59	17.90	17.33	16.71	17.52	17.63	16.42	17.86	18.17
2009 Aug 04	4	3	16.42	17.86	17.41	16.68	17.69	17.63	16.43	17.88	18.18
2009 Aug 12	5	1		18.20	17.89	17.09	17.96	17.92	16.56	18.20	18.23
2009 Aug 12	5	2	16.57	18.18	17.70	17.27	17.79	17.73	16.39	18.15	18.26
2009 Aug 12	5	3	16.57	18.07	17.66		17.95	17.98	16.43	18.11	18.38
2009 Aug 19	6	1	18.61	18.61	17.94	17.52	18.17	18.18	16.70	18.27	18.63
2009 Aug 19	6	2	18.41	18.41	17.83	17.59	18.15	18.33	17.53	18.30	18.62
2009 Aug 19	6	3	18.43	18.43	17.89	17.49	18.24	18.35	15.65	18.37	18.56
2009 Aug 26	7	1	16.84	18.02	18.94	16.34	18.15	18.12	16.91	18.16	18.28
2009 Aug 26	7	2	16.93	18.15	17.05	16.30	18.37	18.15	16.93	18.14	18.27
2009 Aug 26	7	3	16.86	18.18	18.02	16.37	17.77	18.11	16.91	18.23	18.30
2009 Sep 02	8	1	17.47	18.30	18.01	17.34	17.95	18.48	16.69	17.93	18.29
2009 Sep 02	8	2	17.36	18.26	17.90	17.08	17.98	18.15	16.79	18.16	17.97
2009 Sep 02	8	3	17.34	18.12	17.92	18.94	17.79	18.36	16.61	18.10	17.96
2009 Sep09	9	1	17.03	18.78	18.42	17.65	18.31	18.73	17.68	18.29	18.64
2009 Sep09	9	2	17.28	18.88	18.52	17.64	18.32	18.86	17.02	18.46	18.41
2009 Sep09	9	3	17.29	18.70	18.48	17.80	18.11	18.97	16.50	18.52	18.31
2009 Sep 17	10	1	16.96	16.86	16.35	16.04	16.64	16.12	16.68	16.56	16.20
2009 Sep 17	10	2	16.69	16.38	16.85	17.57	16.35	16.27	16.82	16.51	16.66
2009 Sep 17	10	3		16.65	16.59	20.11	16.11	16.06	16.39	16.70	16.69
2009 Sep 24	11	1	16.47	16.87	16.47	16.65	16.28	16.90	15.88	16.54	16.38
2009 Sep 24	11	2	16.50	16.74	16.65	16.39	16.79	16.06	15.85	16.33	16.52
2009 Sep 24	11	3	16.44	16.85	16.73	16.15	16.36	16.79	15.88	16.10	16.86
2009 Sep 29	12	1		17.17	17.20	17.66	17.05	16.99	16.98	17.51	16.90
2009 Sep 29	12	2	17.60	17.12	17.18	17.55	17.09	17.06	16.95	17.49	17.08
2009 Sep 29	12	3	17.64	17.10	17.07	17.53	17.04	17.07	16.88	16.98	17.09

NA = *no airflow*; A1 = *airflow 1*; A2 = *airflow 2*

10 APPENDIX 3 – Chamber Study Raw Data

10.1 Germination

			Germination (%)	
Date	**Week**	**Bin**	**NA**	**A**
2008 Aug 29	0	1	91	90
2008 Aug 29	0	2	92	91
2008 Aug 29	0	3	90	92
2008 Sep 03	1	1	81	84
2009 Sep 03	1	2	86	88
2010 Sep 03	1	3	84	89
2008 Sep 10	2	1	68	78
2009 Sep 10	2	2	67	69
2010 Sep 10	2	3	68	71
2008 Sep 18	3	1	45	48
2009 Sep 18	3	2	56	60
2010 Sep 18	3	3	58	56
2008 Sep 25	4	1	40	42
2009 Sep 25	4	2	32	34
2010 Sep 25	4	3	30	32
2008 Oct 02	5	1	18	20
2009 Oct 02	5	2	16	16
2010 Oct 02	5	3	12	20
2008 Oct 09	6	1	7	12
2009 Oct 09	6	2	8	8
2010 Oct 09	6	3	5	6
2008 Oct 16	7	1	3	5
2009 Oct 16	7	2	4	7
2010 Oct 16	7	3	3	5
2008 Oct 23	8	1	4	5
2009 Oct 23	8	2	2	4
2010 Oct 23	8	3	4	4
2008 Oct 30	9	1	3	4
2009 Oct 30	9	2	2	5
2010 Oct 30	9	3	4	5
2008 Nov 06	10	1	4	4
2009 Nov 06	10	2	1	3
2010 Nov 06	10	3	4	4
2008 Nov 13	11	1	2	3
2009 Nov 13	11	2	2	3
2010 Nov 13	11	3	1	2
2008 Nov 22	12	1	0	0
2009 Nov 22	12	2	1	2
2010 Nov 22	12	3	0	1

NA = *no airflow*; A = *airflow*

10.2 FAV

			FAV Values					
			B1		B2		B3	
Date	Week	Rep	B1 NA	B1 A	B2 NA	B2 A	B3 NA	B3 A
2008 Aug 29	0	1	9.75	8.77	8.78	8.78	10.71	8.78
2008 Aug 29	0	2	9.74	8.78	9.75	9.75	9.74	10.73
2008 Aug 29	0	3	8.77	8.78	8.77	7.80	8.78	8.77
2008 Sep 03	1	1	7.79	9.75	9.74	9.75	3.90	7.80
2008 Sep 03	1	2	7.79	11.68	9.76	7.79	7.79	7.80
2008 Sep 10	2	1	13.65	15.60	11.70	7.80	9.75	9.74
2008 Sep 10	2	2		15.60	13.65	9.74	5.85	9.74
2008 Sep 18	3	1	9.74	11.69	9.75	9.75	11.69	9.74
2008 Sep 18	3	2	7.80	11.70	9.75	11.70	19.48	11.68
2008 Sep 25	4	1	13.64	15.60	10.73	17.55	9.76	12.67
2008 Sep 25	4	2	14.63	16.57	9.75	16.58	9.75	12.66
2008 Sep 25	4	3	13.64	13.65	10.72	16.56	11.70	13.64
2008 Oct 02	5	1	27.31	35.09	29.23	31.19	27.28	29.27
2008 Oct 02	5	2	25.33	33.15	25.33	25.32	35.10	33.15
2008 Oct 09	6	1	37.01	38.97	35.08	37.02	35.10	42.92
2008 Oct 09	6	2	35.11	37.07	37.05	42.88	39.01	40.94
2008 Oct 16	7	1	44.83	39.00	36.07	39.94	39.97	40.00
2008 Oct 16	7	2	40.00	40.97	39.01	39.00	41.95	40.96
2008 Oct 16	7	3	39.98	40.97	38.00	38.04	41.91	39.96
2008 Oct 23	8	1	42.84	48.73	42.85	46.78	44.83	35.09
2008 Oct 23	8	2	44.79	46.79	40.95	46.80	50.72	44.84
2008 Oct 30	9	1	46.31	51.14	50.16	54.10	51.20	44.36
2008 Oct 30	9	2	48.23	49.24	48.27	54.10	51.17	48.24
2008 Nov 06	10	1	54.54	52.65	50.69	48.76	38.01	49.73
2008 Nov 06	10	2	54.61	50.66	51.65	50.64	50.71	48.76
2008 Nov 13	11							
2008 Nov 13	11							
2008 Nov 13	11							
2008 Nov 22	12	1	44.83	46.80	45.79	43.80	10.71	8.78
2008 Nov 22	12	2	45.84	47.75	44.81	44.87	9.74	10.73
2008 Nov 22	12	3	45.84	46.78	45.80	43.87	8.78	8.77

NA = *no airflow*; A = *airflow*

10.3 Moisture content

			MC (% WB)					
			B1		B2		B3	
Date	Week	Rep	B1 NA	B1 A	B2 NA	B2 A	B3 NA	B3 A
2008 Aug 29	0	1	20.87	20.42				
2008 Aug 29	0	2	20.83	20.08				
2008 Aug 29	0	3	20.45	21.06				
2008 Aug 29	0	4	20.44	20.76				
2008 Aug 29	0	5	20.23	20.85				
2008 Sep 03	1	1	20.46	20.69	20.43	20.39	20.38	20.84
2009 Sep 03	1	2	20.53	20.19	20.67	20.61	20.79	20.52
2010 Sep 03	1	4	20.54	21.13	20.68	20.64	20.77	20.63
2008 Sep 10	2	1	20.15	20.75	20.68	20.72	21.01	20.76
2009 Sep 10	2	2	20.66	20.64	20.68	20.67	20.38	20.42
2010 Sep 10	2	4	21.02	20.61	20.16	20.67	20.52	20.71
2008 Sep 18	3	1	20.53	20.67	20.60	20.64	20.63	20.63
2009 Sep 18	3	2	20.51	20.69	20.58	20.70	20.54	20.62
2010 Sep 18	3	4	20.52	20.67	20.56	20.62	20.59	20.61
2008 Sep 25	4	1	20.77	20.53	20.56	20.69	20.48	20.61
2009 Sep 25	4	2	20.58	20.65	20.51	20.69	20.52	20.65
2010 Sep 25	4	4	20.39	20.73	20.53	20.60	20.51	20.74
2008 Oct 02	5	1	21.04	20.89	20.75	20.84	20.93	20.83
2009 Oct 02	5	2	20.80	20.69	20.80	20.71	20.80	20.70
2010 Oct 02	5	3	20.96	20.80	20.84	20.75	20.88	20.82
2008 Oct 09	6	1	20.83	20.87	20.66	20.61	20.66	20.65
2009 Oct 09	6	2	20.70	20.82	20.68	20.78	20.59	20.69
2010 Oct 09	6	3	20.59	20.87	20.59	20.84	20.51	20.73
2008 Oct 16	7	1	20.74	21.01	20.92	20.93	20.79	20.93
2009 Oct 16	7	2	20.65	20.88	20.81	21.00	20.77	20.88
2010 Oct 16	7	3	20.61	20.92	20.78	20.97	20.77	20.93
2008 Oct 23	8	1	20.80	20.88	20.66	20.92	20.78	20.88
2009 Oct 23	8	2	20.73	20.78	20.67	20.80	20.69	20.83
2010 Oct 23	8	3	20.79	20.78	20.49	20.87	20.68	20.97
2008 Oct 30	9	1	20.80	20.65	20.67	20.37	20.91	20.75
2009 Oct 30	9	2	20.79	20.64	20.59	20.77	20.88	20.64
2010 Oct 30	9	3	20.79	20.70	20.67	20.74	20.89	20.88
2008 Nov 06	10	1	20.70	20.63	20.93	20.80	20.61	20.80
2009 Nov 06	10	2	20.74	20.87	20.61	20.92	20.68	20.81
2010 Nov 06	10	3	20.62	20.56	20.57	20.67	20.61	20.91
2008 Nov 13	11	1	20.80	20.74	20.76	20.44	21.02	20.67
2009 Nov 13	11	2	20.74	20.65	20.86	20.94	20.91	20.64
2010 Nov 13	11	3	20.82	20.59	20.80	20.67	20.98	20.67
2008 Nov 22	12	1	20.80	20.61	20.66	20.69	20.87	20.67
2009 Nov 22	12	2	20.50	20.75	20.81	20.69	20.75	20.63
2010 Nov 22	12	3	20.80	20.57	20.63	20.60	20.74	20.72

NA = *no airflow*; A = *airflow*

11 APPENDIX 4 - Determination of Ochratoxin A, B and Zearalenone in Grains by HPLC with Fluorescence Detection

Provided by Mike Roscoe, Canadian Grain Commission (2010)

Analyte:
Ochratoxin - A: C20H18ClNO6 FW 403.8
B: C20H19NO6 FW 369.4
Zearalenone - C18H24O5 FW 320.4

These compounds are part of a family of compounds called mycotoxins. Mycotoxins are secondary metabolites produced by fungi that have adverse biological effects in animals and or man. For example, Ochratoxin A is embryotoxic and teratogenic affecting primarily the kidneys, Zearalenone is estrogenic causing reproductive problems in farm animals.

Substrates Covered:
Cereal, oilseed and pulse crops.

Analytical Approach

The method employed by the Grain Research Lab for the determination of these mycotoxins is based on the method "**High-performance liquid chromatographic determination of zearalenone and ochratoxin A in cereals and feeds",** W. LANGSETH, Y. ELLINGSEN, U. NYMOEN AND E. M. OKLAND, Journal of Chromatography, 478 (1989) 269-274, which involves a diphasic extraction of a 30 G sample with 0.1M H3PO4 and Chloroform in a 250 mL Teflon centrifuge bottle. The sample is shaken for 45 min and centrifuged at 4° C at 3000 rpm for 10 min. The organic layer is filtered and 25 mL of this extract is concentrated to dryness and reconstitued in 10 mL Dichloromethane. This portion of the sample is then cleaned-up on a silica gel Sep-Pak cartridge which once loaded with the sample and eluted with different strength solvents will produce an eluent containing Ochratoxin A,B and Zearalenone. The extract is dried under N2, reconstituted in 50/50 acetonitrile/0.5% acetic acid, filtered with a 0.4um PVDF filters and injected on the HPLC with fluorescence detection.

Compound	GRL Detection Limit ppb
Ochratoxin	
A	1
B	1
Zearalenone	10

Apparatus

(a) **Grinder -** Romer Series II Mill
Lab Mill 3100
Retsch Rotor Beater Mill- SR 300

Adjust grinder control so that at least 80% of the ground grain will pass through a twenty-mesh sieve.

(b) **Rotary Sample Divider**- Materials Sampling Solutions, 1287 Harriet Ave., Driehoek, Germiston, 1400 Gauteng, South Africa.

(c) **Shaker -** Reciprocating, flat bed (Eberback Model 6010 with two speeds: low-189 excursions per min and high-280 excursions per min.)
(d) **Electronic Balances -** (1) Top loader, readability 0.01g (Denver Instruments, Model P2002);
(2) Analytical, readability 0.1mg (A&D Company, Ltd. Model; ER-182A)

(e) **Centrifuge -** Refrigerated, benchtop type capable of spinning at 3000 rpm (Thermo IEC Centra CL3R) horizontal rotor and four buckets fitted with polypropylene bottle sleeves.

(f) **Rotary Evaporator -** Buchi brinkman Model R-124 and R-205. Connect an aspirator pump (Cole-Parmer Model 7049-00) to rotary evaporator to provide vacuum and a Lauda-RM6 (Brinkman) cold recirculating bath connected to the condensor.

(g) **Vacuum manifold for Sep-Pak cleanup -** Supelco Visiprep-D-L model 5-7044

(h) **Membrane Filters -** Nylaflo (Nylon) 0.2um 47mm and Acrodisc LC13 PVDF 0.45um 13mm filters (Gelman Sciences).

(i) **Micropipettes** – 10-100uL and 25-250ul Brand Tech Transferpette, 100-100uL Eppendorf and 5mL adjustable Socorex micropipette.

(j) **Glassware per Sample -**

1) 1- 250mL PPCO centrifuge bottle (Nalgene)
(1-1000mL PPCO centrifuge bottle (Nalgene))
2) 1 glass funnel, 6cm I.D.
3) 1-250mL glass Erlenmeyer flask
4) 1-15mL sample tube

(k) **HPLC System -** Waters Acquity UPLC Binary Solvent Manager, Sample Manager and UPLC Fluorescence detector or LCM1 system equipped with a 715 autosampler and a Waters 474 scanning fluorescence detector.

Reagents

(a) **Chloroform –** Certified ACS, 100.0%

(b) **Methanol -** HPLC grade, 99.9%

(c) **Acetonitrile** - HPLC grade, 99.9%

(d) **Acetic Acid**, glacial - HPLC grade Certified ACS

(e) **Hexane** – Certified ACS, 99.9%

(f) **Toluene** - HPLC grade, 99.99%

(g) **Dichloromethane –** HPLC grade, 99.96%

(h) **Water -** All water used is that from a purification system capable of producing 18.0 MΩ.cm water.

(i) **Phosphoric acid -** H3PO4, A.C.S. 85%

(j) **Celite 545** - (Fisher Scientific Cat# C212-500)

Solutions

Note: Filter all aqueous and organic solutions for the HPLC through Nylaflo 0.2um 47mm nylon membrane filters.

(a) **Moblie Phase A:** (LCM1) Adjust the pH of 2L of Milli-Q water to 3.3 with Acetic Acid, glacial.
(Acquity UPLC) Adjust the pH of 2L of Milli-Q water to 3.9 with Acetic Acid, glacial.

(b) **Mobile Phase B**: (LCM1/Acquity UPLC) Acetonitrile HPLC Grade

(c) **Seal Wash Solution:** (10% methanol/water) Add 50mL to 450mL Milli-Q water and

(d) **0.5% Acetic Acid** (v/v): Add 0.5mL Acetic Acid, glacial, to a 10mL volumetric flash and bring to volume with Milli-Q water.

(e) **0.1M Phosphoric Acid** (w/v): Weigh 11.5g of H3PO4, A.C.S. 85%, into a 1L volumetric flask and bring to volume with Milli-Q water.

(f) **Toluene/Acetic Acid** 9/1 (v/v): Add 10mL Acetic Acid, glacial, to a 100mL volumetric flask and bring to volume with Toluene.

Standards

Ochratoxin A, B and Zearalenone

(1) **Ochratoxin A -** Crystalline, Benzene free, (Sigma P.N. O 1877)

(2) **Ochratoxin B -** (Sigma P.N. O 1382)

(3) **Zearalenone -** Crystalline (Sigma P.N. Z 2125)

(4) **Stock Solutions (200ng/uL)** - Weigh approximately 1 mg of reference standard into a 5mL volumetric flask. Dissolve and dilute to volume with toluene/ acetonitrile 95/5 (v/v). Stock standards are stored at 0° C and made up yearly. Stock standard concentrations are verified using a UV Spectrometer.

(5) **Injection Mixes -** Appropriate aliquots of the stock solutions are dried under N2 and diluted in 1:1 acetonitrile/0.5% Acetic Acid to give working standard concentrations of 0.002-.08 ng/uL. Ochratoxin A and B and 0.02-0.5 ng/uL Zearalenone. A calibration curve is generated for each compound by injecting a minimum of four standards in this concentration range. These standards are prepared daily.

Sample Handling

Ideally store sample in a freezer until required for analysis. Division of grain samples prior to grinding should be carried out with the aid of a sample divider. When subsampling whole grain, if the use of a sample divider is not feasible, the sample should be mixed and small portions removed from different locations throughout the sample container.

Sample Preparation

Grinding

(2-5Kg samples) Romer series II mill, (10Kg samples) Lab Mill 3100 or Retsch SR 300 mill.

Mixing/Dividing

For 2-5Kg samples, the splitting/sub-sampling feature of the Romer series II mill is used, where a 1/10 split is collected which would give you 200-500g ground test sample.
For 10Kg samples, after the entire samples is ground on either the Lab Mill 3100 or Retsch SR 300, it is placed into the Rotary sample divider which will give ten 1Kg sub-samples. This process is done twice. Then one of the 1Kg samples is further split to give ten 100g test samples.

Extraction
Weigh 30g of ground sample directly into a 250mL Teflon centrifuge bottle. Add 5 g Celite, 25mL 0.1M H3PO4 and 150mL chloroform to the bottle. Shake sample on a flatbed shaker at 280 excursions per min. for 45 min.

Centrifugation
Set centrifuge at 4° C, insert centrifuge bottles into the sleeves ensuring that the rotor is properly balanced. Shut door, set rotor speed at 3000 rpm and break on low, set timer for 10 min and engage rotor.

After removing centrifuge bottles from the centrifuge, filter the organic layer through a 6cm funnel in which 2V folded filter papers have been inserted in the base and collect the filtrate. Transfer 25mL of the extract into a 250mL Erlenmeyer flask with 3 rinses of chloroform.

Sep-Pak Clean-up
Concentrate the extract to near dryness on a Rotary Evaporator with the water bath at 50 ° C and the cooling bath at -5° C and then add approximately 6mL dichloromethane. Attach a 10mL reservoir to a Silica Sep-Pak and place it on the vacuum manifold system Condition it with 5mL hexane and then 5mL dichloromethane. Add the sample to the Sep-Pak and rinse the flask with dichloromethane to bring the sample volume to ~10mL.
Apply the sample to the Sep-pak under vacumm at a drop wise rate. (Note- Do not allow the Sep-Pak to go dry) Next rinse the Sep-Pak with 10mL dichloromethane, then 10mL hexane and finally 10mL toluene. Place a 15mL sample tube under the Sep-Pak and elute the sample drop wise with 12mL toluene/acetic acid 9/1 (v/v) allowing all of the solvent to pass through the Sep-Pak. This contains Ochratoxin A,B and Zearalenone.

(Ochratoxin A, B and Zearalenone)

Concentrate the sample under N2 at 50°C to dryness. Add 1mL acetonitrile and vortex for 1 min. Add 1mL of 0.5% Acetic Acid and vortex the sample for 1 min and then sonicate the sample for 15 min. Centrifuge the sample at 3000 RPM at 4°C for 10 min. Filter the sample through a LC13 PVDF 0.45um filter into autosampler vials and run on the HPLC system.

HPLC Analysis

Column 3.9×150 mm Symmetry C18 5 µm steel cartridge column and a Sentry Symmetry column from Waters at a temperature of 30°C at a flow rate of 0.9 mL/min.
Gradient conditions

Time (min)	A%	B%
Int.	70	30
2	70	30
10	40	60
18	40	60
20	70	30
30	70	30

A - Milli-Q water pH 3.3 with acetic acid

B - Acetonitrile

Wavelengths

Time (min)	Excitation (nm)	Emission (nm)
Int.	310	470
10.4	280	465
11.9	340	465
30	310	470

Injection volume – 25 µL
Detector parameters – (a) Gain 100
(b) Filter - 1.5 s
(c) Cell volume 16 µL
Retention times - (a) Ochratoxin B – 10.0 min
(b) Ochratoxin A – 12.7 min
Calculation of analyte in ppb = $A/A^1 \times C/W \times F.V.$

A – Area of parameter in sample
A^1 – Area of parameter in standard
C – Concentration of parameter in standard (ng / µL)
W – Weight of sample used × mL of extract used/mL of solvent added
F.V. – Final value of extract