Feasibility of the Application of Electronic Nose Technology to Monitoring Insect Infestation in Wheat

by

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A Thesis submitted to the Faculty of Graduate Studies of

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ABSTRACT

An Alpha MOS FOX-3000 electronic nose equipped with 12 Metal Oxide Semiconductor (MOS) sensors was used to evaluate the presence of two insects in wheat. Canada Western Red Spring (CWRS) wheat (cv. AC Barrie) infested with rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), or red flour beetle, *Tribolium castaneum* (Herbst), were placed in the glass jars (4 L capacity). Different numbers of insects (0, 1, 2, 10 and 20 insects/kg) were tested for each insect species in combination with three moisture content levels for the grain (14%, 16%, and 18%). The headspace volatiles from infested or non-infested wheat was sampled and injected into the sensor array. Each individual sample collected was analyzed in triplicate and each treatment was tested seven times. The response of gas sensors, in the form of a multi-dimensional matrix, was extracted and interpreted using AlphaSoft V8.0 software. The automated pattern recognition algorithms in the software (Principal Component Analysis, Discriminant Factorial Analysis, and Partial Least Square) were used to evaluate the samples.

Sensors were shown to be sensitive to the moisture content of the wheat. When wheat moisture was low, sensors became more sensitive to the volatiles and metabolites by rusty grain beetle. At high wheat moisture content, wheat volatiles and perhaps some low level mould odours (although no mould was visible during the test duration) might affect the sensor responses.

The electronic nose could detect the presence of red flour beetle (RFB) in wheat with the high infestation level (20 insects/kg) at 14% and 16% moisture content. However, the electronic nose did not detect the presence of rusty grain beetle (RGB) in

wheat at either the low (1 insect/kg and 2 insects/kg) or the high infestation levels (10 insects/kg and 20 insects/kg). It also failed to detect the presence of RFB in wheat with the low (1 insect/kg and 2 insects/kg) and high infestation level (20 insects/kg) at 18% moisture content.

The electronic nose could differentiate 1 RFB/kg infestation level from 20 RFBs/kg infestation level in wheat at 14% and 16% moisture content. However, it did not identify densities for rusty grain beetle in wheat. High percentage of recognition (99%) and high cross-validation (0.99) were achieved for predicting moisture content of non-infested wheat.

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1. INTRODUCTION

In Canada, about 30 million tonnes of cereal grains are produced each year (CGC 2000). Cereal grains, as one of three major food grains (cereals, oilseeds, and legumes), provide the least expensive source of energy and protein for human beings and livestock in the world (Neethirajan et al. 2005). Wheat, corn, rice, barley, oats, millet, rye, and sorghum are the most important cereal grains, and wheat is considered as the main crop in Canada and the yearly output of wheat is valued at billions of dollars (Muir 2001).

There are several reasons for storing grains, which affect the choice of a storage strategy (Muir 2001). Under most climates and in most countries, farmers store grains in grain bins whose capacity can store at least one year's production. However, long-term storage in these structures provides an ideal environment for population development of common stored-product insects. Furthermore, the presence of insects in the stored-grain facilities can contribute to substantial losses during storage. Sinha and Watters (1985) have discussed that the most common insect pests of stored grain in western Canada are the rusty grain beetle (RGB), *Cryptolestes ferrugineus* (Stephens), and red flour beetle (RFB), *Tribolium castaneum* (Herbst), which can survive even in severe environments for long periods. These insects not only consume the grain, but also contaminate it with their excreta, reducing the quality and quantity of the commodity.

An important part of insect control is the early detection of insects in the grain bin. Several methods have been developed to monitor the quality of grain and to take preventative actions to reduce quality and quantity losses. One of early insect detection methods commonly used is to sift the grain on a sieve for visual inspection. This is labor intensive and time consuming. Furthermore, insects may not distribute uniformly through the bulk of grain, and a low number of insects may not be detected. This causes the possibility of large insect infestation in the future. New technologies that can accurately detect the early insect contamination of grains in a given time are needed. Sensor technologies, such as electronic nose, might offer numerous advantages over more traditional stored-product insect detection techniques. The electronic nose technology might be used to analyze interstitial gas in stored grain for specific insect-produced volatiles. However, little information about the effects of insects on changes in grain odour during storage is available (Pederson 1992, Pomeranz 1992). Volatiles produced by a few insects in a relatively large grain bulk are not easy to detect with the human nose. Usually pheromones are the primary odours released by the insects, e.g., sex pheromones produced by female moths and aggregation pheromones from male beetles. According to Seitz and Sauer (1996), red flour beetle causes some off-odour while rusty grain beetle causes little objectionable smell even though the number of the insects is large. Smith et al. (1971) indicated that chemical compounds from red flour beetle infested bread are benzoquinones. Olsson et al. (2000) found that wheat produces different volatiles with changing storage time,

and the medium-polarity volatiles increase gradually while the small-polarity volatiles reduce gradually.

Persaud and Dodd (1982) working at the University of Warwick put forward the concept of the artificial nose system, which was based on the signal collected from the chemical gas sensors. With the development of electronic noses after 1990, several commercial instruments were introduced to research and industry areas. In principle, such instruments adopt an array of non-selective electronic gas sensors, which have different sensitivity to different volatile compounds. Because the electronic nose has great sensitivity to a particular class of volatiles associated with different insect species, it might be possibly used for rapid and automated grain insect detection.

The output of the electronic nose measurements are usually multi-dimensional matrices that are hard to plot and visualize by human observers. Furthermore, the moisture content of wheat in a large grain bulk may change depending on storage conditions. How to extract interpretable information from the output of the electronic nose and whether the wheat moisture would affect the ability of the electronic nose in measuring insect-produced volatiles are the main challenges that need to be investigated. For better understanding of the terms used in the document, the abbreviations are explained in table 1.

Table 1. Definition of terms used in the document

Abbreviation	Definition
RFB	Red Flour Beetle
RGB	Rusty Grain Beetle
CWRS	Canada Western Red Spring
PCA	Principal Component Analysis
DFA	Discriminant Factorial Analysis
PLS	Partial Least Square
SSC	Sensory Panel Score Correlation (linear regression algorithms). This is a term specific to the AlphaMOS AlphaSoft V8.0 software.

2. OBJECTIVES

The aim of this research was to investigate the technical feasibility of using electronic nose technology for early detection of insect infestation in grain storage. The specific objectives were to: (1) detect the presence of insects in wheat at low or high infestation levels at 14%, 16% and 18% moisture content; (2) differentiate the insect species in wheat; (3) identify and predict insect density for each insect species.

3. LITERATURE REVIEW

3.1 Stored Grain Insects

Stored-product insects often damage grain during storage, and grain loss caused by insects can be a severe problem (Seitz and Sauer 1996). Sinha and Watters (1985) have mentioned that the most common and economically important insect pests of stored grain in Canada are the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), and the red flour beetle, *Tribolium castaneum* (Herbst). For these two insect species, even low populations are problems for cereal grains if environmental conditions are favorable for them. Rusty grain beetle (RGB) is regarded as one of the most troublesome insects that attacks stored grain in Canada. There are four life stages for RGB, which are egg, larva, pupa and adult (Anonymous 1981). According to Muir (2001), RGB can survive in dry wheat down to 10% moisture content and can tolerate cold as low as -15°C for two weeks if acclimated. However, it does not develop in dry grain whose moisture content is less than 12%. It only takes 4 weeks for RGB to grow from egg to adult beetle at 30°C.

The red flour beetles (RFB), also has four life stages. It only takes 15 to 20 days for the transition from egg to adult under optimum conditions, and it can also survive on extremely dry grain (Muir 2001). RFB is mainly found in bins where grain is stored for long periods and it feeds on a wide range of foods (Anonymous 1981). Usually long-term grain storage in granaries provides a good environment for insect population development. Furthermore, a single insect infestation in a farm or elevator

bin might contain thousands of actively multiplying insects that potentially could contaminate a significant portion of the grain handling and transporting system. Hence, overlooking insect infestation might lead to large losses.

Insects as living organism in a grain bulk respire. Odours and metabolites produced by insects were usually pheromones and metabolic by-products (Muir 2001). However, little information has been published on the change in grain odour due to the presence of insects during storage (Pederson 1992; Pomeranz 1992). Male beetles produce aggregation pheromones to attract female and male adults, to food or to mate and sometimes the effect of this aggregation pheromone can be increased due to synergistic effects with grain odours (Burkholder 1988; Loschiavo et al. 1986). Seitz and Sauer (1996) mentioned that RFB could produce some off-odour which might be benzoquinone compounds. Further research on pheromones and metabolites from RFB and RGB by using ion chromatograms detected 1-pentadecene, an apparent diene, 4, 8-dimethyldecanal, and another apparent diene from RFB, and macrolides I, II, and III were produced by RGB (Seitz and Sauer 1996).

3.2 Techniques for Insect Detection

Canada maintains a reputation for exporting insect-free grain, which means that all grain entering the grain handling system from farms must contain zero detectable insects (Canada Grain Act 1975). Looking for a better way to detect a few insects in the large volume of grain bulk is a challenge for farmers and grain researchers (Muir 2001). Some new insect detection techniques that have shown their

potentials include: pheromone traps, acoustical methods, near-infrared reflectance (NIR) spectroscopy, X-ray imaging, and electronic nose technology. These new technologies could be used to detect the emergence of insects in the early storage stages and to estimate insect density and populations in the large grain bulk. Therefore, they could provide many opportunities to reduce stored-grain losses and allow the use of effective methods to solve storage problems.

3.2.1 Pheromone traps

Sex pheromones are specific chemicals released by female moths. These chemicals attract males, helping them to find unfertilized females for mating (Karlson and Butenandt 1959). Pheromones of many species had been identified and were synthetically produced for use in stored-product insect detection. These are effective for moths, such as the Indianmeal moth and Mediterranean flour moth. Some pheromones attracte only one type of insect, while others attract several related species. Neethirajan et al. (2005) reported that three stored-product insects would appear together when using pheromones. The efficiency of traps for monitoring insect populations depends on proper trap setting time and placement of traps. Kowalsick (2006) from Cornell University suggested "setting traps at least two weeks before the insect was to emerge and at a proper position in the bin". Pheromone traps were not only intended for determining whether the insects were trapped, when certain insect species existed and whether a population was increasing, peaking, or decreasing, but also for potentially controlling insects. Over several seasons, the researchers could

develop a database for many species indicating especially heavy, light or normal captures of insects. This information is essential in determining the optimal time and frequency of remedial action. The main advantage of a pheromone trap was that it was inexpensive, easy to operate, and the capture areas could be larger compared to probe traps. However, the pheromone trap was not a perfect technique. Disadvantages of the pheromone traps were that the actual usefulness of a pheromone traps varied according to many factors such as the target species, the height of the trap, the prevailing weather conditions, and the time of day (Fields et al. 1992). Also, the chemicals (pheromones) were occasionally expensive and not easily available all the time.

3.2.2 Acoustical methods

Shuman et al. (1997) and Pittendrigh et al. (1997) both discussed the use of acoustical methods with computer and electronic technologies to detect infestations of hidden insects in stored products. This technique provides an immediate answer and does not destroy the grain sample. The acoustic detection method only requires a microphone, low-noise amplifier, and loud speaker or a cathode ray oscilloscope display tube. And the grain can be directly linked to a piezo-electric crystal for detection of insect infestation (Adams et al., 1953; Bailey and McCabe 1965). The method can change the conventional task of manually sampling and visual inspection used by researchers, which is labor intensive, and can improve the efficiency of insect detection while being non-destructive to the grain. Despite its power, the acoustic

detection system can only be used in a relative quiet environment and the ambient and system noise must be determined before a test (Webb et al. 1988). Furthermore, the utility of acoustic tools for detecting insect infestations is limited by several factors related to insect's activity. The development of insects may be reduced under low temperatures (Maier et al. 1996), molting or pupation (Pittendrigh et al. 1997), and after disturbance, depending on the species (Mankin et al. 1999; Miyatake 2001). Vick et al. (1988) thought that "even under optimal temperatures, stored-product insect larvae were quiet in 10-30% of 5 min monitoring periods during growth phases", and Miyatake (2001) and Pittendrigh et al. (1997) thought "quiescent periods could extend from 3-5 min after a disturbance to more than 8 h during molting phases". The reliability of acoustic surveillance will be improved if effective excitatory stimuli can be applied to inactive insects just before monitoring, particularly if the excitation increases the loudness of the sounds produced (Mankin 2002). Therefore, to estimate the real insect densities or populations, representative sampling should be obtained for the acoustic method, or numerous microphones would need to be placed through a granary.

3.2.3 Near-infrared (NIR) spectroscopy

The near-infrared (NIR) spectroscopy has been discussed as a relatively fast, accurate, and economical technique available to the grain industry for compositional analysis. This technique can be used for both qualitative and quantitative analysis. The NIR spectroscopy has been used to identify several *coleoptera* species (Dowell et

al. 1998), to detect parasitized weevils in wheat kernels (Baker et al. 1999), and to detect external and internal insect infestation in wheat (Dowell et al. 1998). This technique can also be applied to distinguish between sound and internally insect-infested seeds in a seed lot of *Cordia africana* Lam (Tigabu and Oden 2002). Therefore, NIR spectroscopy has been adapted for non-destructive and automated detection of pests in grain. Several studies showed that live (Dowell et al. 1998; Ridgway and Chambers 1996) and dead internal insects (Cheewapramong and Wehling 2001) could be detected by using NIR spectroscopy. Maghirang et al. (2003) showed the potential of using NIR spectroscopy to detect a single kernel of wheat containing dead or desiccated insects (rice weevil) at different stages of growth in wheat stored over a period of time. Therefore, though NIR spectroscopy has been used for measuring other quality attributes such as moisture content, protein or oil content, it will be highly beneficial if NIR instruments have the added capability to detect kernels containing live and dead internal insects automatically and non-destructively in future. However, the accuracy of NIR spectroscopy analysis can be affected by several factors, such as moisture content, season, location of plant growth, and variety, as well as the precision of the reference analysis (Osborne et al. 1993; Williams 2001). Dowell et al. (1998) documented that the performance of detecting internal insect larvae in wheat kernels was affected by the variation in moisture among grains.

3.2.4 X-ray imaging

Milner et al. (1950a) thought that X-ray was appropriate for detecting hidden insects at most stages of development. Radiography was developed by several researchers for agricultural applications at Kansas State University (Katz et al., 1950; Milner et al. 1950 b, 1952). The most important thing is that X-ray does not cause any destructive effect on grain (Haff and Slaughter 1999). Many insects might be detected by visual examination of the X-ray radiographs such as the granary weevil, *Sitophilus granarius* L., the rice weevil, *Sitophilus oryzae* L., the maize weevil, *Sitophilus zeamais* Mots., and the Angoumois grain moth, *Sitotroga cerealella* (Olivier) in grain kernels (Schatzki and Fine 1988; Keagy and Schatzki 1991).

Stermer (1972) developed a completely automated X-ray system to monitor grain on a kernel-to-kernel basis that was more reliable and practical for use. His study also resulted in the development of a procedure using fine-grain "mammography" film to obtain a contrast of about 75%, after only 2.5 min exposure time. The film was examined by a low-power binocular microscope with transmitted light. With this technique, Stermer (1972) obtained an accuracy of close to 100% in detecting fully grown larvae and pupae and 80% to 90% with early instars and eggs. However, an automated X-ray imaging machine is incapable of detecting eggs and small larvae. Therefore X-ray is a good technique to estimate mature larvae or pupae in grains (Semple et al. 1992). Karunakaran et al. (2004) used a real-time soft X-ray imaging method to detect infestations caused by *Rhyzopertha dominica* in wheat

kernels, and more than 99% of kernels infested by *R. dominica* larvae were correctly identified by the back propagation neural network (BPNN).

3.3 Electronic Nose Technology

3.3.1 What is an electronic nose?

There has been significant interest in recent years for the development of the electronic nose. An electronic nose as a system to detect odorants in the headspace above a product, typically consists of four basic components: a sampling system which allows automatic sampling of the headspace, an array of gas sensors with different selectivity, an electronic data acquisition system and control system that permits measurement and saving of all the sensor signals and systems parameters, and a software package operated by the computer to produce the results related to the volatile compounds (Schaller and Bosset 1998). The basic architecture of an electronic nose was shown in Figure 1. The function of an electronic nose is conceptually similar to the human olfactory system. When the headspace odorants is sampled by the electronic nose, it passes through the sensor array, whose signal pattern is collected and stored by a computer. Then the data are further processed by a pattern recognition system. Finally data is produced by the computer software in the form of "maps" which relates to the pattern of each of the sample (Gardner and Bartlett 1999). This is different from the human olfactory system in that the signal pattern is generated from the receptor cells in the olfactory bulb (Kandel et al. 1991).

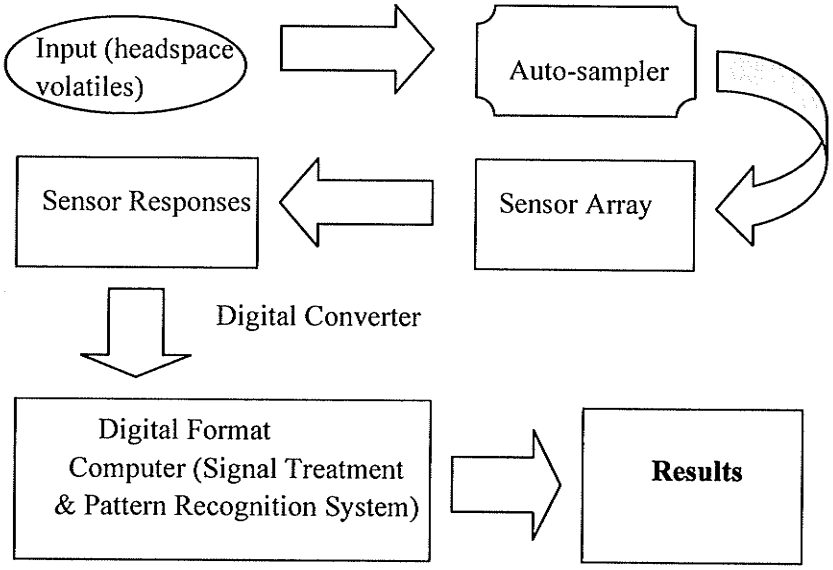

Figure 1. Basic architecture of an electronic nose

3.3.2 The history of the electronic nose

In 1920, Zwarrdemaker and Hogewind proposed the development of a useful machine for recognizing simple or complex odours. However, "due to the lack of suitable electronic instrumentation (Gardner and Bartlett 1999)", this technique for detection of volatiles based on their physical characteristics was delayed. The first real report about the electronic nose technique did not come out until 30 years later. Hartman (1954) and his colleagues tried to make use of various sensors with combination of several electrodes, by changing electric potential to control the instrument simultaneously. Moncrieff (1961) applied a number of different thermistors coated with different non-specific materials to construct a machine which could differentiate a large range of different chemical volatiles. Nearly 20 years later, Persaud and Dodd (1982), and Ikegami and Kaneyasu (1985) put forward the concept

of an artificial nose system at the University of Warwick and Hitachi Research Laboratory in Japan, separately. After that, several commercial instruments were developed and tested in the research setting, with specific applications in the food industry.

To date, the major resources for definition in the field of electronic nose technology are contained in the works by Gardner and Bartlett (1994) and they have generally been accepted by most scholars. One exception is in work by Mielle et al. (1995) who have regarded this analytical system as more a machine than a human nose.

3.4 Gas Sensors in the Electronic Nose

3.4.1 General requirements

The choice of gas sensors in an electronic nose is clearly of fundamental importance. Shiers (1995) thought that "the sensors needed to be ones whose reactions could be measured when they exhibited interactions with different types of volatiles". Due to various types of gas sensors available for the electronic nose presently and sometimes it was hard to select suitable sensors for some specific samples. The sensors chosen to be integrated in an electronic nose should fulfill the following requirements.

The first requirement of the sensors used in an electronic nose is that they must respond to different compounds present in the headspace of the sample. Gardner

and Bartlett (1999) thought that "the individual sensor element should generate a pattern of responses and should not be highly specific to any range of compounds. This requirement was different from other conventional chemical sensors which strived to eliminate effects from interfering species". The first report about using arrays of gas sensors and pattern recognition to quantify the concentration of gases in mixtures was published in the 1990s (Gardner and Bartlett 1999). Different types of sensors were tried for this quantification. For example, Stetter et al. (1986) employed arrays of amperometric sensors to detect hazardous gas, Carey et al. (1986) applied arrays of piezoelectric gas sensors to select adsorbates, Ballantine et al. (1986) adopted arrays of coated surface acoustic wave (SAW) devices to correlate the sensor response with solubility properties and chemical structure, Weimar et al. (1990) used arrays of metal-oxide resistive sensors to analyze gas mixture, and Sundgren et al. (1990) evaluated a multiple gas mixture with arrays of metal-oxide semiconductor field-effect (MOSFET) gas sensors and pattern recognition (Gardner and Bartlett 1999).

Secondly, the sensors should have high sensitivity when volatile compounds flow over them (Bartlett et al. 1993). Sometimes certain gas molecules can only be detected at low concentration while others can be detected at high concentration. Depending on different olfactory thresholds for molecules, the gas sensor should have a wide range so that they can response at an appropriate level. Ideally, the sensor should respond linearly to the concentration of the gas within a certain range. However, most sensors have no response when under the detection limit and response

may saturate at a certain point (Gardner and Bartlett 1999). In theory, Gardner and Bartlett (1999) thought that "the smaller the baseline noise was, the more power supply was needed for the instrument".

Also the sensors should have low sensitivity towards humidity, temperature, and flow rate (Demarne and Sanjines 1992). In reality, the changes of these three factors do affect the performance of the gas sensors. Looking for a better way to eliminate or reduce these effects becomes a major challenge (Hodgins 1995, 1997; Mari and Barbi 1992). Environmental factors considered as inputs in the electronic nose may be solved in the future. Gardner and Bartlett (1999) verified that within the temperature range of 180°C to 550°C, the sensor resistance changed differently for different gas samples. With the change of humidity, the baseline resistance of a tin-dioxide sensor dropped remarkably.

The sensors should also have short reaction and recovery time (Schaller and Bosset 1998). Gardner and Bartlett (1999) mentioned that "the reaction time was defined as the time taken for the device to reach some predetermined fraction of its final output in response to a step change in concentration". Ideally, gas sensors need shorter reaction and recovery time for analysis. Therefore, a quick recovery time may not be adequate for them. As the sensors are designed for industrial purposes, especially as on-line systems, Schaller and Bosset (1998) thought "high stability system with high reproducibility and reliability, robust and easy calibration could present valuable advantages".

3.4.2 Overview of gas sensor types

A gas sensor is a device that can convert a chemical change into an electrical signal. The principle of this conversion is based on the chemical changes of the sensitive layer. Gardner and Bartlett (1999) found that "chemical changes between the layer and the analyte can be reversible or irreversible". In the reversible case, when the external concentration is removed, the analyte molecules are removed from the sensor material. In the irreversible case, a chemical reaction occurs between adsorbed species at the surface of the sensor. The selection of gas sensors that can be used in an electronic nose need to respond to odorous molecules in the gas phase, which are typically volatile organic compounds with different relative molar masses. Gardner and Bartlett (1999) thought that "chemical sensors could be divided into three categories depending on the type of material used". The first category includes a range of inorganic crystalline or polycrystalline materials, such as semiconductors and metal oxide sensor. The second category includes organic materials and polymers. In general, these materials are used as reversible sensor materials. Biological materials such as protein, enzymes, and antibodies belong to the third category of sensor materials. Due to instability, these kinds of materials have not been widely used in the electronic nose. Usually, an array of nonspecific sensors was adopted, and combining of different types of gas sensors into arrays could provide a broad response of volatile substances (Henryk et al. 2003).

The Metal Oxide Semiconductor (MOS) sensor is one of the important gas sensors, which is based on changes of conductivity by chemical reaction with gaseous molecules, and have been widely used in sensor arrays for gas and odour measurements. This type of gas sensor was first applied commercially in the 1960s in Japan manufactured by Figaro Engineering Company, and much of the early work was done with a sensor of this type in the electronic nose field (Abe et al. 1987; Persaud and Dodd 1982; Shurmer et al. 1989). The basic construction of the sensor is similar. It comprises a ceramic substrate heated by a wire and coated by a metal oxide semiconductor film (Schaller and Bosset 1998). The metal oxide semiconductor film can be n-type coating (mainly tin-dioxide) or of the p-type (mainly nickel-oxide). The n-type coating is more sensitive to oxidizing compounds while the p-type responds to reducing compounds (Mielle 1996; Greenwood and Earnshaw 1988; Huheey 1983). The baseline of the sensor is the electrical resistance R_0 of the sensitive layer, which is a function of the carrier (electron) concentration. In the atmosphere, some oxygen atoms are adsorbed on the surface of the sensing material to trap free electrons from the semiconductor. The electron is therefore no longer available for conduction: the conduction becomes lower and the resistance increases (Demarne and Sanjines 1992). Consequently a highly resistive layer is produced in the vicinity of the surface. The adsorption of oxygen atoms on the surface with a negative charge creates an electrical-double layer that acts as the scattering center for conducting electrons. Gardner and Bartlett (1999) thought that "increase in free electrons and decrease in scattering centers resulted in an increase in conductivity". Once the sensor is stable

under oxygenated atmosphere (under air), it reaches a balanced condition with the absorbed oxygen at the surface of the material (Kohl 1992). The mechanism of n-type and p-type semiconductors is the same but is of opposite sign (Pearce et al. 2003).

The most widely used material as a semiconducting MOS is tin oxide (SnO_2) doped with a small amount of impurities and catalytic metal additives. By changing the choice of impurity, catalyst and operating conditions such as operating temperature, different kind of gas sensors using SnO_2 have been developed for detecting various gases (Ivanov et al. 2004; Wang et al. 2001). Then the use of sensor array allows the detection of different species by using the partial selectivity of each sensor (Fleischer et al. 2002). The advantages of the sensors were that they had high sensitivities to a range of organic vapors, a relatively fast response time and they were commercially produced. However, the disadvantages included high operating temperature, and they were highly sensitive to compounds like ethanol, CO_2 or humidity. Currently, MOS sensors are widely marketed, and are available for use in commercialized electronic noses. The system known as Fox (Alpha M.O.S., Toulouse, France), manufactured by Alpha MOS in France, has been sold with different sensor technologies, and is available in several different models.

The conducting polymer (CP) gas sensors are based on changes of resistance by adsorption of gas. However, the mechanism by which the conductivity is changed by this adsorption is very complex and not yet well understood (Schaller and Bosset 1998). This kind of sensor has similar construction to MOS sensors. Usually CP sensors comprise a substrate, and a conducting organic polymer such as polypyrrole,

polyaniline or polythiophene used as a sensing element (Mielle 1996; Amrani et al. 1995). Because of their remarkable transduction matrices, Nanto and Stetter (2004) thought that "CP sensors were sensitive to gases and vapors, and their conductance change due to the modulation of their doping level". The use of organic conducting polymer as gas sensor is very promising, because they show good sensitivities (Gerard et al. 2002). A device incorporating an array of 14 different conducting polymer sensors which produced an odour profile relating to the volatile compounds demonstrated the ability of the device to detect the mites in wheat at the concentrations of relevance to the cereal trade (Ridgway et al. 1999). However, short lifetime and high sensitivity to moisture were their disadvantages.

The acoustic wave gas sensors are operated based on the change of mass, which may be shown as a change in resonance frequency. In general, the sensors are made of tiny discs, usually quartz, lithium niobate or zinc oxide, coated with materials such as chromatographic stationary phases, lipids or any non-volatile compounds that are chemically and thermally stable (Nieuwenhuizen and Nederlof 1992; Guilbault and Jordan 1988). To date, crystals have been applied to bulk acoustic wave (BAW) devices and surface acoustic waves (SAW) devices. The only difference between them is their structure. BAW device is based on 3-dimensional waves which travel through the crystal while SAW device is based on 2-dimensional waves. When an alternating voltage is applied at room temperature, the crystal oscillates at its resonant frequency, defined by its mechanical properties. Upon exposure to vapor molecules, the sorbent coating increases the mass of the sensing layer crystal and affects the

propagation of the acoustic wave. Consequently, Gardner and Bartlett (1999) thought that "the effect of sensor changed in the wave velocity, frequency, and amplitude of oscillation might be monitored and related to the volatile present".

The field-effect gas sensors are based on a change of electrostatic potential. In general, this kind of sensor comprises three layers, a catalytic metal gate, a silicon oxide insulator and a silicon semiconductor. Gardner and Bartlett (1999) thought "there were two basic configurations for catalytic metals: semiconductor field-effect transistor (MISFET) and metal-insulator-semiconductor capacitor (MISCAP), which differed by the method of measurement". The metal usually operates as the gate contact that controls the current through the transistor. The semiconductor and insulator acts to allow the current in and out. When the external voltage is applied on the metal and semiconductor, it creates an electric field, which affects the conductivity of the transistor. If any volatile compounds interact with the metal gate, the surface potential will be changed and sensed by the device. Lundstrom et al. (1991) applied a two-dimensional 'image' technique with the device to sense three different gases: ammonia, hydrogen, and ethanol. The results showed that the device could generate different patterns for these three gases.

3.5 Applications

Some factors such as the nature of the test, the measurement conditions, and the nature of the measurand have to be considered for the electronic nose instrumentation when applied to both simple and complex odours in some fields of

application (Gardner and Bartlett 1999). The factors that influence the successful application of an electronic nose can vary considerably. It depends on the different situations and conditions. Selection of data analyses depends on the different purpose of the application. For example, the electronic nose can be used to discriminate two simple volatiles, or to identify an unknown volatile from known volatile compounds. Unsupervised pattern recognition techniques, such as principal component analysis, can be performed for the first step, while supervised pattern recognition technique can be applied for the further analysis. The condition under which the application is applied also determines the experimental success and the ability of the device to detect complex volatile compounds. For example, temperature and humidity can cause significant changes in the signals of the sensors, and the variations in flow rate of the gas sample can influence sensor output. Furthermore, the nature of the measurand also has to be considered in the application of the electronic nose. Factors such as sample stability, and the number of the components in the test must be considered.

3.5.1 Electronic nose in the grain storage system

Zhang and Wang (2007) applied a commercial electronic nose (PEN2, Win Muster Airsense Analytics Inc., Germany) with 10 metal-oxide semiconductor (MOS) sensors to successfully discriminate five storage ages of wheat and 15 groups of different degrees of insect infested wheat. In their research, principal component analysis (PCA) and linear-discriminant analysis (LDA) were applied to the signals of

sensor array, and both of these two multivariate data analysis techniques provided a good indication of differences in stored wheat with different degrees of insect infestation.

Electronic noses have also been applied for the identification of grain spoilage by fungi. Keshri et al. (1998) analyzed six headspaces of spoilage fungi (four *Aspergillus* species, a *Penicillium* sp. and *Wallemia sebi*) with the help of an electronic nose consisting of 14 polymer sensors. By visual plotting of discriminant function analyses (DFA) technique, they achieved 97% separation for five spoilage fungi (three *Eurotium* species, a *Penicillium* sp. and *Wallemia sebi*) during 48 h visible growth at 25°C. Olsson et al. (2000) also investigated the application of an electronic nose to quantify ergosterol and colony forming units (CFU) of naturally contaminated barley samples, while Schnurer et al. (1999) already found that this approach was able to predict CFU of artificially infected grain samples with different moisture contents. In their experiment, based on the effect of changes in the relative humidity of gas headspace over the sensor, Schnurer et al. (1999) adopted a partial least square model (PLS) by adding moisture content as an X-variable and the sensor signal as an Y-variable. The result showed that the PLS model could give highly accurate predictions of ergosterol while low accuracy predictions of CFU. With the combination of gas chromatography-mass spectrometry (GC-MS) it was possible to identify and quantify volatile compounds of interest for the electronic nose; also it could be helpful to predict the ergosterol levels in stored barley grains.

The early detection of mites is also an important part of managing grain storage systems. Hand-sieving with visual inspection as a conventional method is slow and subjective and is not suitable for some grain products such as flour. Curtis et al. (1981) applied gas chromatography to determine the headspace of mite-specific volatiles, and tridecane is determined to be linked to *Acarus siro* (L.) counts in bin-stored wheat. Thind and Wallace (1984) found that even at low density of mite which was of relevance to the cereal trade, the electronic nose could detect the major mite grain pest *A. siro* with the classification errors of 20%. The use of electronic nose with transient flow sampling to detect mite infestation in wheat was reported by Ridgway et al. (1999). They used a Bloodhound BH114 (Blood-hound Sensors Ltd, Leeds, UK) unit comprising 14 different semi conducting polymer sensors in their research.

3.5.2 Applications of the electronic noses in other fields

The applications of the electronic nose have also been reported in other fields. Naresh et al. (2001) presented three case studies on an electronic nose system which could recognize spoilage bacteria and yeasts in milk. The first one was using discriminant function analysis and the sensor array, and he found it was possible to separate unspoiled milk and spoiled milk containing spoilage bacteria or yeasts. Furthermore, they explored the relationship between bacterial concentration and sensitivity of detection in milk. The results showed that the system could effectively differentiate three different concentrations of *Pseudomonas aureofaciens* (10^6,

3.5×10^8, 8×10^8 CFUs/ml). The second one was that by using an initial inoculum of about 10^3–10^4 CFUs/ml, the system was also possible to discriminate between unspoiled milk, yeasts and bacterial species. The last one was applied to the potential for distinguishing between bacterial and yeast contamination by analyzing the derived spoilage volatile patterns using an electronic nose system. He found that cross validation using labeled individual replicates of treatments as unknowns was possible to recognize and differentiate between species, the butanol and milk medium controls. Although the examples above demonstrated that electronic nose technology could provide a useful tool in the early detection and species involvement in dairy spoilage, it was not an efficient way to do a quick determination for dairy products due to certain conditions (temperature and time) required for this application.

Lozano et al. (2005) used tin dioxide multisensor array based electronic nose to classify 29 typical aromas in white wine. For the practical application of this technique, they used the headspace technique to extract aroma of the wine and multivariate analysis to discriminate several aromatic compounds from different families of main aromas of white wine. The probabilistic neural network theorem was adopted to compare the sensitivity, selectivity and accuracy for different aromas in wines. Lozano et al. (2005) concluded that "wines from different areas and grape varieties exhibited different aromatic profiles and the system could be used for the classification of origin and grape variety of white wines".

Falasconi et al. (2005) adopted electronic nose to detect the presence of fungi among the contaminated maize and to classify *Fusarium verticillioides* strains in corn.

The electronic nose they used was equipped with an array of six metal-oxide semiconductor (MOS) sensors and PCA was employed to extract information from data. Falasconi and his research group tried the experiments with the two headspace sampling techniques: static headspace and dynamic headspace autosampler. The results showed that with the static headspace analysis, results were very reproducible measurements and were independent of the error from human interference. However, there also existed some disadvantages such as variability among replicates due to the small amount of the sample; low response and sensitivity due to the small headspace, while dynamic headspace method could solve those problems. Therefore, both of the sampling techniques were good for the development stage of detecting contaminated maize and classifying the toxigenic behavior of the investigated strains by sensors.

3.6 Data Analysis in Electronic Nose

3.6.1 General introduction of electronic-nose signal

In general, the input sample to an electronic nose is either a simple odour compound or a mixture of odour compounds (Gardner and Bartlett 1999), and it can be usually presented by a time-varying column vector (Enescu and Koivunen 2000). Gardner and Bartlett (1999) explained a specific class of compounds characterized by a set of concentrations of m odour components sample using the following formula:

$$c_j(t) = \begin{bmatrix} c_{1j} \\ c_{2j} \\ .... \\ c_{mj} \end{bmatrix} \quad (1)$$

where $c_j(t)$ = the input odour sample of j'th class at a certain time t, and

c_{mj} = the input odour sample of the m'th component in the j'th class at a certain time t.

This described the situation where only one type of the input odour made up of many compounds

The signal processing of the electronic nose is normally divided into four steps. The headspace of a sample is collected and transferred to an array of sensors. Depending on the different sensor material, which is usually either organic, such as conducting polymer, or inorganic material, such as semi-conducting oxide, the sensor responses are measured in the form of a change in the electrical resistivity or conductivity of the material. Sometimes, a change in mass or electrical potential can be measured for certain materials, e.g., a coated BAW device or a Pd-gate MOSFET (Bergveld et al. 1998). Then the sensor response is converted into an electrical signal by sensor electronics (e.g., voltage or current change, or peak to peak signal) in the second step of signal processing (Garcia et al. 2003). The electrical signal is then transferred to a sensor pre-processor system, which can convert an analogue signal into a digital one. Each digital pre-processing signal from different sensors comprises an array of sensor signals, which is normally a column vector. Gardner and Bartlett (1999) described this column response vector X_j in form of an equation as follows:

$$X_j = \begin{bmatrix} x_{1j} \\ x_{2j} \\ .... \\ x_{nj} \end{bmatrix} \quad (2)$$

where X_j = the output of a sensor array for odour of class j, and

n = the number of the sensor.

3.6.2 Pre-processing of array signals

The signal from the sensor array is a multi-dimensional column vector. Figure 2 shows the gas sensor responses in relative resistance change, which displays time and selected sensor value.

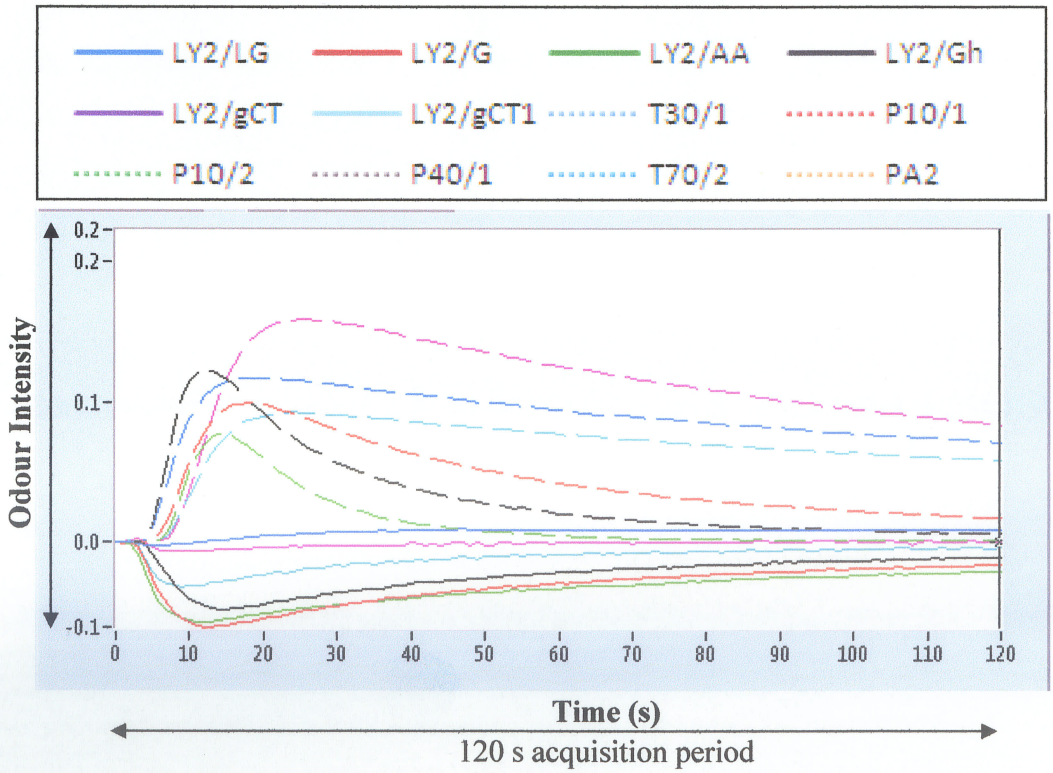

Figure 2. Example of pre-processing sensor response curves from the Alpha Mos Fox 3000 electronic nose, showing 12 sensors' relative changes in resistance during 120 s period.

Gardner and Bartlett (1999) thought that "error in the array vector associated with the errors on the responses of the individual sensors, which might come from systematic sensor drift or randomize sensor errors on the response of sensor array". Variations of the environment parameters could cause sensor drift and reduce the robustness of statistical models. Therefore, the responses from the sensor array might be considered as non-stationary.

To minimize the sensor error, mathematical transformations and chemical sensor-drift compensation method could be used (Alpha MOS SA 2002). Normalisation of array response as one of the statistical sensor-drift compensation method became necessary before pre-processing the data, which could eliminate the effect of fluctuations in the compounds' concentration (Gardner and Bartlett 1999). The process is to normalise the array vector by placing vectors of different lengths on the surface of an (n-1) dimensional unit hypersphere. Normalized transformation can be expressed according to the equation (Gardner and Bartlett 1999) below:

$$x_{ij}^{'} = \frac{x_{ij}}{\sqrt{\sum_{i=1}^{n} x_{ij}^{2}}} \qquad (3)$$

where x_{ij} is the initial sensor response, and

$x_{ij}^{'}$ is the normalized response vector.

3.6.3 Signal processing challenges

Practically, due to the variation of the interfering signal, the output noise from the sensor array was induced on the data for further analysis and reduced the

robustness of the statistical models. Gardner and Bartlett (1999) presented this modification in the form of following equation:

$$y'_{ij}(\mathrm{t}) = y_{ij}(t) + \varepsilon_t(t) \tag{4}$$

where $y'_{ij}(\mathrm{t})$ is the real output of an odour of class j from sensor i,

$y_{ij}(t)$ is an ideal output of the odour of class j from sensor i, and

$\varepsilon_t(t)$ is interfering signal.

In most cases, the interfering signals came from the variation of the operation temperature and humidity of sensor ambient environment. Gardner and Bartlett (1999) verified the effect of the sensor temperature and humidity changes on the sensor responses in their reproduced tests from Ihokura and Watson (1994). In his test, with increase of temperature from 180°C to 550°C, the sensor resistance changed differently for different gas samples. Also when humidity changed slightly, the baseline resistance of a tin-dioxide sensor type dropped remarkably. Ideally, it has been suggested by Gardner and Bartlett (1999) to keep each sensor at the constant temperature and humidity condition to maintain the same threshold for the sensor response. However, practically it is difficult to keep the sensor at the same ambient temperature and humidity. Some approaches have been adopted to compensate for the variability of sensor conditions. One approach is to apply some mathematical algorithms, which can be used for compensation of sensor baseline drift or unwanted concentration effect. Another method is to do periodical diagnostic compensation,

which can maintain the condition of the sensors constantly (Gardner and Bartlett 1999).

3.6.4 Statistical data processing techniques for electronic nose

The data obtained from the electronic nose is usually stored as a multi-dimensional matrix. To extract and interpret the pertinent information contained in a data set, multivariate statistic processing techniques are useful to compare data sets as a whole and can visually display the difference in two or three dimension maps for various samples.

The Principal Component Analysis (PCA) as an unsupervised learning technique can be used to explore the data and to assess discrimination performances (Gardner and Bartlett 1999). This method changes the dimensionality of numerical data sets from n-dimensional input to a two or three dimension space, and furthermore, Zhang et al. (2007) thought that "it could explore the data structure, assess the similarities and relationship between objects, and summarize the set of variables by a small number of synthetic variables". Therefore, a multi-dimensional problem can be solved by reducing the number of variables, and eliminating the redundancy. In the analysis of signal from the sensor, PCA is a tool to summarize the information contained in the initial database (Raychaudhuri et al. 2000). Henryk et al. (2003) evaluated the possibility of the PCA for differentiation of profiles of volatile compounds from sound wheat and malodours wheat in a wheat data set. The first component (PC) explained 73.51% of the variation, the second 18.06%, and the third

7.31%. A discrimination index of 96.0% was achieved by this approach. Zhang et al. (2007) explored the possibility of PCA to the optimized sensor array signal from an electronic nose (PEN 2), which comprised ten metal oxide semiconductor sensors. In their experiment, five different storage times of wheat were classified. Therefore, PCA can be used to classify age of wheat. In Olsson et al.'s (2000) experiment, 10 metal oxide semiconductor field effect transistor sensors combined with 6 SnO_2-based Taguchi sensors were exposed to the headspace of 10 barley samples with normal and 30 with different degrees of off-odour samples. By applying both PCA and PLS, they concluded that "ochratoxin A (OA) could be detected and classified below or above the limit of 5 μm /kg". Carey et al. (1986) applied PCA to the response of piezoelectric sensor to reduce a data set containing piezoelectric crystal frequency shifts, which described almost 95% of the variance in the original data set of 27 coatings. Rose-Pehrsson et al. (1988) applied PCA to the responses of the coatings to all the vapours, including two-component mixtures of vapours representing different chemical classes and concentrations. Garcia et al. (2006) applied PCA to reduce 16-dimensional data to 2-dimensional data with achievement of 100% of the data variance to classify two hams ("Montanera" and "Spoiled"), and 98% of the variance for 4 hams ("Montanera", Fodder, Spoiled and Parma ham).

A more common type of statistical data analysis is based on a supervised learning technique, in which mathematical models can be developed based on the training data to predict unknown samples. The Partial Least Squares (PLS) as a supervised learning technique is slightly different from PCA. PLS is used to predict

quantitative values. Usually, two matrices X and Y are introduced into the PLS analysis. X can be defined as sensor measurement, while Y could be the matrix containing the predictive values (Höskuldsson 1988; Sjöström et al. 1983). The purpose of building PLS model is to find a B matrix that can minimize distances between Y and the predictive value of Y', which can be simply expressed as Y'= X.B. Phatak and Jong (1997) explained the geometry of the PLS that the covariance between matrix X and Y should be maximised by calculating the principal component in the model. In building the PLS model, two different methods could be adopted by choosing two quantitative methods (Alpha MOS SA 2002). The first method adopted linear regression algorithm to analyse the raw data and the algorithm allowed putting null or negative values for the X matrix. Another method also used a linear regression algorithm. However, raw data have to be transformed by log algorithm before building the model (Alpha MOS SA 2002). In this method, null or negative values would not be allowed for the model. Sjöström et al. (1986) "constructed a dummy variable to represent the sample odour (e.g., normal= -1, off-odour = 1), then PLS discriminant analysis was used to classify and describe which variables in X which stood for each sample odour". Henryk et al. (2003) performed the PLS analysis to correlate the results of sample recognition from an electronic nose with the sensory panel score. The PLS model achieved a correlation of 0.9484 between electronic sample recognition and sensory panel score for grain sample, 0.9497 for musty sample, and 0.9102 for earthy sample.

Discriminant Factorial Analysis (DFA) is another supervised learning technique that distinguishes between classes of objects. The difference between DFA and PCA was summarized by their different mathematical algorithms used to separate individuals. DFA is focused on maximizing the difference between values of the dependent, whereas PCA maximizes the variance in all the variables accounted for by the factor. DFA is mainly used in a second step to build a classification model which will be applied for the identification of unknown sample (Nicolas et al. 2000). Gardner and Bartlett (1999) explained one method by applying the discriminant function Z approach to address the problem of separating two or more groups of individuals as accurately as possible. Another way is to use multivariate distance method to calculate the distance of a single observation from the center of a known group to the observation. Mahalanobis (Gardner and Bartlett 1999) gave an equation for the distance calculation:

$$d^2 = \sum_{r=1}^{r=n} \sum_{s=1}^{s=n} (x_r - \mu_r)\ \nu^{rs}(x_s - \mu_s) \qquad (5)$$

where d^2 is the eponymous Mahalanobis distance,

$x^T = \{x_1,\ x_2,...,\ x_n\}$ is the vector for a single multivariate observation,

$m^T = \{\mu_1,\ \mu_2,...,\ \mu_n\}$ is the vector for the population mean values,

ν is the population co-variance matrix, and

ν^{rs} is the element in the rth row and sth column of the inverse co-variance matrix ν^{-1}.

To quantify the performance of the model prediction, a validation of the model should be applied. Alpha MOS SA (2002) introduced two validation methods, which

were generally used recently. The first is called the leave-one-out (LOO) method (Duda et al. 2001), which is calculated by removing each data from the model and regards it as an unknown sample. The second one is called the bootstrap method, in which half of samples are randomly removed from the data and identified in one of the predefined groups. This operation is repeated until a convergence occurs. The discrimination score is obtained by calculating the average of the scores (Alpha MOS SA 2002). Aishima (1991) investigated the importance of MOS gas sensors by applying DFA to 4 coffee samples. The results indicated that using only one sensor, DFA could discriminate the coffees with discrimination score of 78.3%, while employing three sensors, DFA could discriminate the coffees with a score of 100%.

4. MATERIALS AND METHODS

4.1 Experimental Design

The purpose of the experiment was to detect the presence of a few insects in a large volume of grain by using the electronic nose. To simulate a real condition, a large lid-sealed glass jar with a hand-tight plastic lid with special fitting was designed. Canada Western Red Spring (CWRS) wheat infested with different insect infestation levels (0 insect/kg, 1 insect/kg, 2 insects/kg, 10 insects/kg, and 20 insects/kg) by the rusty grain beetle (RGB) and the red flour beetle (RFB) were used. A comprehensive table of all the codes used in this study for the wheat moisture content/insect-infestation level/insect combinations/storage time was given in Appendix A. Headspace volatiles of the sample collected in Tedlar bags was transferred to the sensor array by the flowcell sampling method (Alpha MOS SA 2002).

4.1.1 Description of experimental holding chambers

Glass jars (4 L capacity, 24 cm high, 13 cm exterior diameter with a hand-tight plastic lid) were used as sample containers (Figure 3). Two kg of wheat were placed into each container with the designated number of insects to simulate different insect infestation levels. The wheat occupied 2/3 of the total space in the container. Two holes (6.35 mm interior diameter) were drilled on the diameter of the lid, approximately 1.5 cm from the edge of the lid. Two glass tubes were inserted into the container as an air inlet (0.3 m long) and an air outlet (0.08 m long). The ends of two tubes were extended about 0.06 m high above the container. The inlet tube was

inserted to the bottom of the jar and compressed air was flushed through the intergranular space of the wheat from the bottom to the top (Figure 3). The tops of the inlet and outlet tubes were both capped with rubber septa so that the headspace of volatiles in the container could be maintained. All the containers were stored at room temperature (22°C) for 24 h before sampling. Before collecting volatile compounds, two sections of Teflon tubing (6.35 mm interior diameter) were used as connectors. Teflon tubing was chosen as they did not release odour nor did they adsorb odour from the headspace volatiles. One Teflon tube was connected at the port of the compressed air to the glass inlet tube, and a flow-meter was placed in between to control the air flow rate. Another section of Teflon tubing was used to connect a 3-L Tedlar bag (St. Croix Sensory, Inc., Stillwater, Minnesota) to the glass outlet tube (Figure 3).

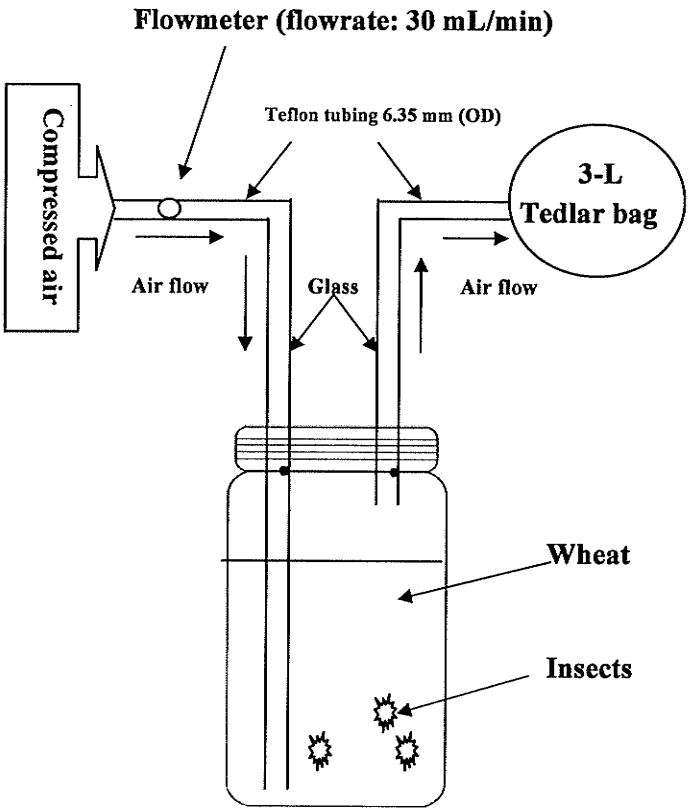

Figure 3. Overview of the sample container design used to collect headspace volatiles from infested or non-infested wheat into a gas-tight Tedlar bag.

A flow rate for the compressed air of 30 mL/min for a 10 min flushing period was used to collect the headspace volatiles above the wheat samples in the holding containers. The time and volume were manually controlled using a Teflon valve. The total volume of air (30 mL/min ×10 min=0.3 L) flushed into the container was smaller than the intergranular spaces filled with air in the container (4 L×1/3=1.33 L) so the compressed air would result in only minor dilution to the headspace volatiles of the

wheat sample. Before starting to collect the headspace sample, the flow meter was used to make sure that the flow rate of the compressed air was stable and there was no leakage from any of the connectors. When sample collection started, the side stem of the Tedlar bag was opened and a timer was used to monitor the 10-min flushing time. On completion, the shut-off valve on the Tedlar bag sealed.

4.2 Preparation of Wheat Samples

Canada Western Red Spring (CWRS) wheat (cv. AC Barrie) harvested in September, 2006 was provided by Dr. N.D.G White of the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB. The determination of initial wheat moisture content was done using the air oven method (ASAE S352.2, 2003) and the moisture content measurement report was attached in Appendix B. The grain was randomly sampled from the supplied wheat and was weighed with a Mettler PE 160 analytical balance (DeltaRange Corp., Zurich, Switzerland). Ten grams of wheat was placed in an aluminum dish. Three replicates were used and the samples were dried in a mechanical-convection oven (Model 70DM, Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 130°C for 19 h (ASAE S352.2, 2003). After drying, all dishes were covered and placed in a desiccator to cool. The initial moisture content was calculated as the weight loss of the wheat sample (Muir et al. 2001) according to the formula as follows:

$$M_i = \frac{W}{G} \times 100\% \quad (6)$$

where M_i = the initial moisture content (% wet basis),

W = the mass of water in sample, g,

G = mass of initial sample, g.

To investigate whether the wheat moisture would affect the ability of the electronic nose in measuring insect-produced volatiles, and obtain certain concentrated volatiles that could be detected by the electronic nose from insects, samples of three different moisture contents were prepared (approximately 14, 16, and 18%). Wheat at 22°C and 14, 16, and 18% moisture content was in equilibrium with a relative humidity in surrounding air of 70%, 80%, and 90% (Sinha and Muir 1973). Based on the initial and targeted moisture contents, the mass of water to be added was calculated as (Muir et al. 2001).

$$\Delta W = \frac{G(M_f - M_i)}{1 - M_f} \quad (7)$$

where ΔW = the mass of water to be added, g,

M_i = the initial wet mass moisture content level, %,

M_f = the designated wet mass moisture content level, %,

G = mass of initial sample, g.

The calculated amount of distilled water was added and then mixed with wheat in a grain rotating mixer for 2 h. The conditioned wheat was held in a sealed plastic bag for 48 h at 2.5°C for moisture equilibration (Abramson et al. 2005). All samples were stored at 4°C in a walk-in refrigerator until used.

4.3 Description of Sample Treatments

Only adults of these two species were selected and all the insects were obtained from the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, MB. Four different infestation levels were selected for each insect species in combination with three moisture content levels (14%, 16%, and 18%). To minimize the interference by possible emergence of fungi, the high moisture content samples (18%, 16% moisture) were analyzed first. According to the Canada Grain Act, zero live insects were allowed in grain bulks in Canada, and in the USA 2 live insects/kg were allowed in the grain (Muir 2001). To investigate the ability of the electronic nose to detect a low level of insect infestation and the response of sensors to different numbers of insects, 0 insect/kg, 1 insect/kg, 2 insects/kg, and 20 insects/kg were used for each of these two moisture contents. However, preliminary data analysis showed that the difference between 1 and 2 insects/kg was not obvious when performing the data analysis from the electronic nose, therefore 10 insects/kg was selected instead of 2 insects/kg for 14% moisture wheat. All the sample codes for the experiment were listed in appendix B and the description of sample codes used in the electronic nose analysis was shown as follows (Table 2).

Table 2. Structure of sample codes used in the electronic nose analysis

Position 1	Position 2	Position 3	Position 4
Insect Source	Moisture Content (MC)	Infestation Level	Holding Time in Tedlar Bag
P = Control	4 = 14% MC	0 = 0 insect/kg	0 = 0 h
F = Red Flour Beetle (RFB)	6 = 16% MC	2 = 2 insects/kg	1 = 24 h
G = Rusty Grain Beetle (RGB)	8 = 18% MC	4 = 4 insects/kg	2 = 48 h
M = Mixture of RFB & RGB		T = 10 insects/kg	3 = 72 h
		M = 20 insects/kg	4 = 96 h
			5 = 120 h
			6 = 144 h

e.g. P401: the Red Spring Wheat at 14% moisture content with no insects introduced and hold for 24 h in the Tedlar bag before testing.

Note: The holding period (Position 4) maybe omitted at certain time in the discussion.

4.4 Electronic Nose Methodology

A FOX 3000 electronic nose manufactured by Alpha-MOS (Alpha M.O.S., Toulouse, France) equipped 12 metal oxide semiconductor gas sensors was used. A nitrogen generator (N2Flow, In House Gas (Manufacturing) Ltd., Killearn, Scotland)

was used to provide a dynamic flow of nitrogen. Sensor arrays located in the two chambers are 6 n-type and 6 p-type metal oxide sensors. Usually n-type sensors responded to oxidizing compounds and p-type sensors to reducing compounds (Gardner and Bartlett 1999). The electronic nose used is located at Richardson Center for Functional Foods and Nutraceuticals, University of Manitoba. The unit was maintained weekly with diagnostic testing to validate the condition of the sensor set. To get headspace volatiles to which the sensors would response, the large-volume syringe (5.0 ml) was installed in the autosampler for transportation from Tedlar bags to the sensors. Standard practice for this system was that the syringe temperature was set at least 5°C higher than that of the headspace of the gas sample to prevent condensation in the syringe. When using Tedlar bags, the syringe temperature was set at 35°C, which was the lowest possible setting that was approximate 13 – 14°C above room temperature. The Alpha MOS recommended 120 s acquisition time and 0.5 s acquisition periods for the high performance chambers (Alphasoft, Toulouse, France). This was tested and found appropriate for these samples. Delay time, the time between testing samples was set for this particular procedure at 20. The parameters for the method of the experiment were created using the software (Alphasoft) and are given in Table 3.

Table 3. The parameters set in the Alphasoft

Parameters	Settings
Syringe Type	5.0 mL
Syringe Temperature	35°C
Flushing Time	120 s
Acquisition Time	120 s
Acquisition Period	1.0 s
Delay Time	18 min
Gas Flow Rate	150 mL/min
Syringe Injection Volume	2.5 mL/s
Syringe Injection Speed	2.5 mL/s

All the samples collected were analyzed in triplicate and during the same sample set. The samples were randomized throughout each sample set. For the purpose of database construction, each moisture content of the wheat sample including eight different qualities (non-infested wheat, RFB or RGB-infested wheat at 3 insect-densities, and a mix of RFB and RGB-infested wheat) were run 7 times.

4.4.1 Flowcell setting

FOX 3000 system provided two ways to run an analysis with the headspace sample. One was taken from 10 mL glass vials with septum cap sealed, and another

one was from the flowcell sampler (Figure 4). This study used only the second procedure to allow the use of Tedlar bag. As previously described, a 4 L glass jar was used as sample container to model an environmental condition in which wheat was infested by low and high insect populations. Tedlar bags were used to collect and transfer the gas sample to the electronic nose directly. A schematic of the flowcell set-up was given in Figure 4. Two Teflon tubes (6.35 mm exterior diameter, 0.5 m long) were connected to the inlet and outlet of the flowcell. A portable air sampler pump (Gilair 5, Gilian Instrument Corp., Clearwater, FL, USA) was located behind the outlet point in order to avoid any contamination of the headspace sample by the pump. The sample was taken from the point shown in Figure 4 by the syringe and moved to the injection port of the electronic nose.

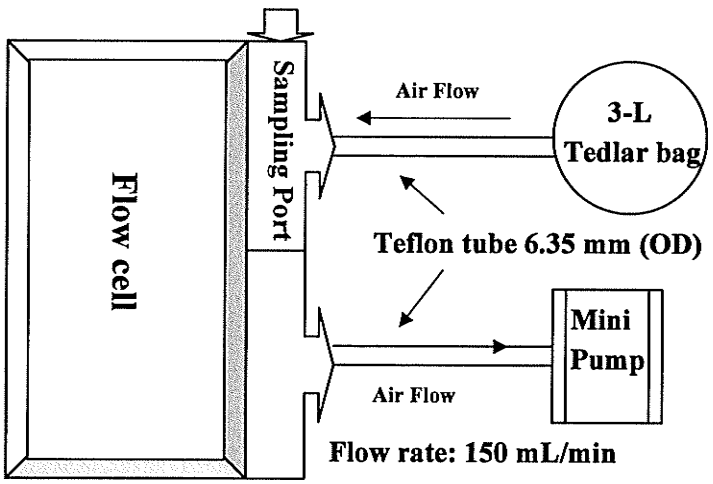

Figure 4. Overview of the flowcell design used to insufflate volatiles into the electronic nose

4.5 Electronic Nose Data Pre-processing

The results for each sample were the relative changes in resistance of the 12 sensors during 120 s. The acquisition files were first loaded in the pre-processing window of Alphasoft V8.0 (Alpha M.O. S., Toulouse, France). Then the sensor responses were visualized and compared. To further process multivariate statistics, the libraries or databases were built by selecting all the data. Within the software, automated pattern recognition algorithms (e.g., Principal Component Analysis, Discriminant Factorial Analysis and Partial Least Square) could be selected to do quick comparison and identification of samples.

4.5.1 Multivariate approaches

All data analyses were performed using Alpha Soft V8.0 (Alpha M.O.S., Toulouse, France). For differentiation and classification of sensor responses were evaluated by Multivariate Statistics available in the AlphaSoft V8.0 software (Alpha M.O.S., Toulouse, France). Unsupervised learning techniques, such as Principal Component Analysis (PCA) were used to reduce the multi-dimensional data of sensor responses to a 2-dimensional matrix which was easy to explore the data and to assess discrimination performances in a 2-dimensional plot. The discrimination performance could be shown through separation or overlap between groups and expressed by the discrimination index. Outliers could also be detected by visual inspection in the PCA plot. If a sample was not grouped with the other samples of the same treatment group,

or it was located evidently far away from the other samples of the same group, it was possible to be regarded as an outlier. Any sample whose sensor responses were in different plot area from others was thought to be an outlier and was removed.

Once the PCA (discrimination) was completed, supervised pattern recognition techniques, Discriminant Factorial Analysis (DFA) and Partial Least Squares (PLS) were performed. Based on the computation of the distance to gravity center of each group, a DFA model was built and used to separate different groups. To validate the DFA model, cross-validation by the full leave-one-out method over all samples was used (Duda et al. 2001). By applying this validation, each sample was removed from the data set and considered as an unknown sample; the total score of all samples was computed. Generally, the model would be valid if a score of 90% was obtained in the validation process. To predict unknown samples, the recognition scores were calculated based on the calculation of the distance to the gravity center of each group from unknown sample. Unknown sample was grouped into a certain group if its distance was less than the model Mahalanobis distance and its residual error was lower than the model residual error.

Partial Least Square (PLS) algorithm in the Alpha Soft V8.0 software based on the linear regression technique was used to predict the value of the quantitative information on the unknown sample (i.e., insect infestation level or moisture content). The graph plotted the quantitative training value (X-axis) versus the predicted values

(Y-axis). For a training set, the dots should lie very close to the Y=X line. However, due to variations from sample to sample, some spread was occurred on the Y-axis. The model will be valid only when the correlation coefficient is more than 0.90 and the angle of the line is closed to 45 degree.

4.6 Evaluation of Holding Times for Headspace Samples in Tedlar Bags

Gas samples should not be stored in Tedlar bags for more than 48 h based on the product operating instructions. Due to the working hour limit, some gas samples could not be analyzed by electronic nose in 24 h and sometimes they were stored in the Tedlar bags for more than 48 h. The essential sample codes for the gas sample with different storage times were described in Appendix A.

5. RESULTS AND DISCUSSION

5.1 Effect of Storage Time of Gas Sample in Tedlar Bags

To determine the effect of long storage time on a gas sample in Tedlar bags, Principle Component Analysis (PCA) algorithm was used. The results were shown as follows (Table 4) and all PCA plots were shown in Appendix C.

The performance of PCA was evaluated by the Discrimination Index (DI) value, which was a numerical result varying from -∞ to +100%. DI gives an evaluation of the discrimination quality on the selected plan from the surface between groups and the size of each group. DI is positive or close to +100% if groups in the PCA plot are small and separated, while DI is negative if groups are larger or overlapped in the plot. DI can be calculated by the equations (Alpha MOS SA 2002) shown as follows:

$$DI = \left[1 - \frac{area(1) + area(2) + ... + area(n)}{Total\ area}\right] \times 100 \quad \text{(No overlaps)} \qquad (8)$$

$$And\ DI = -\left(\frac{\sum (Intersection\ area)}{Total\ area} \times 100\right) \quad \text{(Overlaps occurred)} \qquad (9)$$

where area (n) is the minimun area that all the data from the same sample are grouped in,

the intersection area is the minimun area that all groups are overlapped together, and the total area is the minimun area when all the data in the plot are grouped in.

Table 4. Performance of different sample-holding periods in Tedlar bags prior to the electronic nose analysis for insect-infested CWRS wheat at 14% and 18% MC.

Moisture Content	Insect Species	Infestation levels	Holding time	Discrimination Index (DI)	Conclusion
14%	RFB	1 insect/kg	96 h	-279	No differentiation among samples is shown in this plot as can be seen by the overlapping maps
14%	RFB	10 insects/kg	96 h	-132	F4T0, F4T1, F4T2, and F4T3 are overlapped with each other, while F4T4 is separated from them
14%	RFB	20 insects/kg	96 h	-122	F4M0, F4M1, F4M2, and F4M3 are overlapped with each other, while F4M4 is separated from F4M3 in the map
14%	RGB	1 insect/kg	96 h	-242	No differentiation among samples is shown in this plot as can be seen by the overlapping maps
14%	RFB	10 insects/kg	96 h	-330	No differentiation among samples is shown in this plot as can be seen by the overlapping maps

Moisture Content	Insect Species	Infestation levels	Holding time	Discrimination Index (DI)	Conclusion
14%	RFB	20 insects/kg	96 h	-343	No differentiation among samples is shown in this plot as can be seen by the overlapping maps
14%	RFB& RGB	2 insects/kg	96 h	-216	No differentiation among samples is shown in this plot as can be seen by the overlapping maps
14%	Control	0 insect/kg	96 h	-222	No differentiation among samples is shown in this plot as can be seen by the overlapping maps
18%	RFB	1 insect/kg	144 h	-395	No differentiation among samples is shown in this plot as can be seen by the overlapping maps
18%	RFB	2 insects/kg	144 h	-314	F840, F841, F842, F844, F845 are overlapped with each other.
18%	RFB	20 insects/kg	144 h	-404	F8M0, F8M1, F8M2, F8M4, F8M5 are overlapped with each other, while F8M3 is separated from them

Moisture Content	Insect Species	Infestation levels	Holding time	Discrimination Index (DI)	Conclusion
18%	RGB	1 insect/kg	144 h	-341	No differentiation among samples is shown in this plot as can be seen by the overlapping maps
18%	RFB	2 insects/kg	144 h	-346	G840, G841, G842, G844, G845 are overlapped with each other
18%	RFB	20 insects/kg	144 h	-229	G8M0, G8M1, G8M2, G8M4, G8M5 are overlapped with each other
18%	RFB& RGB	2 insects/kg	144 h	-242	M840, M841, M842, M844, M845 are overlapped with each other
18%	Control	0 insect/kg	144 h	-265	P840, P841, P842, P844, P845 are overlapped with each other

For gas samples from 14% moisture content wheat, the longest holding time in Tedlar bags was 96 h. The same holding times for samples were grouped in the same area. Thereby, five groups of the different holding time were shown in the PCA plot (Appendix C). When discriminating 96 h holding time in the Tedlar bag for samples from

infested wheat at 20 RFBs/kg, negative DI was obtained and the groups for 0, 24, 48, and 72 h holding times were overlapped with each other while another group of holding times more than 72 h were separated from others. For the sample from infested-wheat at 20 RGBs/kg, all five groups of different holding time were overlapped with each other, which meant that there was no significant change in 96 h for gas samples from RGB-infested wheat at high insect densities.

For 18% moisture content wheat, the longest holding time in the Tedlar bag was 144 h. From RGB-infested wheat at 20 insects/kg and the groups of holding times less than 72 h were overlapped with each other while more than 72 h groups were separated. For samples from infested-wheat at 20 RFBs/kg, all the groups were overlapped with each other.

For 14% and 18% moisture content wheat, some changes occurred for gas samples stored in Tedlar bags more than 72 h. Therefore, the storage time in Tedlar bags needed to be considered for further analysis. Based on the product operating instructions, only data from up to 48 h holding time in Tedlar bags were used for further data analysis. Data from up to 72 h holding time in Tedlar bags were only shown in Appendix section. Therefore, for holding in Tedlar bags more than 48 h, 13% of data at 14% moisture were considered as invalid and for 18% moisture content wheat, 24% of data were deleted for further analysis. For holding in Tedlar bags more than 72 h, 6% of data at 14% moisture were considered as invalid and for 18% moisture content wheat, 19% of data were deleted for further analysis.

5.2 Sensor Array Response to Wheat Samples

During the sample acquisition period, the responses of 12 sensors were recorded automatically by the data acquisition system in the AlphaSoft V8.0 software. The signals of 12 sensors to non-infected, low insect-infested and high insect-infested wheat at 14%, 16%, and 18% moisture were shown in the pre-processing window (Appendix D). Each curve in the plot represents a different transient sensor response to the gas sample. A transient sensor response is displayed by the value of the sensors in relative resistance change. i.e., (R0-R)/R0, where R0 is the resistance of sensor at t = 0 s (baseline resistance) and R is the resistance at the selected time. Total of 120 s was selected for the acquisition time.

Table 5 showed that the response of sensors LY2/LG, T30/1, P10/1, P10/2, P40/1, T70/2, and PA2 all increased sharply after an initial period with positive signs and it started to decrease after peaking, while sensors LY2/G, LY2/AA, LY2/Gh, LY2/gCT1, and LY2/gCT resulted in negative signs. To compare different sensor responses between two gas samples, two plots of sensor signals were loaded in the same window (Appendix D). The average values of peak response for data from up to 48 h holding time in Tedlar bags for each of 12 sensors to the headspace volatiles were shown in Table 5.

Table 5. The average values of the peak response for each of 12 sensors to the headspace volatiles

Sample	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
P40	0.008	-0.058	-0.055	-0.047	-0.032	-0.008	0.103	0.091	0.077	0.116	0.079	0.142
P60	0.076	-0.460	-0.438	-0.393	-0.316	-0.038	0.282	0.266	0.213	0.335	0.260	0.330
P80	0.210	-1.357	-1.322	-1.189	-1.017	-0.102	0.623	0.541	0.238	0.561	0.621	0.679
F42	0.014	-0.100	-0.086	-0.081	-0.056	-0.010	0.125	0.102	0.081	0.125	0.098	0.166
F4T	0.023	-0.158	-0.125	-0.127	-0.088	-0.012	0.151	0.113	0.083	0.135	0.117	0.192
F4M	0.035	-0.236	-0.190	-0.193	-0.135	-0.017	0.202	0.137	0.089	0.156	0.159	0.241
G42	0.009	-0.061	-0.058	-0.049	-0.033	-0.008	0.104	0.092	0.078	0.117	0.080	0.145
G4T	0.009	-0.067	-0.063	-0.054	-0.037	-0.008	0.106	0.093	0.079	0.118	0.082	0.149
G4M	0.009	-0.074	-0.071	-0.060	-0.040	-0.009	0.112	0.096	0.080	0.121	0.086	0.157
F62	0.022	-0.162	-0.143	-0.128	-0.088	-0.014	0.125	0.128	0.115	0.172	0.106	0.163
F64	0.021	-0.159	-0.141	-0.126	-0.087	-0.014	0.124	0.127	0.114	0.171	0.105	0.162
F6M	0.037	-0.198	-0.152	-0.161	-0.118	-0.018	0.127	0.124	0.118	0.171	0.107	0.157
G62	0.034	-0.180	-0.164	-0.143	-0.098	-0.018	0.176	0.181	0.204	0.262	0.148	0.221
G64	0.034	-0.177	-0.162	-0.141	-0.096	-0.018	0.174	0.179	0.203	0.260	0.146	0.219
G6M	0.033	-0.172	-0.157	-0.137	-0.094	-0.018	0.174	0.179	0.202	0.259	0.147	0.217
F82	0.220	-1.436	-1.401	-1.258	-1.081	-0.108	0.652	0.567	0.247	0.588	0.652	0.709
F84	0.214	-1.420	-1.386	-1.244	-1.069	-0.106	0.646	0.563	0.245	0.584	0.645	0.704
F8M	0.209	-1.414	-1.376	-1.240	-1.088	-0.105	0.637	0.556	0.247	0.580	0.635	0.697
G82	0.182	-1.197	-1.160	-1.048	-0.892	-0.091	0.558	0.488	0.220	0.514	0.553	0.615
G84	0.187	-1.248	-1.213	-1.091	-0.930	-0.094	0.582	0.508	0.226	0.531	0.578	0.639
G8M	0.179	-1.226	-1.191	-1.073	-0.925	-0.092	0.579	0.504	0.225	0.528	0.573	0.638
M44	0.008	-0.062	-0.058	-0.050	-0.034	-0.008	0.105	0.093	0.078	0.118	0.081	0.147
M64	0.021	-0.158	-0.141	-0.125	-0.087	-0.014	0.124	0.127	0.114	0.171	0.105	0.163
M84	0.201	-1.348	-1.314	-1.178	-1.008	-0.100	0.619	0.538	0.237	0.560	0.616	0.676

Refer to Table 5, the responses of all p-type sensors (LY2/LG, LY2/G, LY2/AA, LG2/Gh, LY2/gCT1, LY2/gCT) and n-type sensors (T30/1, P10/1, P10/2, P40/1, T70/2, PA2) to the non-infested wheat increased when wheat moisture was higher. It was consistent with the point presented by Gardner and Bartlett (1999) that MOS sensors were more sensitive to humidity. It was found that most of sensor curves increased sharply during the initial period. After that period, all sensor responses dropped slowly until they were close to the baseline (the baseline is 0). Maximum value for each sensor response during the acquisition period was selected for sensor signal comparison. Mielle (1996) and other researchers showed that n-type coating was more sensitive to oxidizing compounds while p-type responded to reducing compounds. In this case, four p-type sensors were more sensitive to the moisture content changed from 14% to 16%. Therefore, it seemed that the quantity of the reducing compounds changed when moisture increased from 14% to 16%. For example, the signal of sensor LY2/G increased its relative value from |-0.058| to |-0.460|, or 7.9 times at 14%; sensor LY2/AA 7.9 times; sensor LY2/Gh 8.4 times, and sensor LY2/gCT1 9.9 times. It was speculated that all due to changes in reducing compounds while a little change in oxidizing compounds happened when wheat moisture changed. Furthermore, four of 6 n-type sensors' responses changed slightly when moisture changed from 14% to 16%. For example, sensor T30/1 signal increased from 0.103 to 0.282 in its resistance change, sensor p10/1 from 0.091 to 0.266, sensor p 40/1 from 0.116 to 0.335, sensor T70/2 from 0.079 to 0.260 and sensor PA2 from 0.142 to 0.330. Comparing the sensor responses to headspace volatiles from wheat at 16% and 18% moisture content, all n-type and p-type sensors increased their relative resistance when moisture was higher. Four of the sensors

(LY2/AA, LY2/G, LY2/gCT1, and LY2/gCT) were more sensitive to the change in moisture of wheat from 16% to 18%, for example sensor LY2/AA increased its peak value 3.02 times at 16% more than that at 18% moisture, LY2/G 2.95 times, LY2/gCT1 3.22 times, and LY2/Gh 3.03 times.

For headspace volatiles from 14% moisture content wheat, sensor responses had minor changes between the non-infested wheat and infested-wheat by RGB at 1 insect/kg level. Wheat produce different volatiles depending on the storage time, and aldehydes, ketones, and alcohols are considered as the main volatiles (Zhang and Wang 2007). Seitz and Sauer (1996) mentioned that red flour beetle could produce some off-odour which might be quinine compounds. Seitz and Sauer (1996) used ion chromatograms to determine the pheromones and metabolites from RFB and RGB. They detected 1-pentadecene, an apparent diene, 4, 8-dimethyldecanal, and another apparent diene were from RFB, and macrolides I, II, and III were produced by RGB. However, it seemed that these compounds were produced at such low levels that they could not be detected by the sensors. Alternatively, both RFB and RGB might not be active enough at low moisture content to cause production of the volatile compounds.

For headspace volatiles from 16% moisture content wheat, sensor responses were compared for low and high insect infestation levels between RFB-infested and RGB-infested-wheat samples. Sensor responses between control and RGB-infested wheat had minor change between RFB and RGB infested-wheat at the low infestation level. However, sensor responses to RFB infested-wheat became higher than that to RGB infested-wheat at the high insect density. Since glass jars (the sample jars) were air-tight

and wheat were stored inside for more than 24 h, it could be regarded as non-ventilated systems containing wheat of 16% initial moisture content. Wheat volatiles and perhaps low level mould odour might affect or even overwhelm the volatiles from insects at low density levels. With an increase in insect density in 16% moisture content wheat, the volatiles produced by RFB became stronger and could be detected by the sensors. Since RGB did not like dry conditions (under lower than 12% moisture content wheat) or less than 40% relative humidity conditions (Anonymous 1981), it would not develop. Therefore, sensor responses to volatiles and metabolites by RGB were low even at the high infestation level.

For sensor responses to samples at 18% moisture content, the changes in sensor responses to different infestation levels were not significant. Possibly wheat volatiles occurred in the high moisture content wheat and the sensors were more sensitive to them. Though mycotoxins were not found and ergosterol was low at 16% moisture content wheat, Ochratoxin A and citrinin levels increased when moisture was higher (Abramson et al. 2005). Though 1-octen-3-ol and 3-octanone were less frequently produced, they were correlated with infection by microflora in the high moisture content wheat in non-ventilated bins (Tuma et al. 1989). It seemed that the sensor responses were more sensitive to the compounds produced by wheat at high moisture content than volatiles from insects. Therefore, for 18% moisture wheat, sensor signals had minor changes on non-insect and insect infested-wheat at different density levels.

Sensor responses to the mixture of infested-wheat by RFB and RGB at three moisture contents were compared. The peak values increased for each sensor when

moisture was higher and there was minor changes compared with the infested-wheat by RFB at low density at the same moisture content. Sensory panels from the Department of Federal Grain Inspection (USA) assessed odour from RFB and RGB infested sorghum at 14% moisture content (Seitz and Sauer 1996), and they found RFB caused some off-odour while RGB caused little or no objectionable odour even though density was high. It was consistent with the results that volatiles and metabolites by RGB were too low to be detected by the electronic nose. Therefore, for 14% and 16% moisture content wheat, the compounds that could be detected by sensors in the mixture of infested-wheat might be from RFB, while for 18% moisture wheat, the odours might be from wheat volatile compounds.

In summary, the sensors were more sensitive to the different moisture contents of the wheat. Furthermore, they were more sensitive to the volatiles and metabolites by RFB at low moisture content wheat (14% and 16% moisture), while more sensitive to wheat volatiles and perhaps some low level mould odours (although no mould was visible during the test duration) at high wheat moisture content (18% moisture).

5.3. Wheat Moisture Content Analysis

As discussed above, wheat moisture was an important factor that might affect the ability of the electronic nose to detect insects in a wheat bulk. Principal Component Analysis (PCA) was used to visualize the sensor response to the volatiles from different moisture contents of wheat. Data from up to 48 h holding time in Tedlar bags were shown in Figure 5, and data from up to 72 h holding time in Tedlar bags were shown in

Appendix E. From the PCA and DFA plot, no significant difference was found between data from up to 48 h and 72 h holding time in Tedlar bags.

In Figure 5, three groups were located in different regions. However, three samples were located far away from the other samples of the 18% moisture content group, which caused overlap with 16% moisture content group and a low discrimination index (DI). Those samples were possibly outliers. The suspect samples were isolated and evaluated as sensor signals as shown in the example in Appendix F. The sensor responses to those samples were compared with other samples among the same quality group. The comparisons showed that the sensor responses from those three samples done on August 23rd and 24th were different from the others. Furthermore, the peak values of 12 sensors for those three suspect outliers were significant smaller than the average values of the same quality (Table 6). Therefore they were regarded as outliers and excluded in analysis. Since there were no changes in methodology, outliers might be caused by an occasional incorrect sample injection or preparation.

A PCA plot was shown in Figure 6 after removal of outliers, and there was no overlap among three groups.A clear separation among three groups based on the different wheat moisture contents was shown in the PCA plot (Figure 6). Supervised pattern recognition algorithms were used for further model training and prediction. Two models (DFA and PLS) based on the training data of clean wheat were validated. The DFA model was validated by the leave-one-out cross validation method. The training scores and testing scores for the PLS model were shown in Appendix G. The independent test data in black were shown in Figures 7 and 8.

For the DFA analysis, 99% of the validation score was obtained between the training data and test data. And for PLS analysis, good correlation (0.99) between the expected moisture content of the wheat and predicted moisture content of the wheat was obtained after cross-validation of the PLS model. Therefore, the validation was valid in this case.

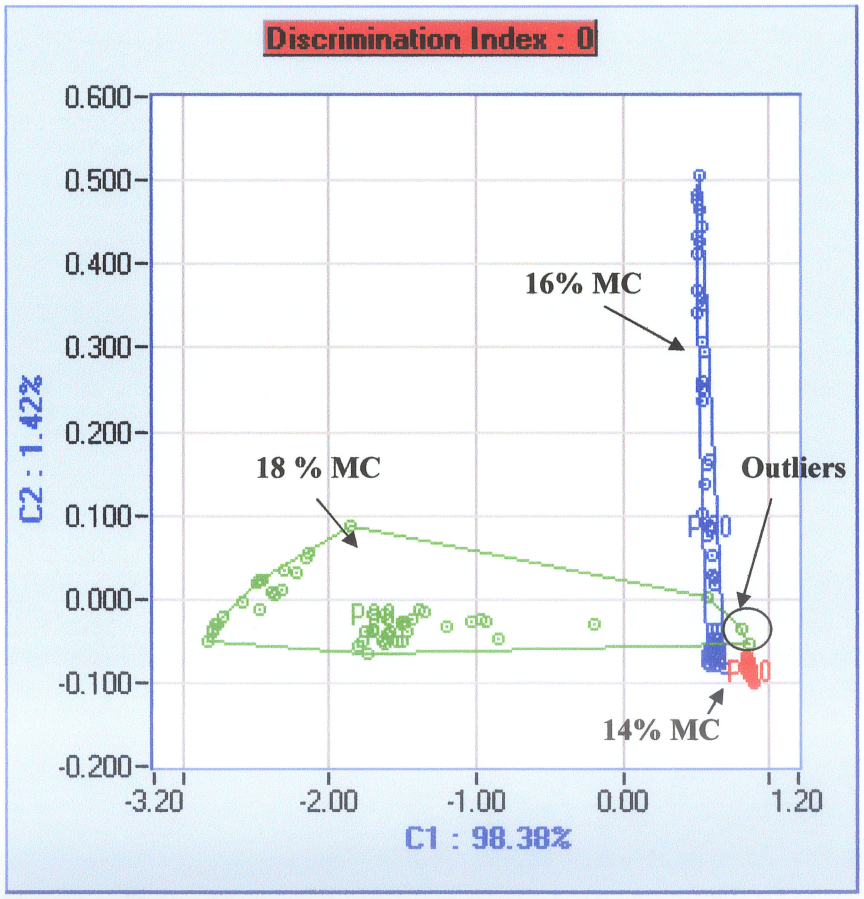

Figure 5. PCA plot of clean wheat at 14%, 16% and 18% MC, and data are from up to 48 h holding in Tedlar bags.

Note: 16% and 18% maps are overlapped with each other.

Table 6. The peak values of 12 sensors to the headspace volatiles from the suspect outliers compared with average values

Sample	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
P80 (Average)	0.210	-1.357	-1.322	-1.189	-1.017	-0.102	0.623	0.541	0.238	0.561	0.621	0.679
P80A_34.dat	0.020	-0.194	-0.176	-0.154	-0.108	-0.017	0.218	0.167	0.107	0.189	0.177	0.274
P80B_42.dat	0.012	-0.074	-0.067	-0.059	-0.043	-0.011	0.137	0.111	0.099	0.146	0.107	0.171
P80C_50.dat	0.011	-0.055	-0.048	-0.046	-0.033	-0.011	0.112	0.099	0.098	0.134	0.088	0.145

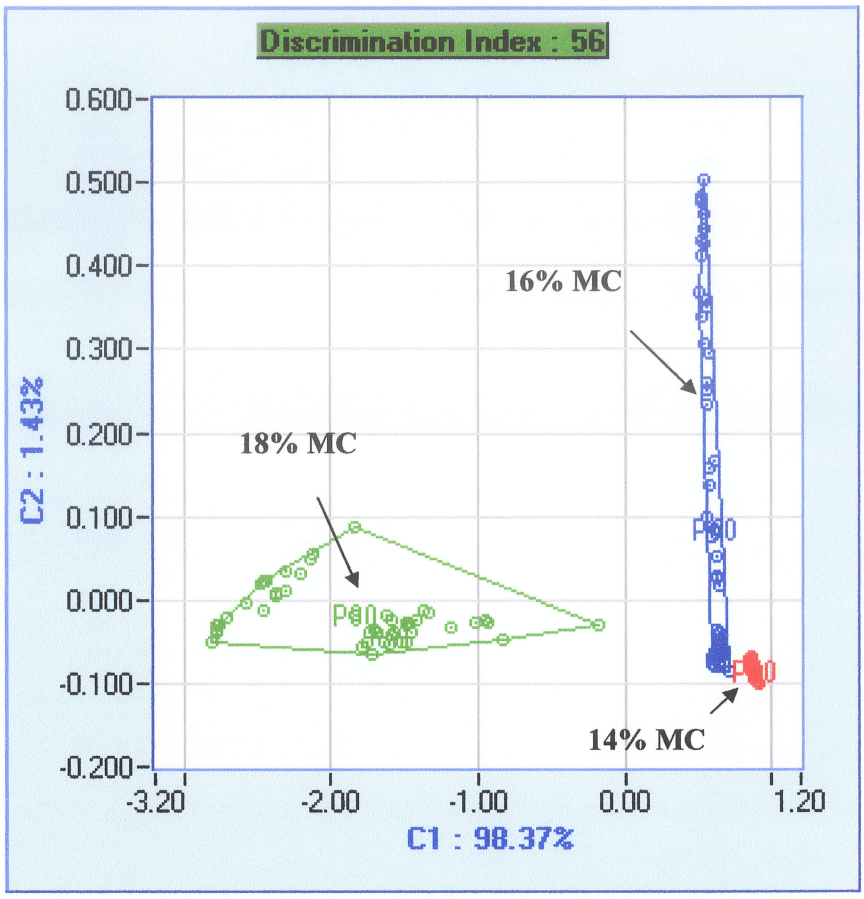

Figure 6. PCA plot of clean wheat at 14%, 16% and 18% moisture content after the removal of outliers, and data are from up to 48 h holding in Tedlar bags.

Note: 16% MC and 18% group are separated due to removal of the outliers.

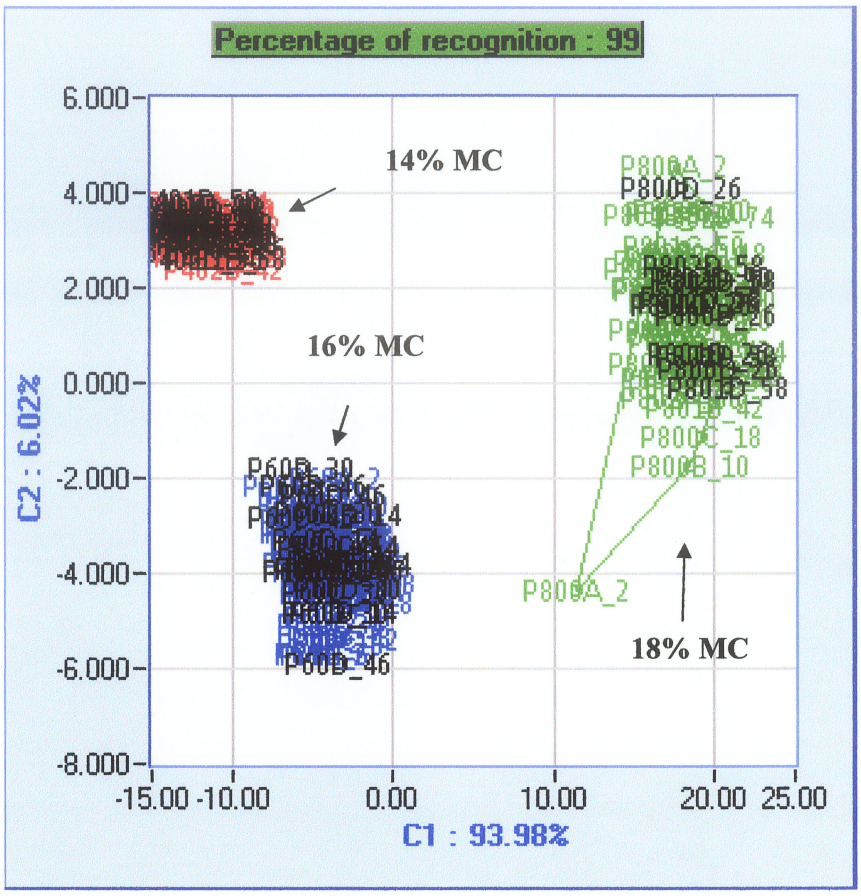

Figure 7. DFA plot of non infested-wheat at 14%, 16% and 18% moisture contents.

Note: Three moisture contents of wheat groups are separated showing no differentiation among the groups.

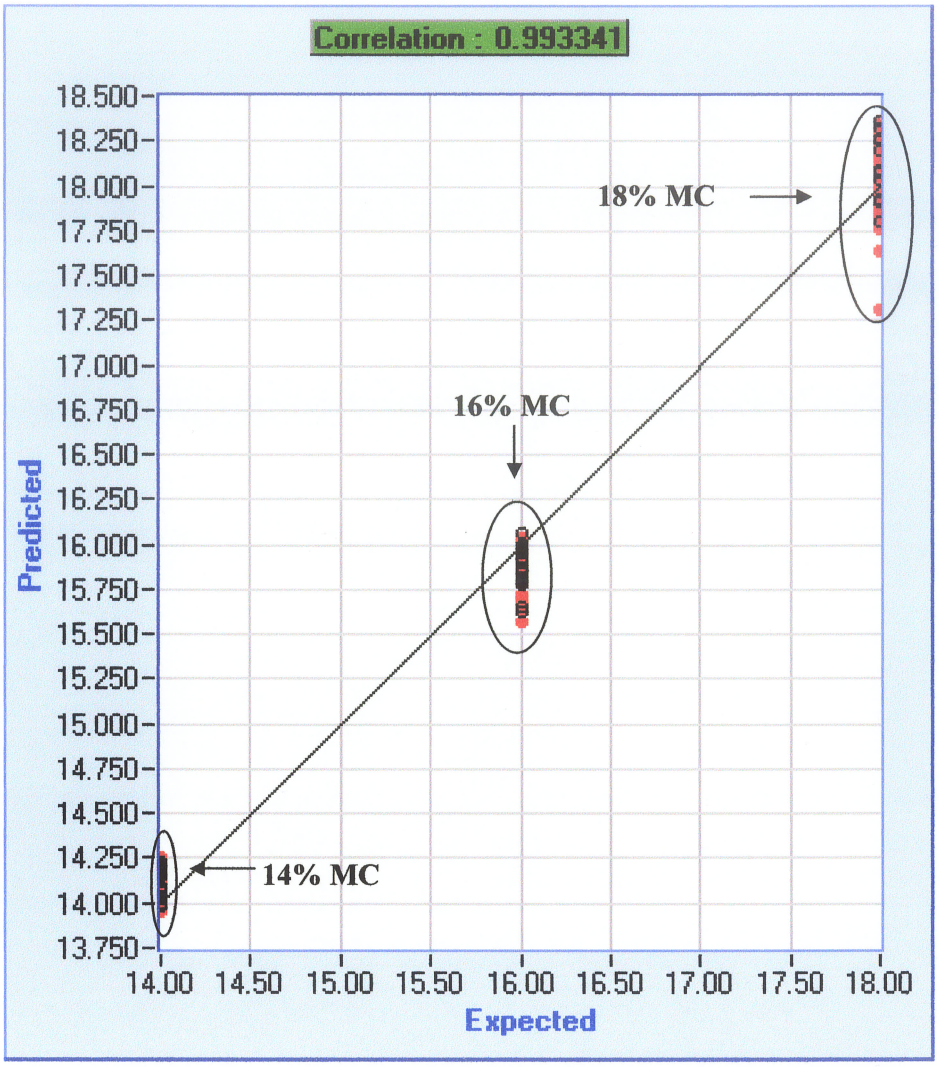

Figure 8. PLS plot of non infested-wheat at 14%, 16% and 18% moisture contents.

Note: The graph plots the training values (X-axis) versus the predicted (Y-axis).

5.4. Insect Detection Analysis

5.4.1 Detection of the presence of insects and insect species differentiation

For 14%, 16% and 18% moisture content wheat, samples were analyzed by selecting the sensor responses to low insect infestation levels from batch A, B, and C (training set). Usually the first sample was run for "warm up" and its sensor signals were not used for further analysis. The batch D data (testing set) were used to validate the model and for prediction of unknown samples. The PCA was performed in the multivariate statistic window from AlphaSoft V8.0 software (Alpha MOS, Toulouse, France). The responses of the sensor array to low density insect infested and non-infested wheat samples at 14%, 16% and 18% moisture content were interpreted and demonstrated in the PCA plots. Plots from up to 48 h holding time in Tedlar bags were shown in Figure 9 – 14, and plots from up to 72 h holding time in Tedlar bags were shown in Appendix H.

From Figures 9 to 11, RFB and RGB infested-wheat at low density of insects and clean wheat were overlapped with each other when three different moisture contents were tested. Therefore, the electronic nose did not detect the presence of either RFB or RGB in wheat at low infestation levels (1 insect/kg, 2 insects/kg) and it also failed to differentiate the RFB from RGB when insect density was low.

The responses of the sensor array to the high density of insect infested and non-infested wheat at 14%, 16% and 18% moisture content were interpreted and demonstrated in the PCA plots (Figures 12 – 14).

From Figures 12 to 14, maps of RGB infested-wheat were overlapped with clean wheat at 14%, 16% and 18% moisture content, while RFB infested-wheat was totally separated from clean wheat at 14% moisture content. Furthermore, both RFB and RGB infested-wheat with the high insect density were overlapped with clean wheat at 18% moisture content. Therefore, the electronic nose did not detect the presence of RGB in wheat at both low and high infestation levels at three different moisture contents. It was consistent with the conclusion that there was low or less objectionable odour present from RGB infested-grain sorghum (Seitz and Sauer 1996). Since there was no superimposition between high density of RFB infested-wheat and clean wheat at 14% moisture content and partial superimposition between the high density of RFB infested-wheat and clean wheat at 16% moisture content, DFA was performed to classify the two groups (Figures 15 – 16).

In Figures 15 and 16, two territories were defined for two groups. The percentage of recognition was 97% in Figure 15, which meant that 97% of the samples could be classified as two categories correctly during the cross validation for 14% moisture content wheat, and for 16% moisture content wheat, 99% of the samples were classified during cross validation (Figure 16).

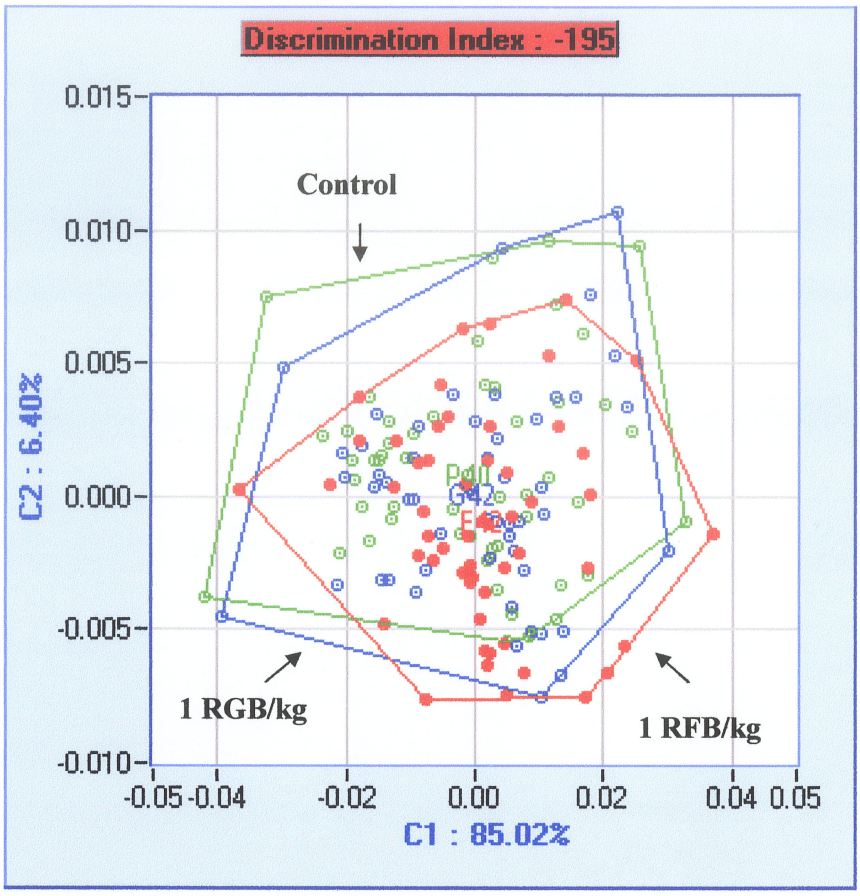

Figure 9. PCA plot of control and low infested-wheat by RFB and RGB at 14% moisture content.

Note: Three groups are overlapped with each other showing no differentiation among the groups.

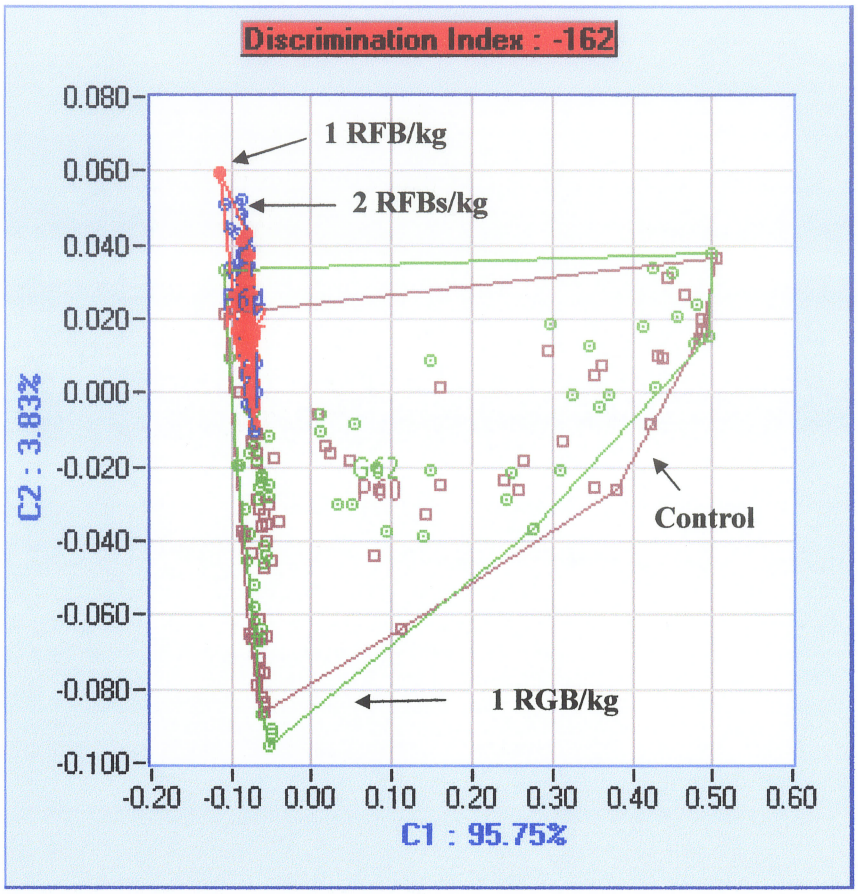

Figure 10. PCA plot of control and low infested-wheat by RFB and RGB at 16% moisture content.

Note: Four groups are overlapped with each other showing no differentiation among the groups.

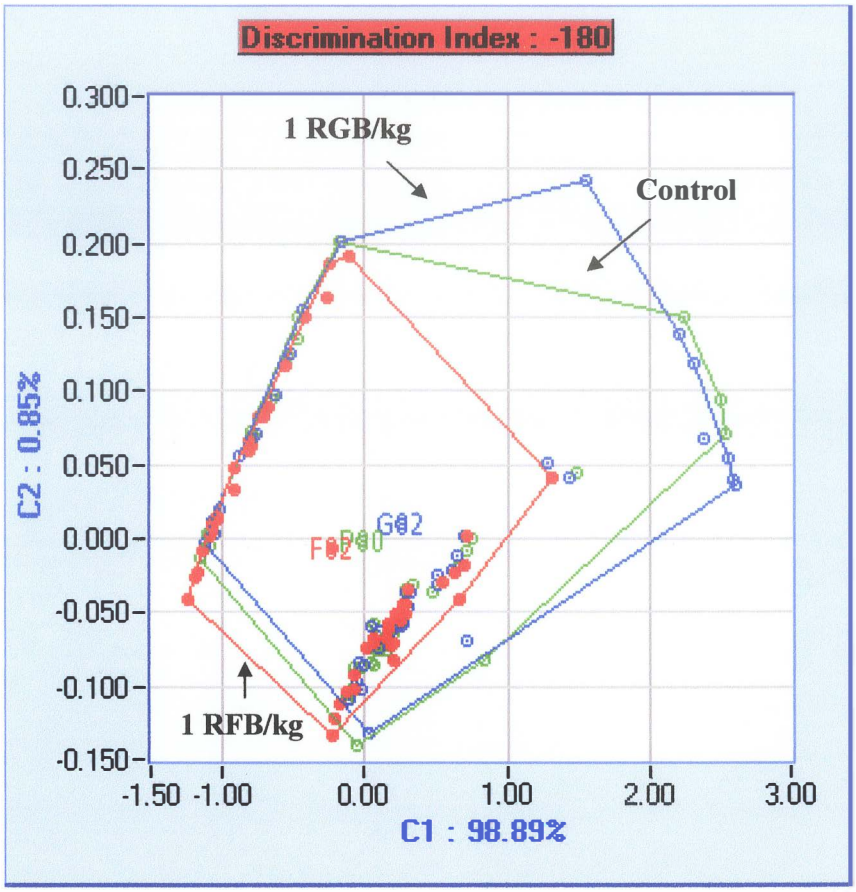

Figure 11. PCA plot of control and low infested-wheat by RFB and RGB at 18% moisture content.

Note: Three groups are overlapped with each other showing no differentiation among the groups.

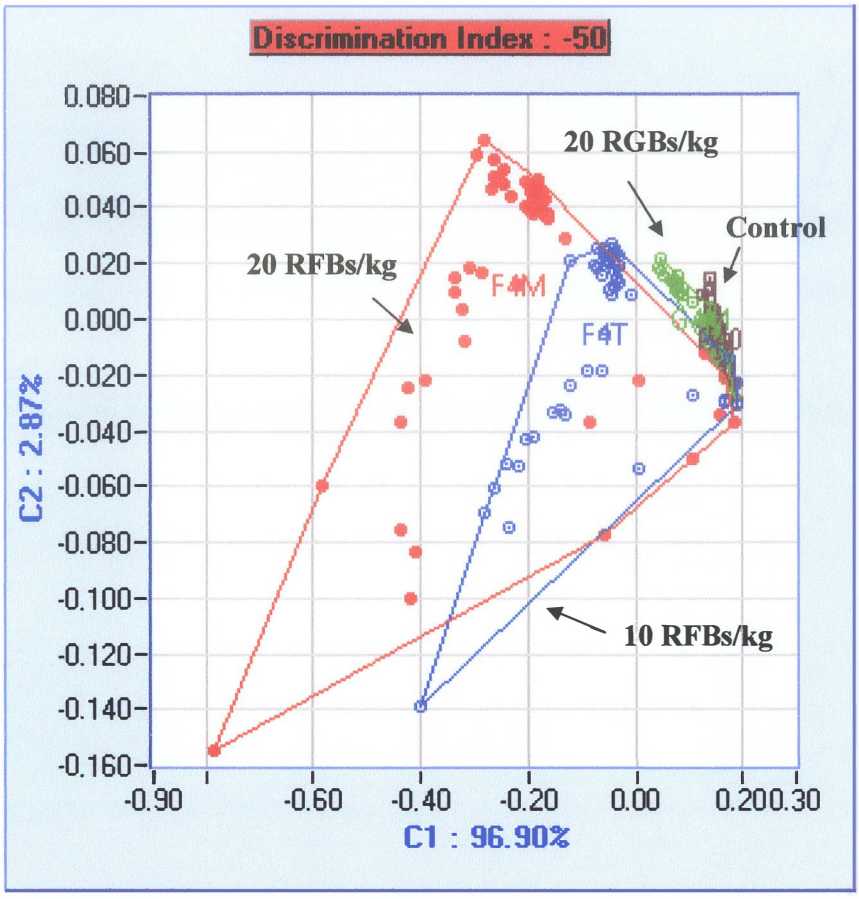

Figure 12. PCA plot of control and high infested-wheat by RFB and RGB at 14% moisture content.

Note: 20 RFBs/kg is separated from Control.

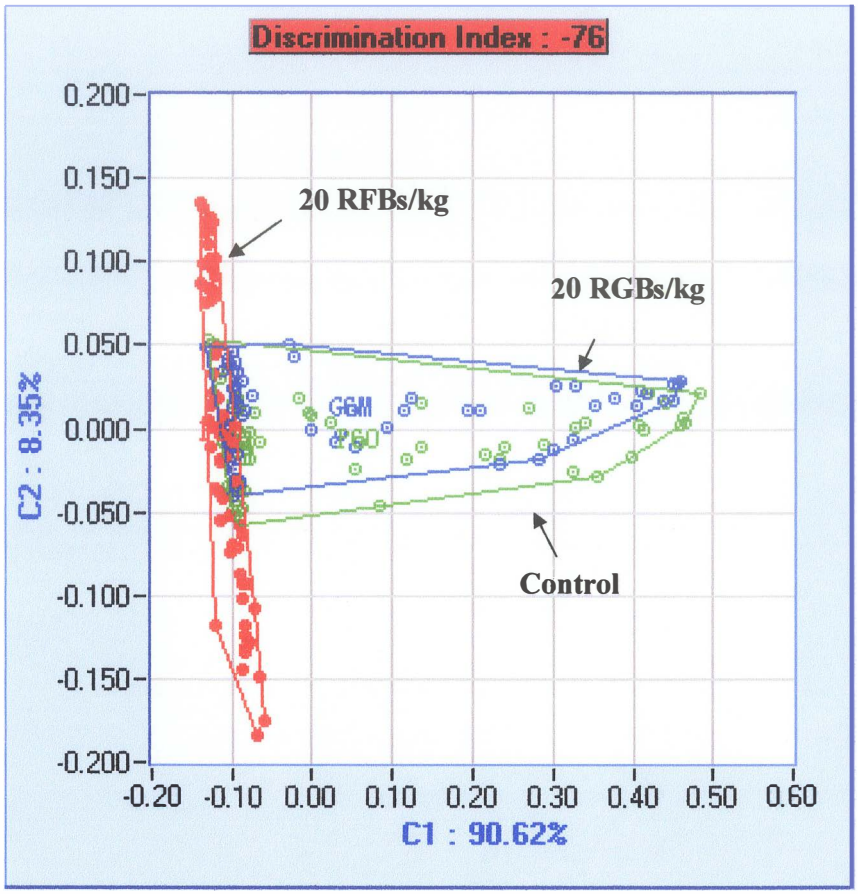

Figure 13. PCA plot of control and high infested-wheat by RFB and RGB at 16% moisture content.

Note: 20 RGBs/kg and Control group are overlapped with each other.

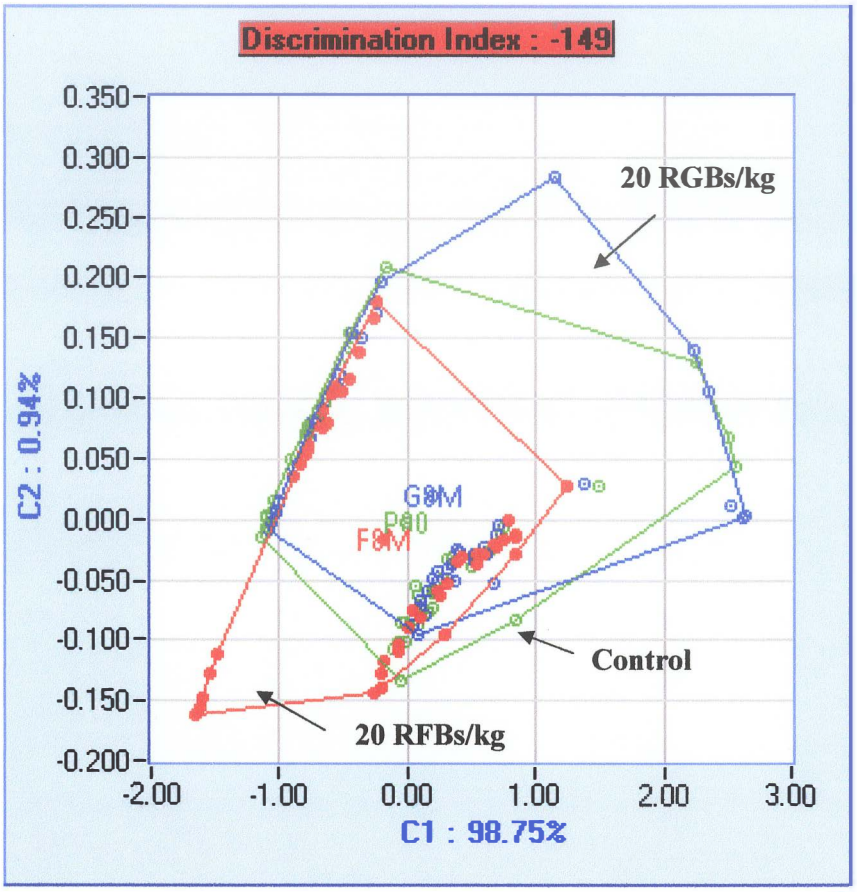

Figure 14. PCA plot of control and high infested-wheat by RFB and RGB at 18% moisture content.

Note: Three groups are overlapped with each other showing no differentiation among the groups.

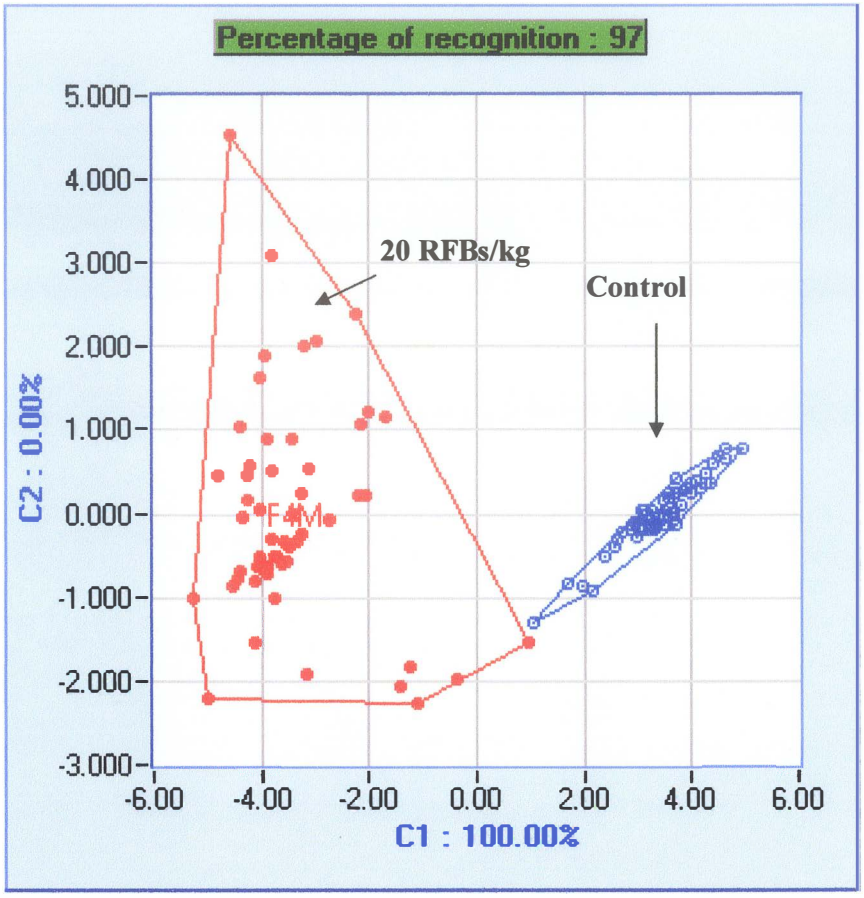

Figure 15. DFA plot of control and infested-wheat by RFB at 14% moisture content.

Note: Two groups are separated showing the difference between the two groups.

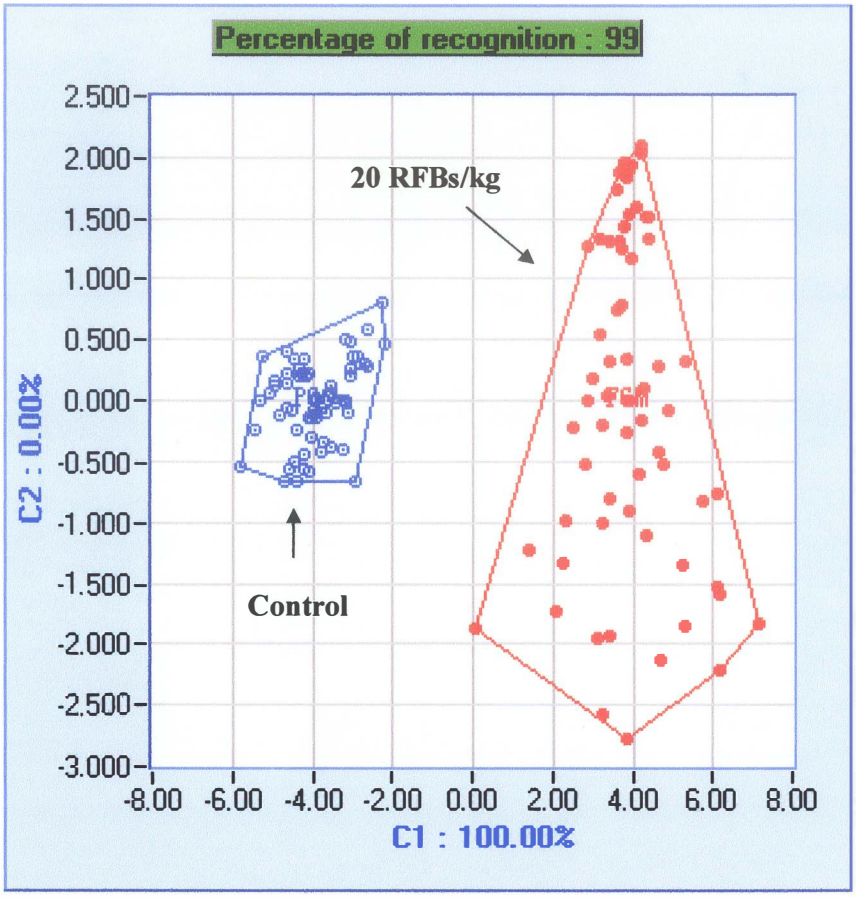

Figure 16. DFA plot of control and infested-wheat by RFB at 16% moisture content.

Note: Two groups are separated showing the difference between the two groups.

5.4.2 Insect infestation level identification

As discussed above, there were overlaps between low and high infestation level of RGB in 14%, 16% and 18% moisture content wheat. Furthermore, low DI was obtained -149 for 18% moisture content wheat (Figure 14), there was no difference between low and high infestation level of RFB in 18% moisture content wheat. Thus, the electronic nose did not identify the degree of RGB infestation in 14%, 16% and 18% moisture content wheat and RFB infestation level in 18% moisture content wheat. Only RFB in 14% and 16% moisture content wheat were discussed. Plots from up to 48 h holding time in Tedlar bags were shown in the PCA and DFA (Figures 17 – 20), and plots from up to 72 h holding time in Tedlar bags were discussed in Appendix I.

Figure 17 showed the results of applying PCA to visualize the difference between groups of different infestation levels. In the PCA plot, the data were defined by the first two principal components (99.8% of the total variance). Though 10 RFBs/kg and 20 RFBs/kg were overlapped with each other, a clear separation of 1 RFB/kg and 20 RFBs/kg or 1 RFB/kg and 10 RFBs/kg was found. The results obtained by DFA analysis provided a similar classification. Therefore, 1 RFB/kg and 20 RFBs/kg infestation levels could be identified in wheat at 14% moisture by the electronic nose. Figure 18 showed that two high infestation level groups were overlapped while the low infestation level group was separated from them.

In Figure 19 and 20, both PCA and DFA plots showed overlaps between two low infestation levels, while high infestation level was separated from others. Therefore, 1 RFB/kg and 20 RFBs/kg infestation levels could be identified in wheat at 16% moisture

by the electronic nose. However, due to low cross-validation scores obtained, the DFA models were invalid to predict unknown samples.

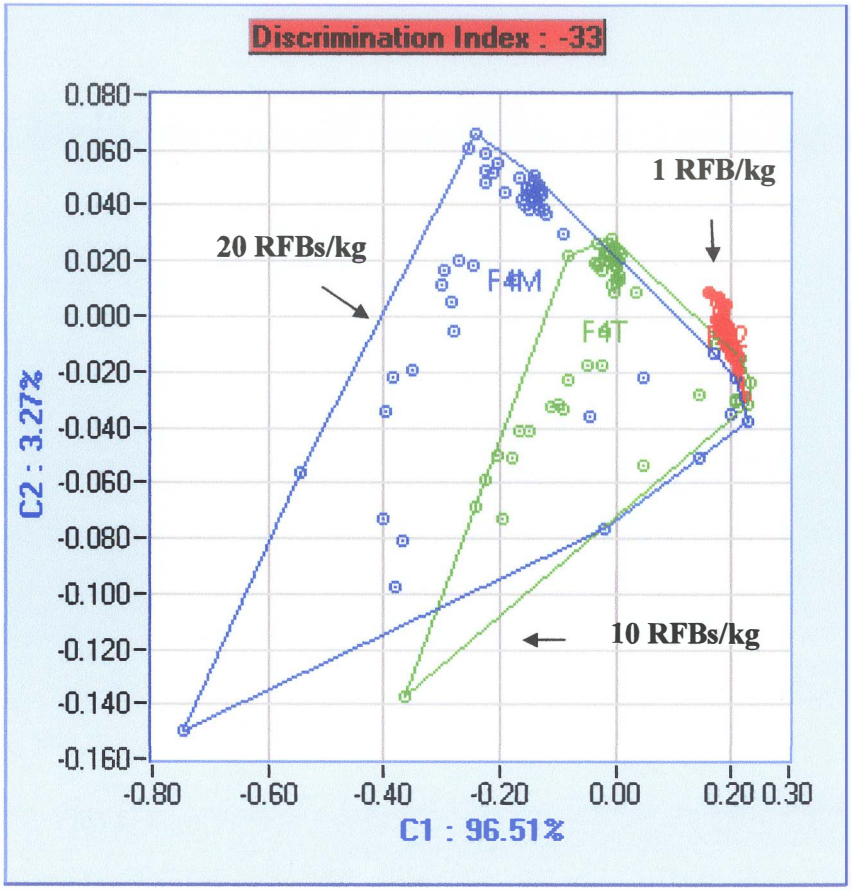

Figure 17. PCA plot of 14% moisture content wheat infested by RFB at three infestation levels.

Note: 20 RFBs/kg and 10 RFBs/kg group are overlapped each other.

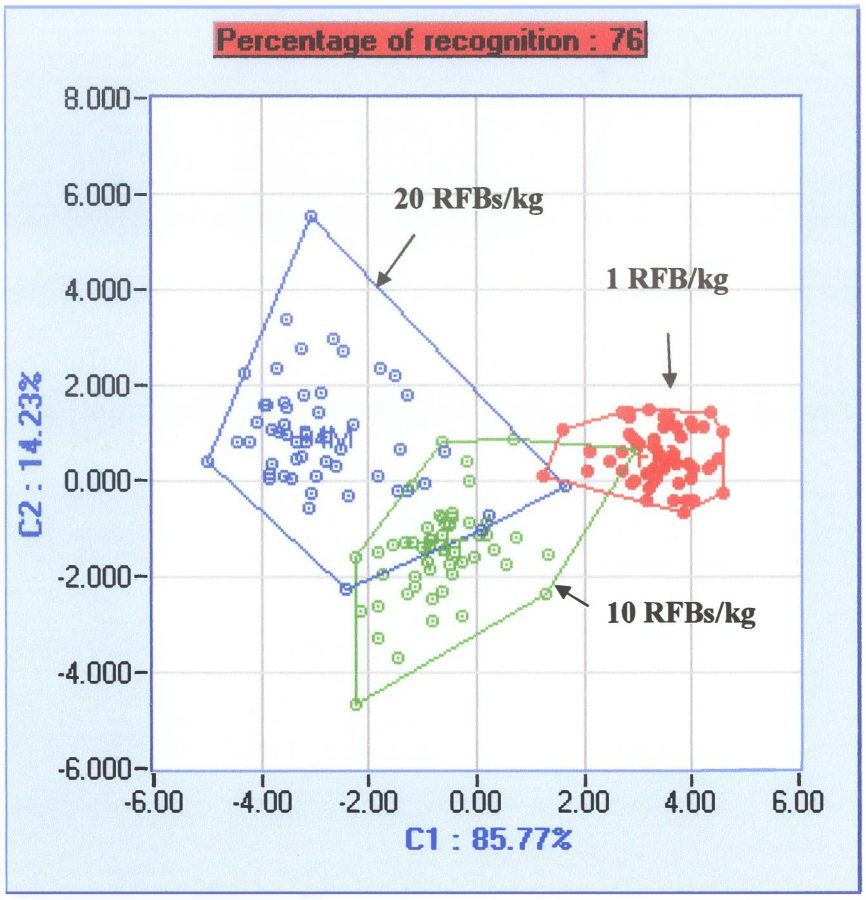

Figure 18. DFA plot of 14% moisture content wheat infested by RFB at three infestation levels.

Note: 20 RFBs/kg and 10 RFBs/kg group are overlapped with each other.

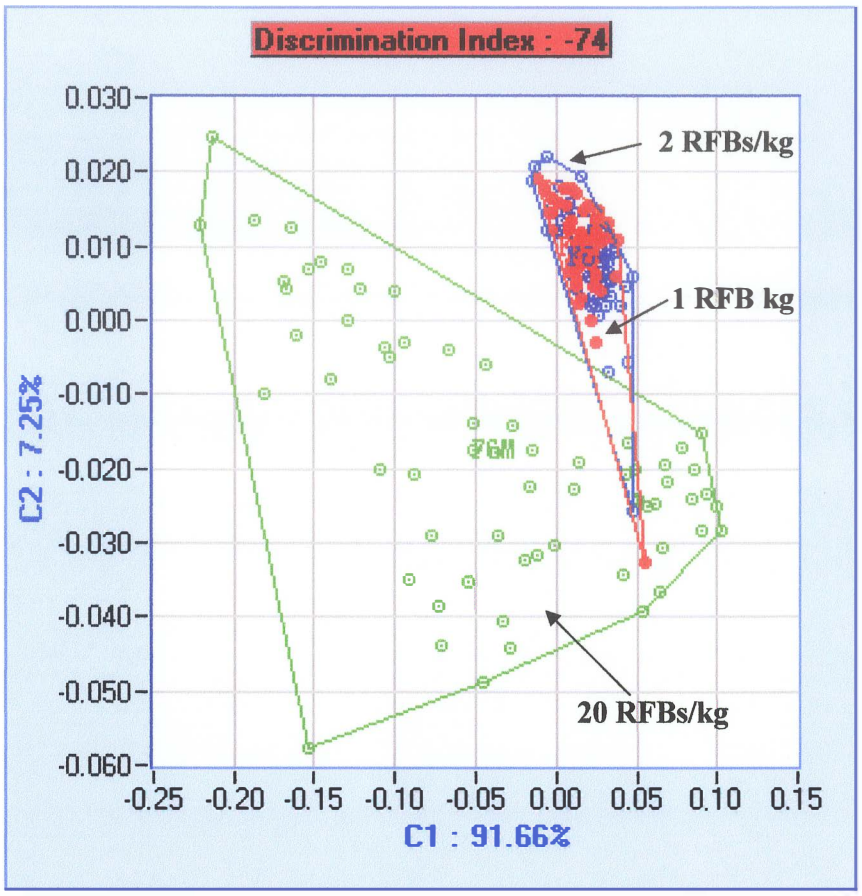

Figure 19. PCA plot of 16% moisture content wheat infested by RFB at three infestation levels.

Note: 1 RFB/kg and 2 RFBs/kg group are overlapped with each other.

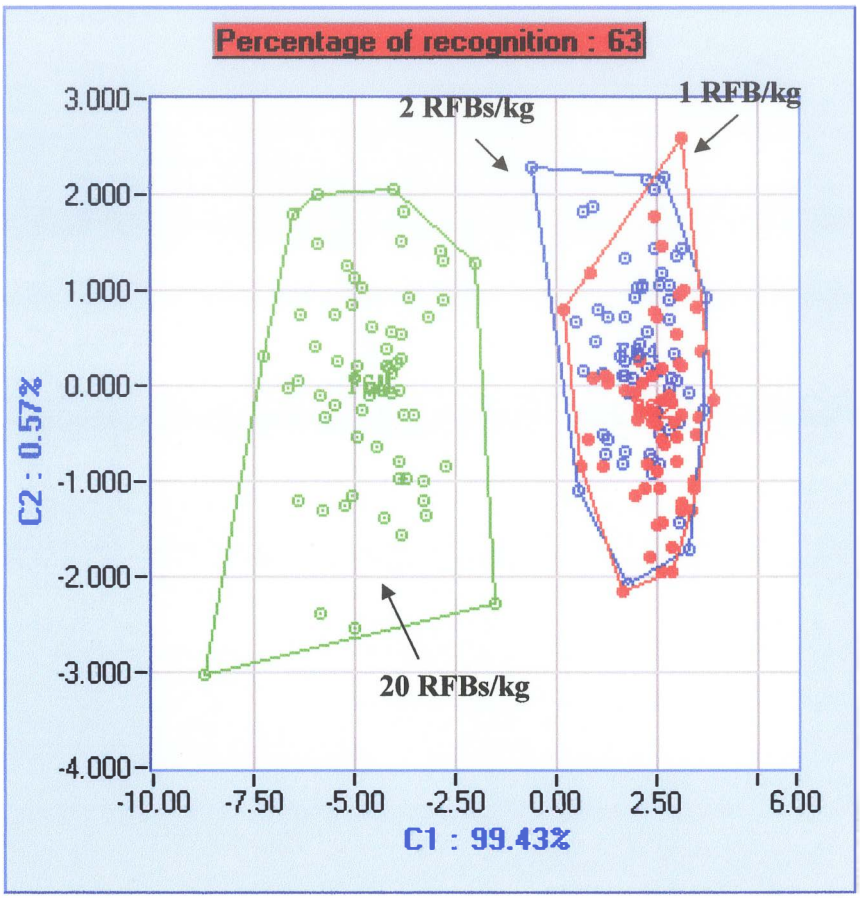

Figure 20. DFA plot of 16% moisture content wheat infested by RFB at three infestation levels.

Note: 1 RFB/kg and 2 RFBs/kg group are overlapped with each other.

6. CONCLUSIONS

An Alpha MOS FOX-3000 electronic nose was used to evaluate the presence of RFB and RGB in wheat. Different numbers of insects (0, 1, 2, 10 and 20 insects/kg) were tested for each insect species in combination with three moisture content levels (14%, 16%, and 18%). The headspace volatiles from infested or non-infested wheat were injected into the sensor array. The automated pattern recognition algorithms in the software, such as Principal Component Analysis (PCA), Discriminant Factorial Analysis (DFA), and Partial Least Square (PLS) were used to evaluate the samples. The following conclusions were drawn from the study:

1. Multivariate statistical methods were useful tools to extract interpretable information from the output of the electronic nose, and PCA, DFA and PLS were effective for the application of electronic nose to detect insects in wheat.
2. Storage time of gas sample in Tedlar bag was a factor that needed to be considered for further analysis. There was no significant difference between holding time in Tedlar bags up to 48 h and 72 h. Therefore, it was suggested to use sample whose holding time was less than 72 h in this research though holding time less than 48 h was recommended by manufactures.
3. Sensors were shown to be sensitive to the moisture content of the wheat. When wheat moisture was low (14% and 16%), sensors became more sensitive to the volatiles and metabolites by red flour beetle (RFB), and when wheat moisture was high (18%), wheat volatiles and perhaps, some low level mould odours (although

no mould was visible during the test duration) might affect the sensor responses.

4. Discriminant Factorial Analysis and Partial Least Squares algorithms were effective in the application of electronic nose to predict wheat moisture content. Both high percentage of recognition (99%) and high cross-validation (0.99) were achieved for predicting moisture content of wheat at non-infestation level.
5. In this research, electronic nose could detect the presence of red flour beetle (RFB) in wheat with the high infestation level (20 insects/kg) at 14% and 16% moisture content. However, the electronic nose did not detect the presence of rusty grain beetle (RGB) in wheat at either the low (1 insect/kg and 2 insects/kg) or the high infestation levels (10 insects/kg and 20 insects/kg). It also failed to detect the presence of RFB in wheat with the low (1 insect/kg and 2 insects/kg) and high infestation level (20 insects/kg) at 18% moisture content.
6. The electronic nose was able to differentiate 1 RFB/kg infestation level from 20 RFBs/kg infestation level in wheat at 14% and 16% moisture content. However, it did not identify densities for rusty grain beetle in wheat at three different moisture contents (14, 16%, and 18%).

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Appendix A

The sample codes for the effect of storage time of gas sample in Tedlar bags.

Table A.1. The sample codes for the effect of storage time of gas sample in Tedlar bags.

Sample Code	Description
F420	0 day in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F421	1 day in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F422	2 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F423	3 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F424	4 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F4T0	0 day in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F4T1	1 day in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F4T2	2 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F4T3	3 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F4T4	4 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F4M0	0 day in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F4M1	1 day in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F4M2	2 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F4M3	3 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F4M4	4 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F400	0 day in Tedlar bag for gas sample from clean wheat at 14% MC
F401	1 day in Tedlar bag for gas sample from clean wheat at 14% MC
F402	2 days in Tedlar bag for gas sample from clean wheat at 14% MC
F403	3 days in Tedlar bag for gas sample from clean wheat at 14% MC
F404	4 days in Tedlar bag for gas sample from clean wheat at 14% MC
G420	0 day in Tedlar bag for gas sample from wheat at 14% MC and 1 RGB/kg
G421	1 day in Tedlar bag for gas sample from wheat at 14% MC and 1 RGB/kg
G422	2 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RGB/kg
G423	3 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RGB/kg
G424	4 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RGB/kg
G4T0	0 day in Tedlar bag for gas sample from wheat at 14% MC and 10 RGBs/kg

Sample Code	Description
G4T1	1 day in Tedlar bag for gas sample from wheat at 14% MC and 10 RGBs/kg
G4T2	2 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RGBs/kg
G4T3	3 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RGBs/kg
G4T4	4 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RGBs/kg
G4M0	0 day in Tedlar bag for gas sample from wheat at 14% MC and 20 RGBs/kg
G4M1	1 day in Tedlar bag for gas sample from wheat at 14% MC and 20 RGBs/kg
G4M2	2 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RGBs/kg
G4M3	3 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RGBs/kg
G4M4	4 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RGBs/kg
M440	0 day in Tedlar bag for gas sample from the mixture of RFB&RGB-infested wheat at 14% MC and 2 insects per kg wheat
M441	1 day in Tedlar bag for gas sample from the mixture of RFB&RGB-infested wheat at 14% MC and 2 insects per kg wheat
M442	2 days in Tedlar bag for gas sample from the mixture of RFB&RGB-infested wheat at 14% MC and 2 insects per kg wheat
M443	3 days in Tedlar bag for gas sample from the mixture of RFB&RGB-infested wheat at 14% MC and 2 insects per kg wheat
M444	4 days in Tedlar bag for gas sample from the mixture of RFB&RGB-infested wheat at 14% MC and 2 insects per kg wheat
F820	0 day in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F821	1 day in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F822	2 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F823	3 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F824	4 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F825	5 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F826	6 days in Tedlar bag for gas sample from wheat at 14% MC and 1 RFB/kg
F8T0	0 day in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F8T1	1 day in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg

Sample Code	Description
F8T2	2 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F8T3	3 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F8T4	4 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F8T5	5 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F8T6	6 days in Tedlar bag for gas sample from wheat at 14% MC and 10 RFBs/kg
F8M0	0 day in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F8M1	1 day in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F8M2	2 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F8M3	3 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F8M4	4 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F8M5	5 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F8M6	6 days in Tedlar bag for gas sample from wheat at 14% MC and 20 RFBs/kg
F800	0 day in Tedlar bag for sample from clean wheat at 14% MC
F801	1 day in Tedlar bag for sample from clean wheat at 14% MC
F802	2 days in Tedlar bag for sample from clean wheat at 14% MC
F803	3 days in Tedlar bag for sample from clean wheat at 14% MC
F804	4 days in Tedlar bag for sample from clean wheat at 14% MC
F805	5 days in Tedlar bag for sample from clean wheat at 14% MC
F806	6 days in Tedlar bag for sample from clean wheat at 14% MC
G820	0 day in Tedlar bag for sample from wheat at 14% MC and 1 RGB/kg
G821	1 day in Tedlar bag for sample from wheat at 14% MC and 1 RGB/kg
G822	2 days in Tedlar bag for sample from wheat at 14% MC and 1 RGB/kg
G823	3 days in Tedlar bag for sample from wheat at 14% MC and 1 RGB/kg
G824	4 days in Tedlar bag for sample from wheat at 14% MC and 1 RGB/kg
G825	5 days in Tedlar bag for sample from wheat at 14% MC and 1 RGB/kg
G826	6 days in Tedlar bag for sample from wheat at 14% MC and 1 RGB/kg
G8T0	0 day in Tedlar bag for sample from wheat at 14% MC and 10 RGBs/kg

Sample Code	Description
G8T1	1 day in Tedlar bag for sample from wheat at 14% MC and 10 RGBs/kg
G8T2	2 days in Tedlar bag for sample from wheat at 14% MC and 10 RGBs/kg
G8T3	3 days in Tedlar bag for sample from wheat at 14% MC and 10 RGBs/kg
G8T4	4 days in Tedlar bag for sample from wheat at 14% MC and 10 RGBs/kg
G8T5	5 days in Tedlar bag for sample from wheat at 14% MC and 10 RGBs/kg
G8T6	6 days in Tedlar bag for sample from wheat at 14% MC and 10 RGBs/kg
G8M0	0 day in Tedlar bag for sample from wheat at 14% MC and 20 RGBs/kg
G8M1	1 day in Tedlar bag for sample from wheat at 14% MC and 20 RGBs/kg
G8M2	2 days in Tedlar bag for sample from wheat at 14% MC and 20 RGBs/kg
G8M3	3 days in Tedlar bag for sample from wheat at 14% MC and 20 RGBs/kg
G8M4	4 days in Tedlar bag for sample from wheat at 14% MC and 20 RGBs/kg
G8M5	5 days in Tedlar bag for sample from wheat at 14% MC and 20 RGBs/kg
G8M6	6 days in Tedlar bag for sample from wheat at 14% MC and 20 RGBs/kg
M840	0 day in Tedlar bag for sample from the mixture of RFB&RGB-infested wheat at 18% MC and 2 insects per kg wheat
M841	1 day in Tedlar bag for sample from the mixture of RFB&RGB-infested wheat at 18% MC and 2 insects per kg wheat
M842	2 days in Tedlar bag for sample from the mixture of RFB&RGB-infested wheat at 18% MC and 2 insects per kg wheat
M843	3 days in Tedlar bag for sample from the mixture of RFB&RGB-infested wheat at 18% MC and 2 insects per kg wheat
M844	4 days in Tedlar bag for sample from the mixture of RFB&RGB-infested wheat at 18% MC and 2 insects per kg wheat
M845	5 days in Tedlar bag for sample from the mixture of RFB&RGB-infested wheat at 18% MC and 2 insects per kg wheat
M846	6 days in Tedlar bag for sample from the mixture of RFB&RGB-infested wheat at 18% MC and 2 insects per kg wheat

Appendix B

Experimental Report - Moisture Content

Project Code		Method File Name	
Sample Source			
Oven temperature		Heating time	
Sample size		Date of Analysis	
Analyst		Date of this Report	

MC Test Conditions

No.	Dish Weight without lid (g)	Dish + Grain (Before drying) g	Dish + Grain (After drying) g	Moisture Content (%)	Average (%)

Appendix C

PCA plot of different sample-holding periods in Tedlar bags prior to the electronic nose analysis for non insect-infested and insect-infested CWRS wheat

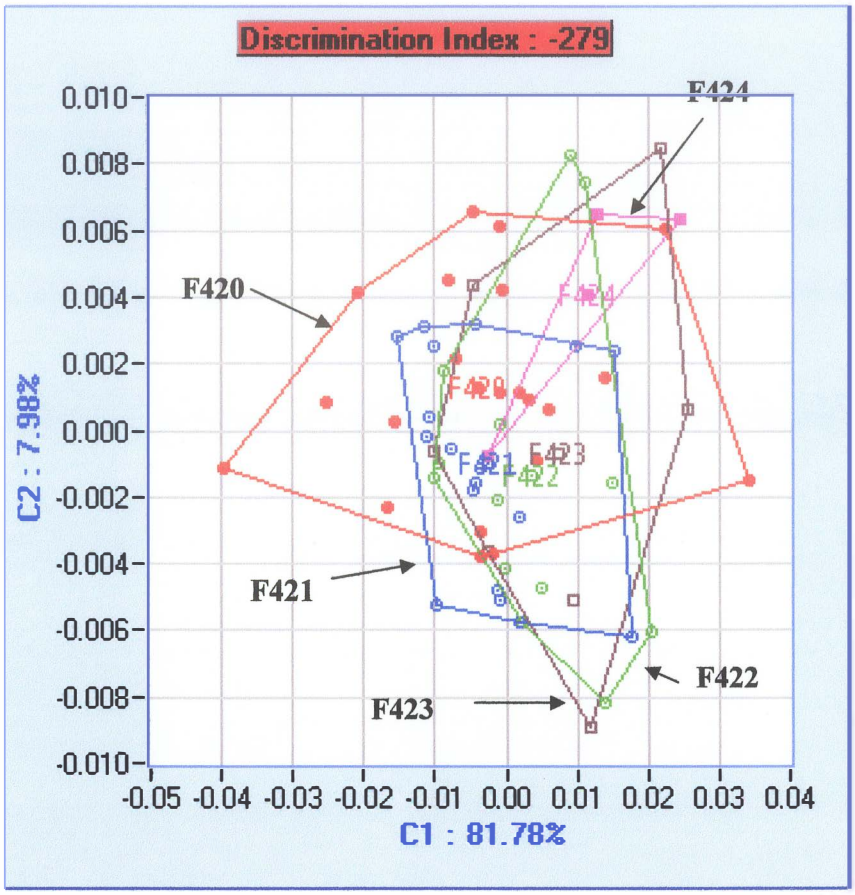

Figure C.1. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RFB-infested CWRS wheat at 14% moisture content (MC) and 1 insect per kg.

F420: 22 samples
F421: 18 samples
F422: 13 samples
F423: 7 samples
F424: 3 samples

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

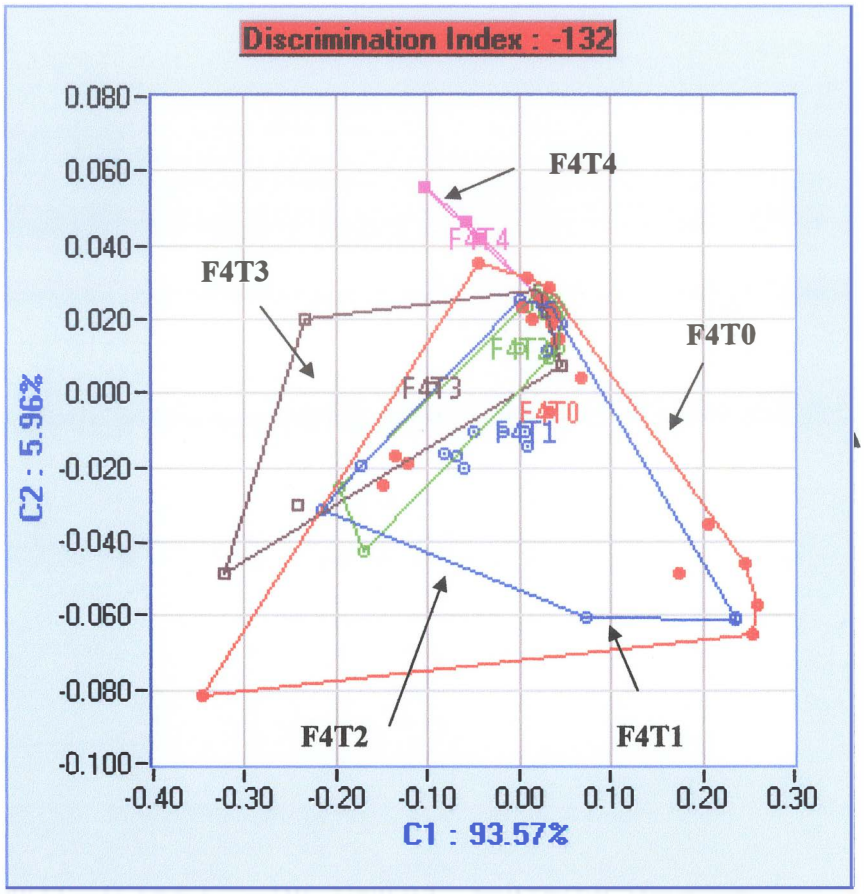

Figure C.2. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RFB-infested CWRS wheat at 14% MC and 10 insects per kg.

F4T0: 22 samples
F4T1: 18 samples
F4T2: 13 samples
F4T3: 7 samples
F4T4: 3 samples

Note: F4T0, F4T1, F4T2, and F4T3 are overlapped with each other, while F4T4 is separated from them.

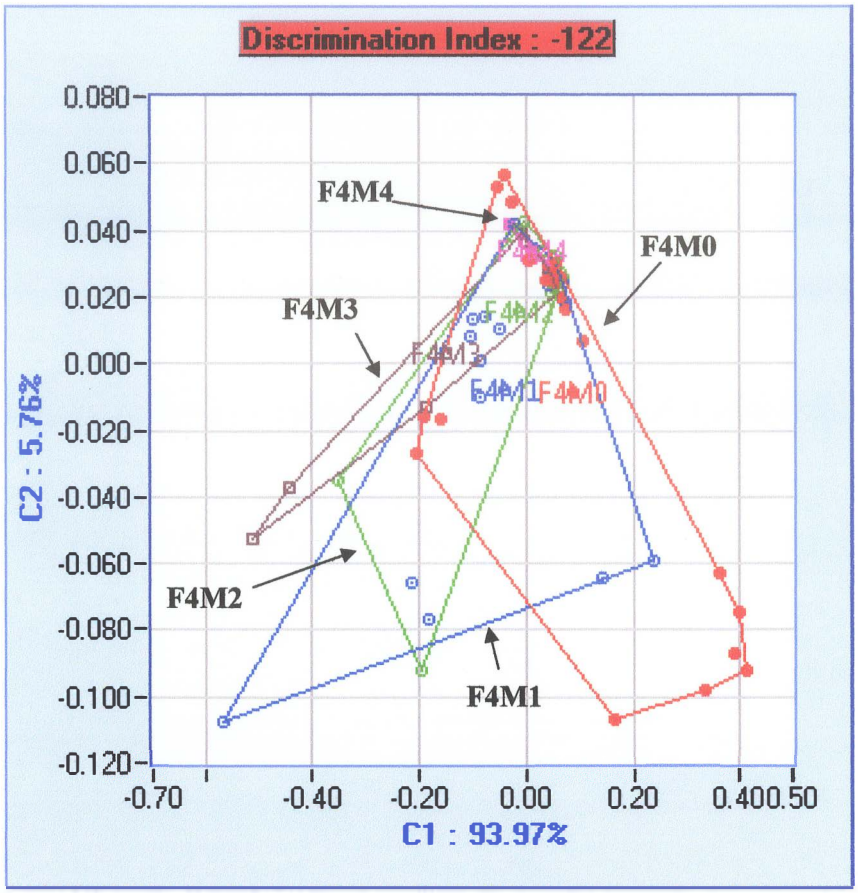

Figure C.3. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RFB-infested CWRS wheat at 14% MC and 20 insects per kg.

F4M0: 22 samples
F4M1: 18 samples
F4M2: 13 samples
F4M3: 7 samples
F4M4: 3 samples

Note: F4M0, F4M1, F4M2, and F4M3 are overlapped with each other, while F4M4 is separated from F4M3 in the map.

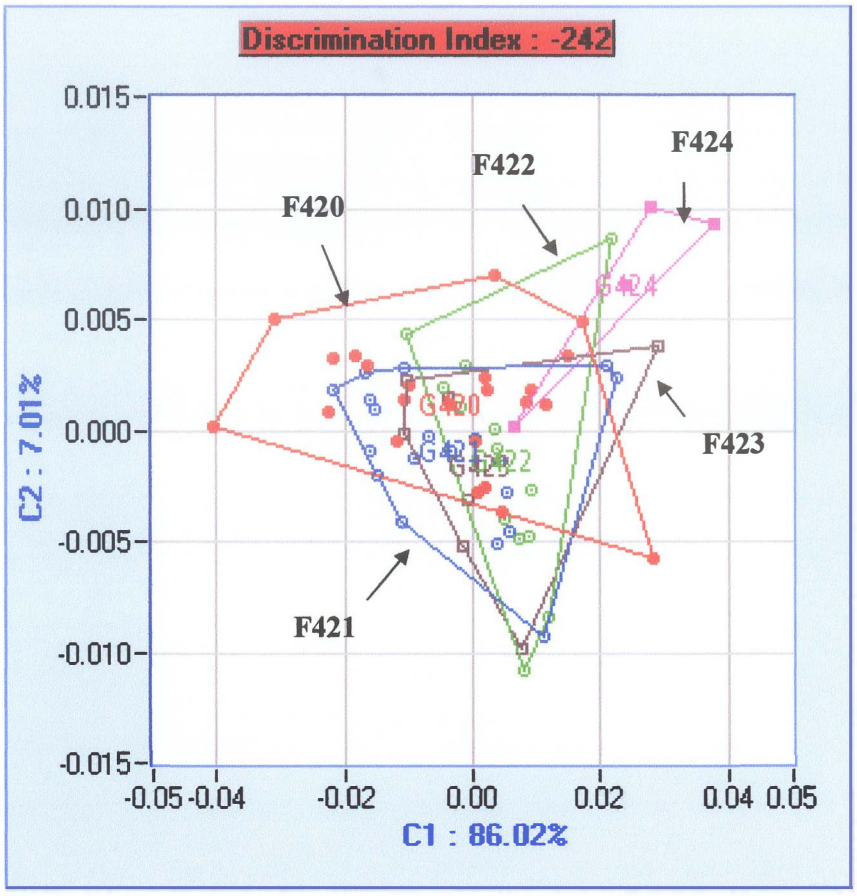

Figure C.5. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RGB-infested CWRS wheat at 14% MC and 1 insect per kg.

G420: 22 samples
G421: 18 samples
G422: 13 samples
G423: 7 samples
G424: 3 samples

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

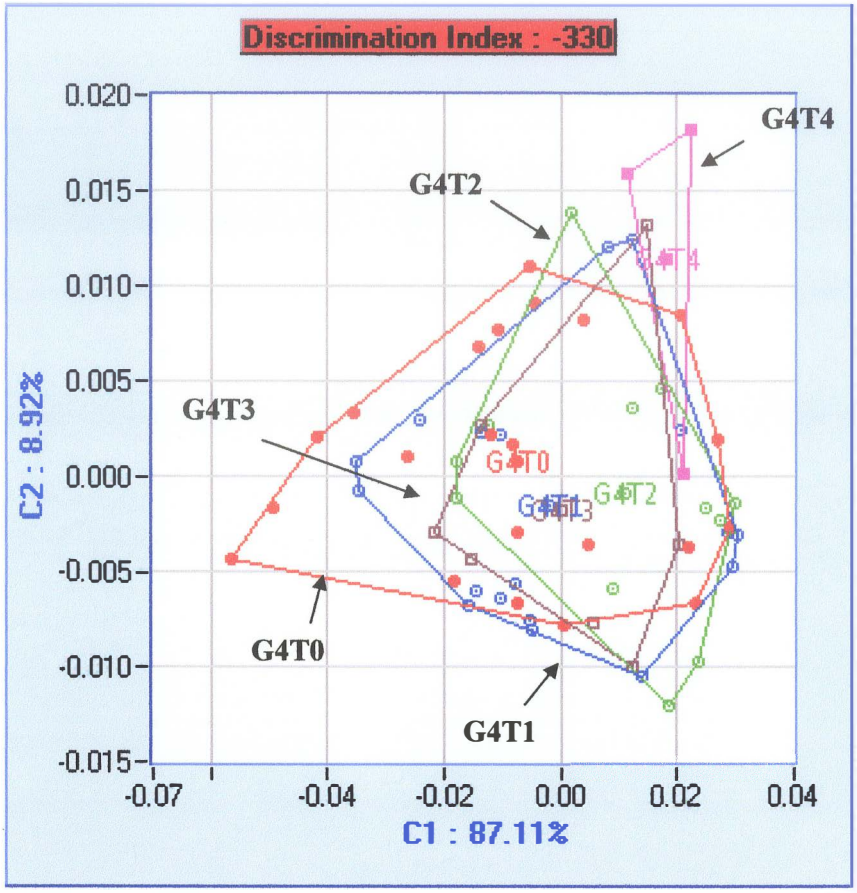

Figure C.6. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RGB-infested CWRS wheat at 14% MC and 10 insects per kg.

G4T0: 22 samples
G4T1: 18 samples
G4T2: 13 samples
G4T3: 7 samples
G4T4: 3 samples

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

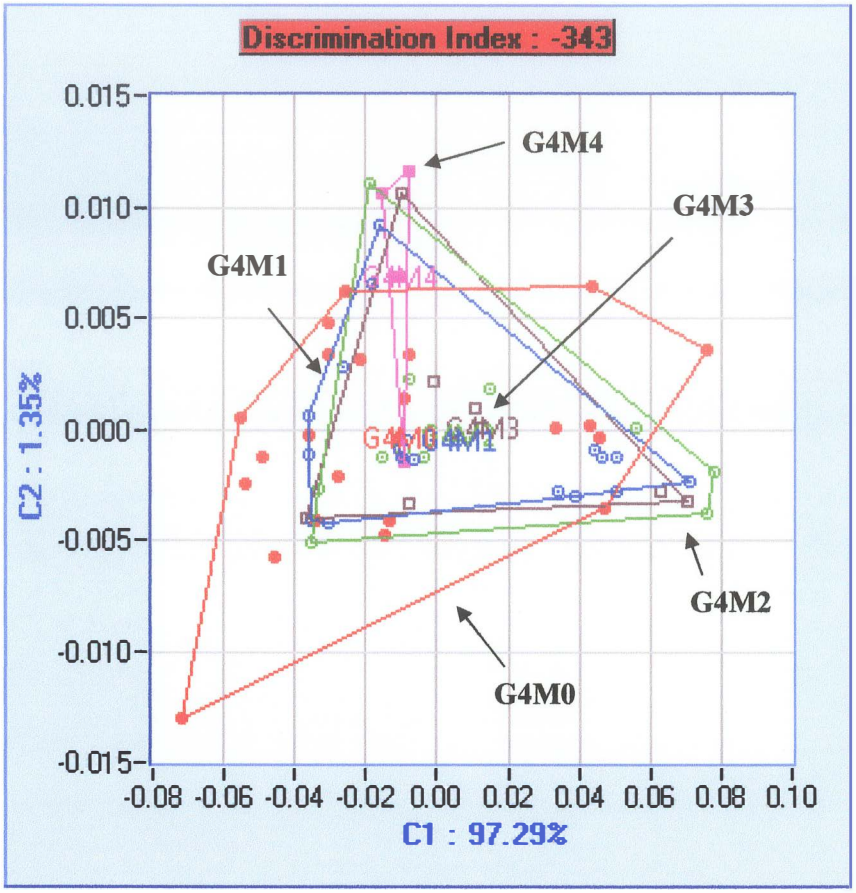

Figure C.7. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RGB-infested CWRS wheat at 14% MC and 20 insects per kg.

G4M0: 22 samples
G4M1: 18 samples
G4M2: 13 samples
G4M3: 7 samples
G4M4: 3 samples

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

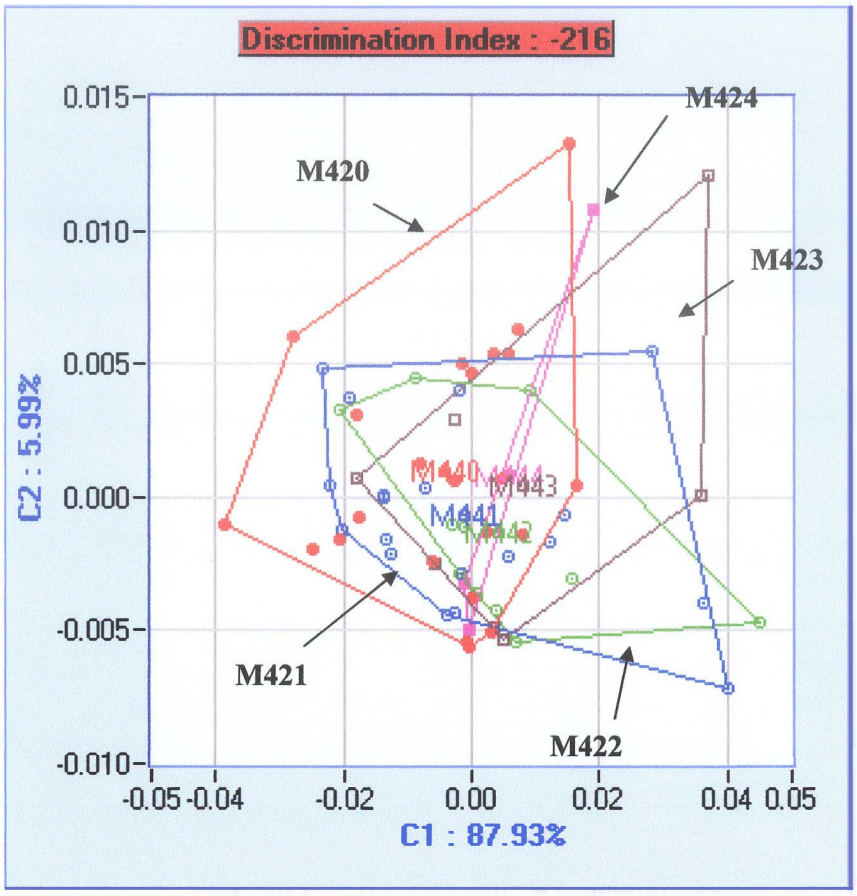

Figure C.8. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for mixture of RFB&RGB-infested CWRS wheat at 14% MC and 2 insects per kg.

M420: 23 samples
M421: 19 samples
M422: 11 samples
M423: 7 samples
M424: 3 samples

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

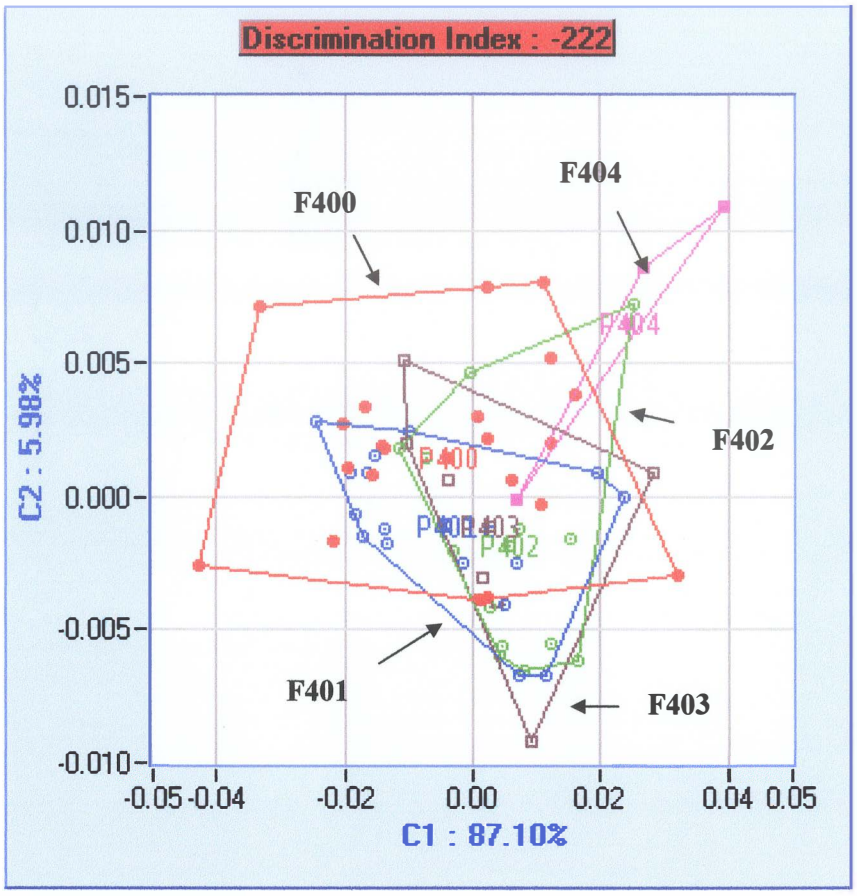

Figure C.4. PCA plot of 5 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for clean CWRS wheat at 14% MC.

F400: 22 samples
F401: 18 samples
F402: 13 samples
F403: 7 samples
F404: 3 samples

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

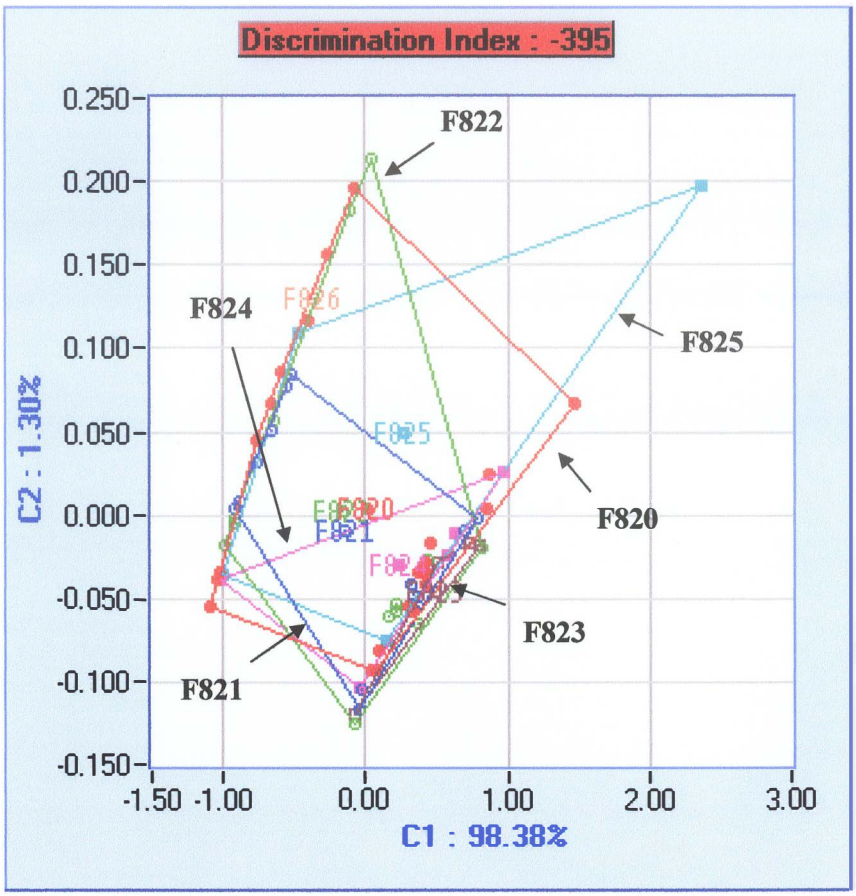

Figure C.9. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RFB-infested CWRS wheat at 18% MC and 1 insect per kg.

F820: 22 samples
F821: 13 samples
F822: 14 samples
F823: 4 samples
F824: 5 samples
F825: 4 samples
F826: 1 sample

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

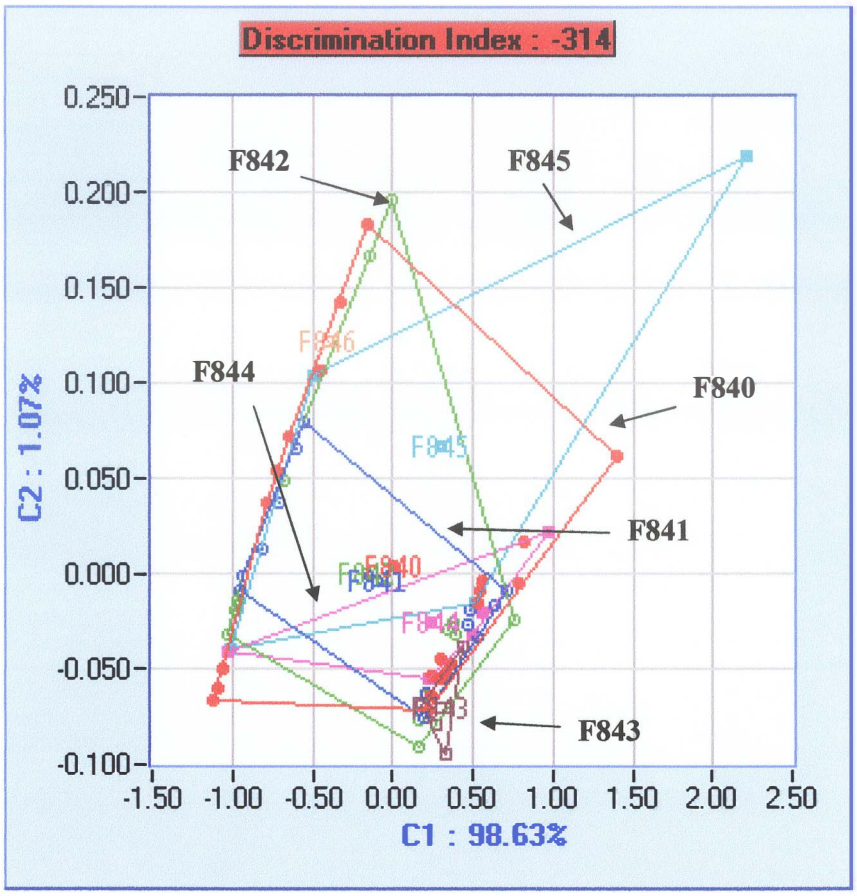

Figure C.10. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RFB-infested CWRS wheat at 18% MC and 2 insects per kg.

F840: 22 samples
F841: 13 samples
F842: 14 samples
F843: 4 samples
F844: 5 samples
F845: 4 samples
F846: 1 sample

Note: F840, F841, F842, F844, F845 are overlapped with each other.

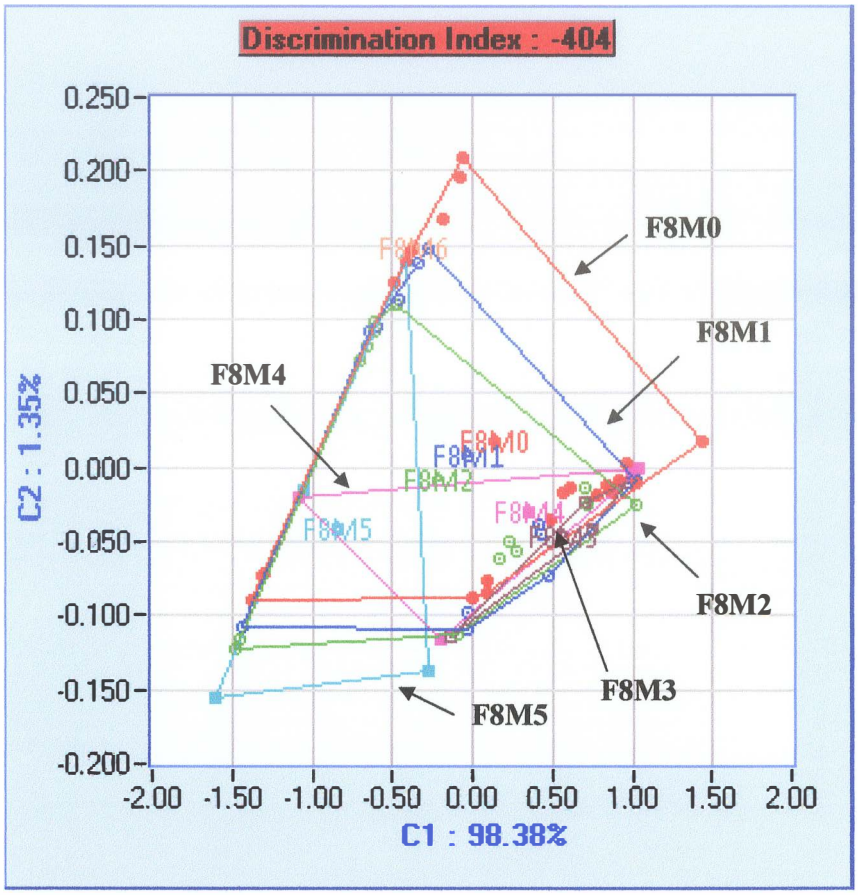

Figure C.11. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RFB-infested CWRS wheat at 18% MC and 20 insects per kg.

F8M0: 22 samples
F8M1: 13 samples
F8M2: 14 samples
F8M3: 4 samples
F8M4: 5 samples
F8M5: 4 samples
F8M6: 1 sample

Note: F8M0, F8M1, F8M2, F8M4, F8M5 are overlapped with each other.

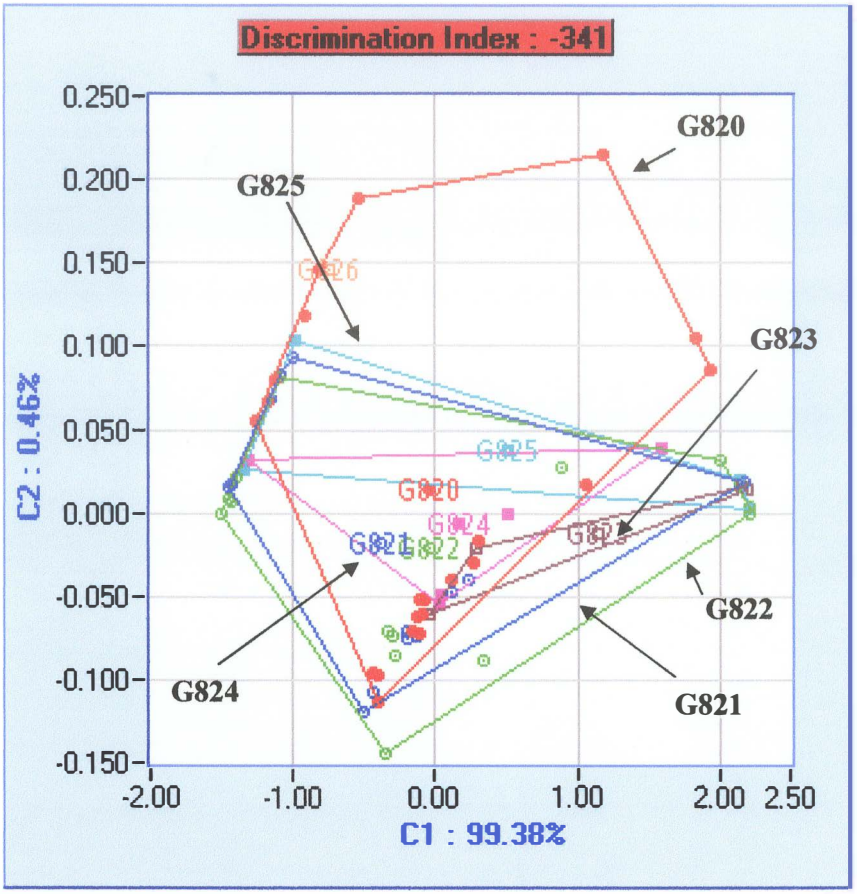

Figure C.12. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RGB-infested CWRS wheat at 18% MC and 1 insect per kg.

G820: 22 samples
G821: 13 samples
G822: 14 samples
G823: 4 samples
G824: 5 samples
G825: 4 samples
G826: 1 sample

Note: No differentiation among samples is shown in this plot as can be seen by the overlapping maps.

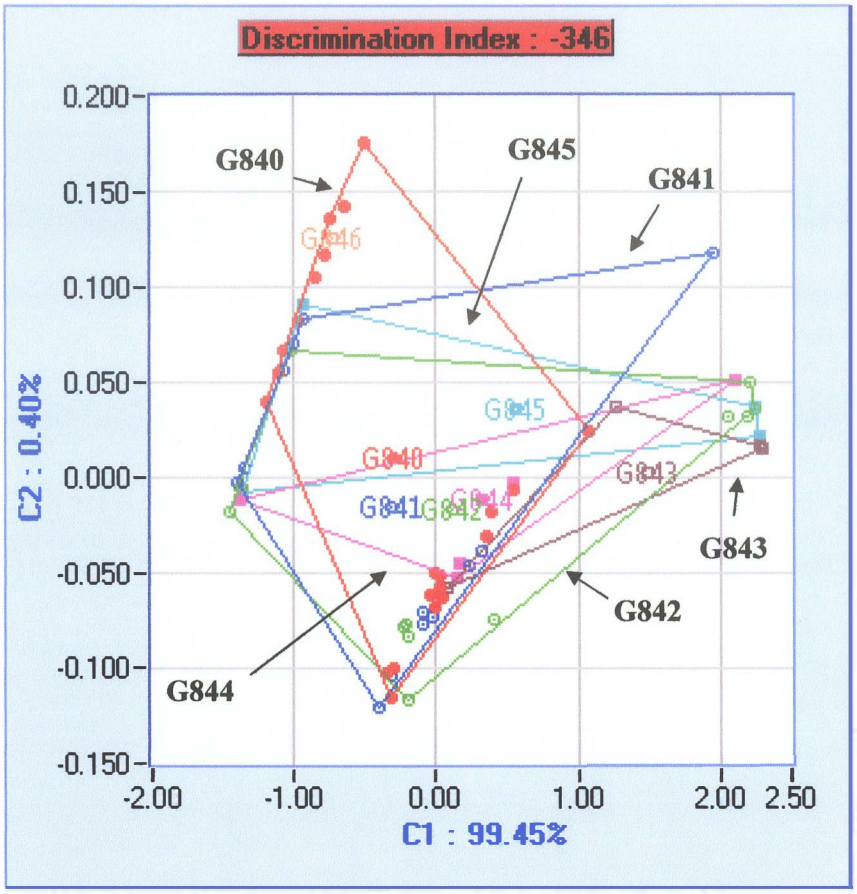

Figure C.13. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RGB-infested CWRS wheat at 18% MC and 2 insects per kg.

G840: 22 samples
G841: 13 samples
G842: 14 samples
G843: 4 samples
G844: 5 samples
G845: 4 samples
G846: 1 sample

Note: G840, G841, G842, G844, G845 are overlapped with each other.

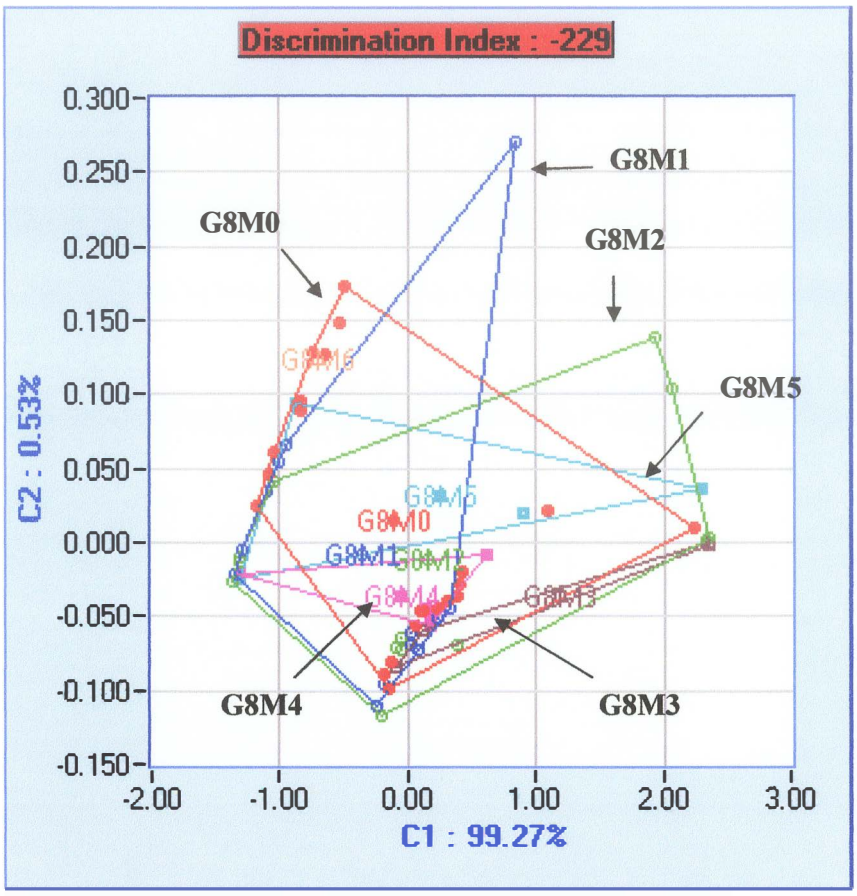

Figure C.14. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for RGB-infested CWRS wheat at 18% MC and 20 insects per kg.

G8M0: 22 samples
G8M1: 13 samples
G8M2: 14 samples
G8M3: 4 samples
G8M4: 5 samples
G8M5: 4 samples
G8M6: 1 sample

Note: G8M0, G8M1, G8M2, G8M4, G8M5 are overlapped with each other.

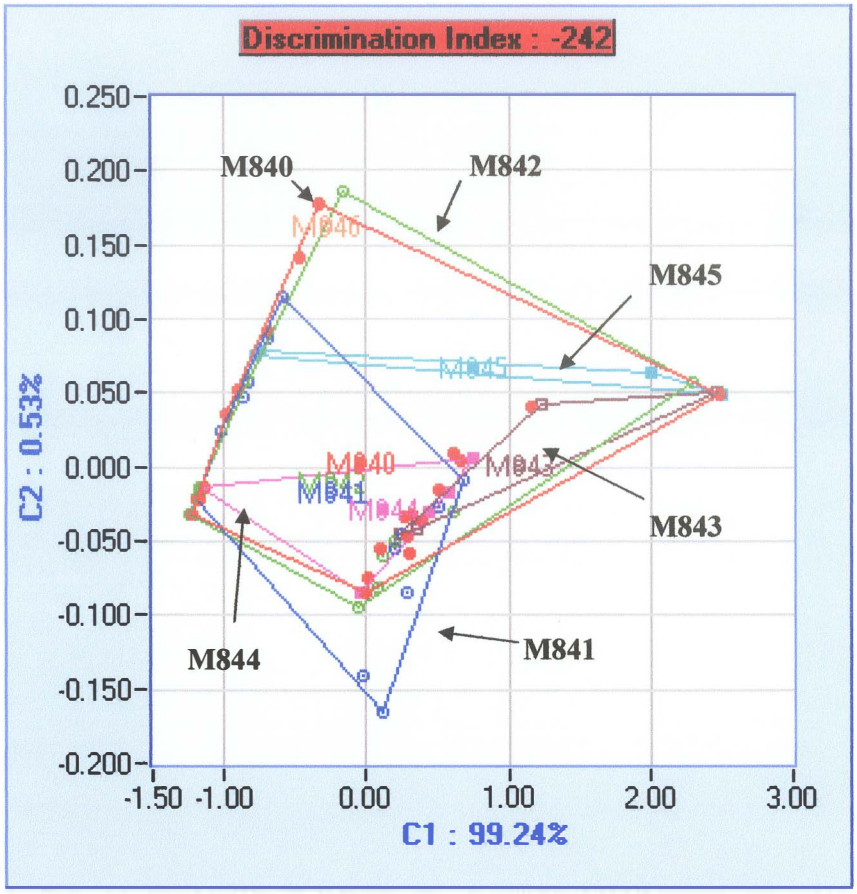

Figure C.15. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for mixture of RFB&RGB-infested CWRS wheat at 18% MC and 2 insects per kg.

M840: 22 samples
M841: 13 samples
M842: 14 samples
M843: 4 samples
M844: 5 samples
M845: 4 samples
M846: 1 sample

Note: M840, M841, M842, M844, M845 are overlapped with each other.

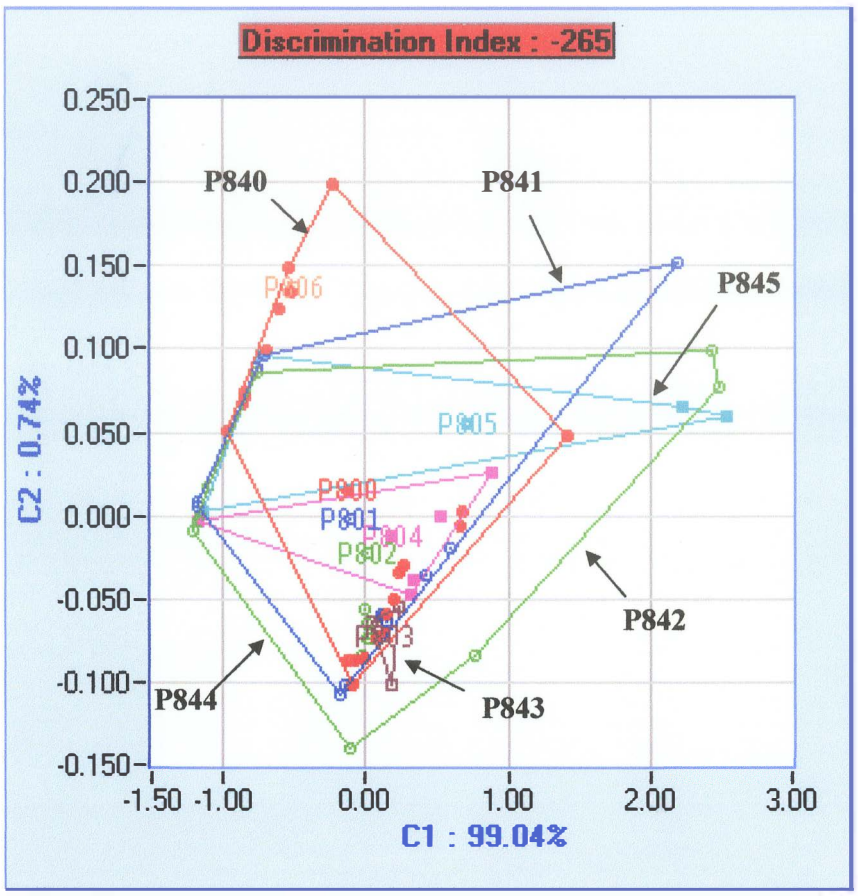

Figure C.16. PCA plot of 7 different sample-holding periods in Tedlar bags prior to the electronic nose analysis for clean CWRS wheat at 18% MC.

P840: 22 samples
P841: 13 samples
P842: 14 samples
P843: 4 samples
P844: 5 samples
P845: 4 samples
P846: 1 sample

Note: P840, P841, P842, P844, P845 are overlapped with each other.

Appendix D

Comparison of 12 sensor reactions to uninfected, low insect-infested and high insect-infested wheat at 14%, 16%, and 18% moisture content

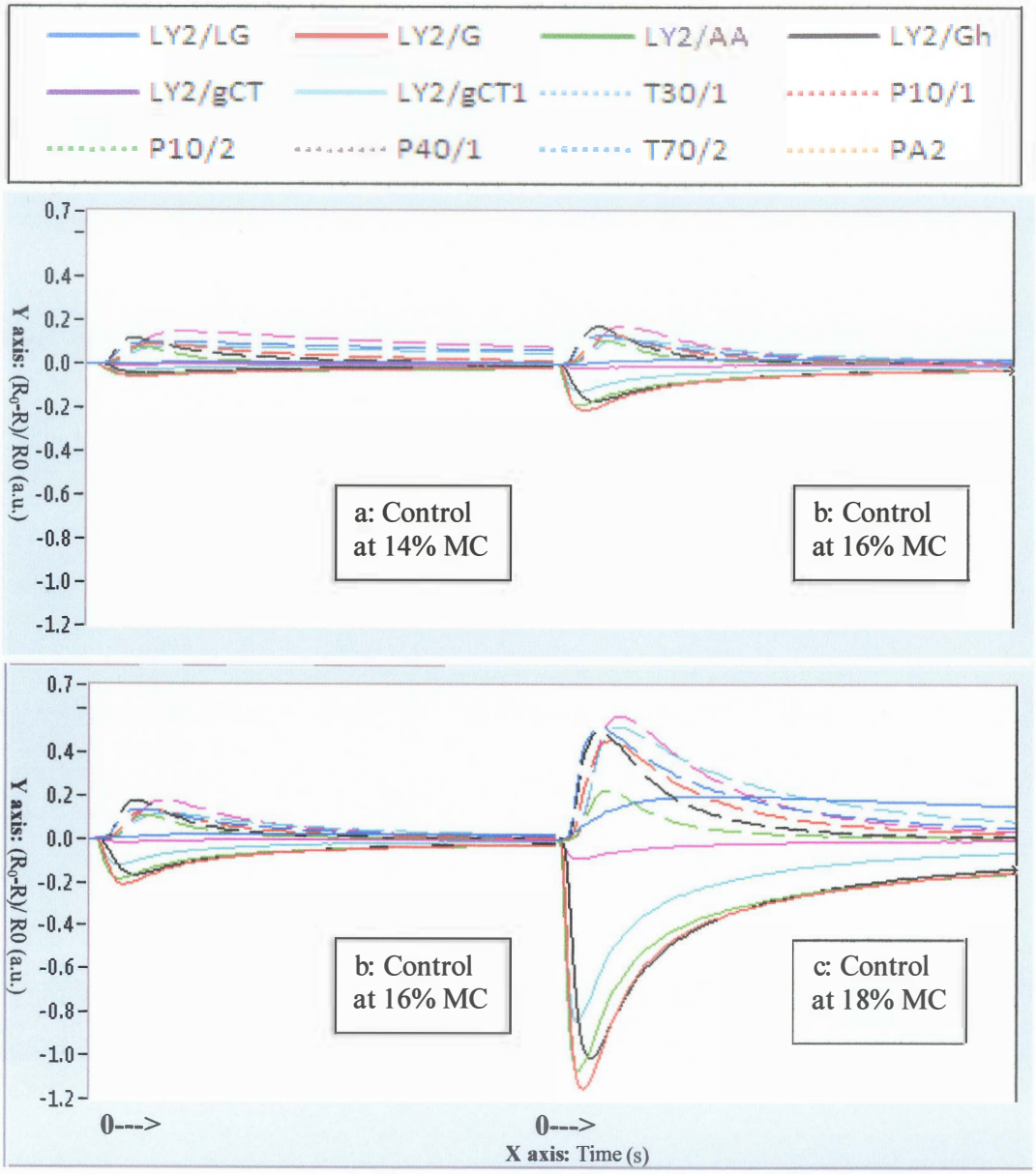

Figure D.1. Comparison of sensor reaction curves to control sample of wheat at 14, 16 %, and 18% moisture content, showing the sensor array responses when moisture was higher.

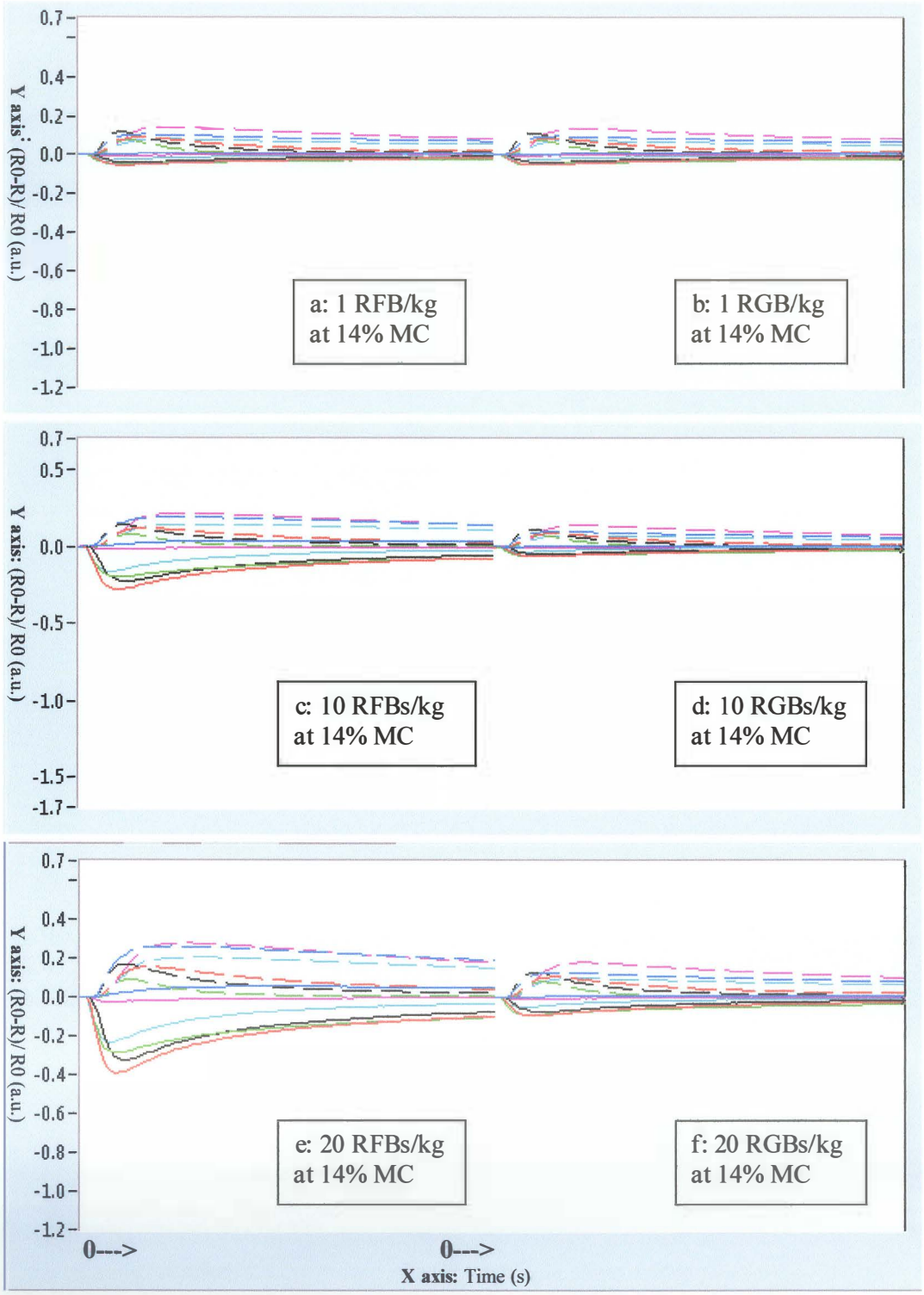

Figure D.2. Comparison of sensor reaction curves to RFB and RGB infested-wheat at three densities and 14% moisture content wheat, showing the sensor array responses to different insect species and insect infestation levels at 14% MC.

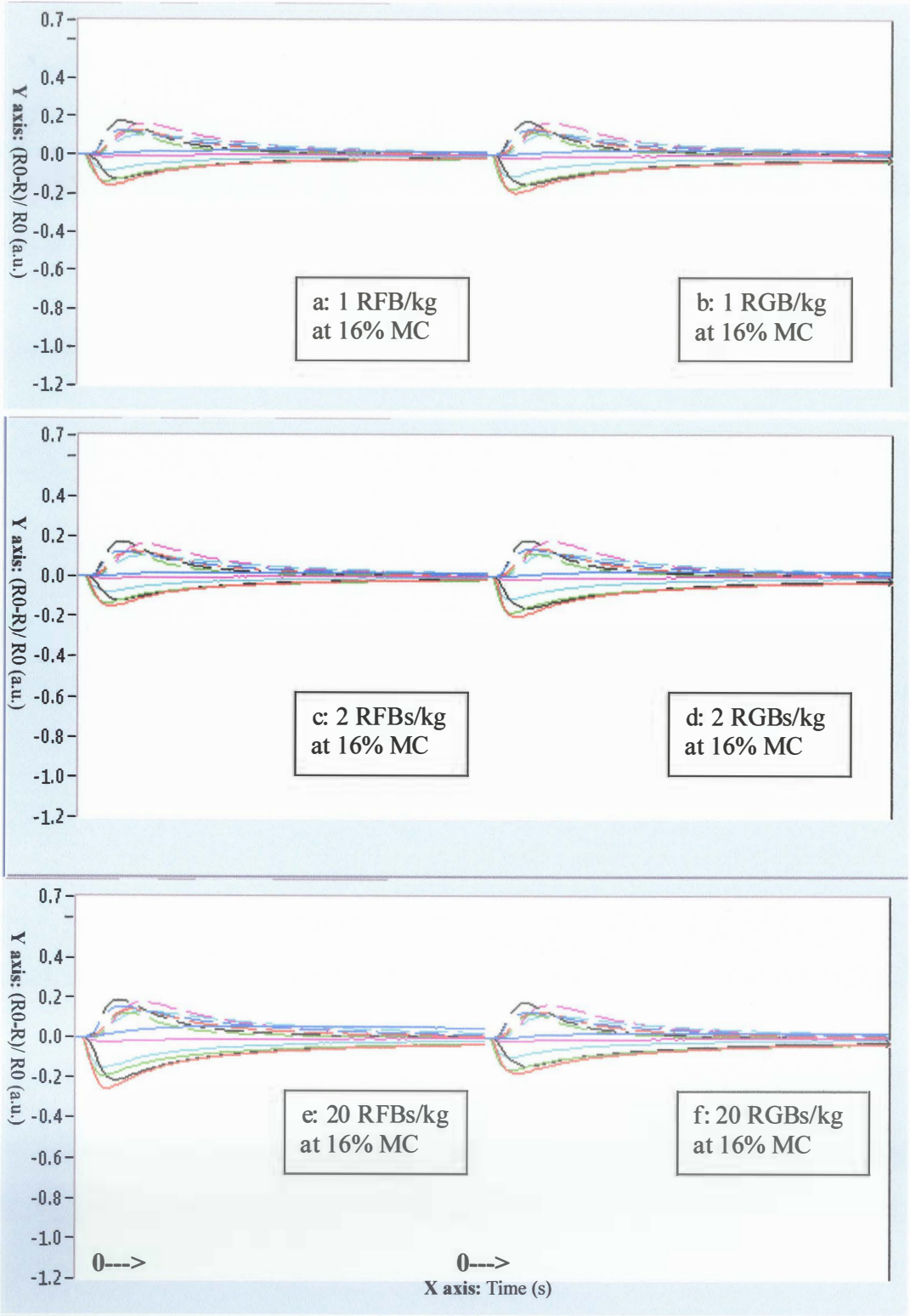

Figure D.3. Comparison of sensor reaction curves to RFB and RGB infested-wheat at three densities and 16% moisture content wheat, showing the sensor array responses to different insect species and insect infestation levels at 16% MC.

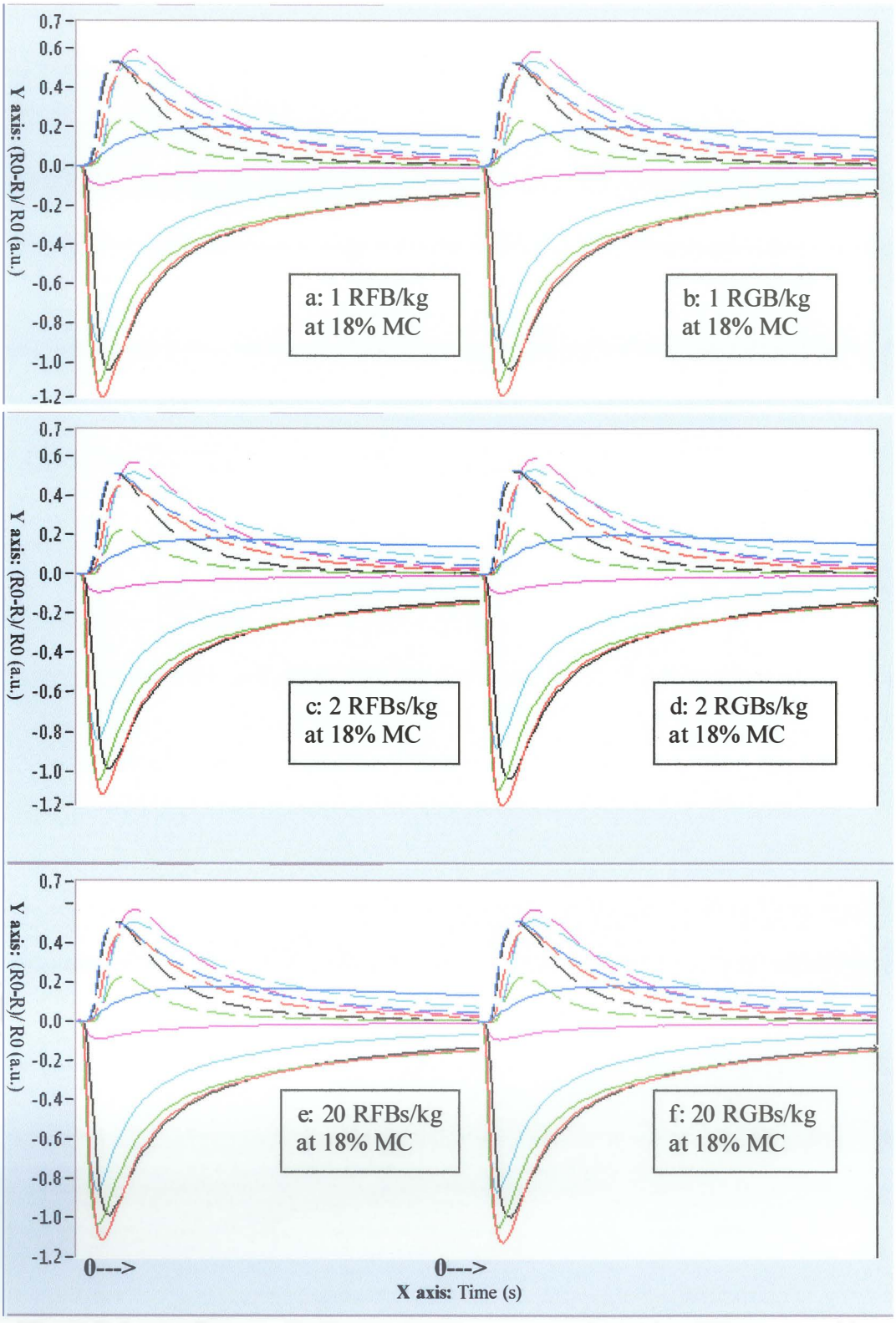

Figure D.4. Comparison of sensor reaction curves to RFB and RGB infested-wheat at three densities and 18% moisture content wheat, , showing the sensor array responses to different insect species and insect infestation levels at 18% MC.

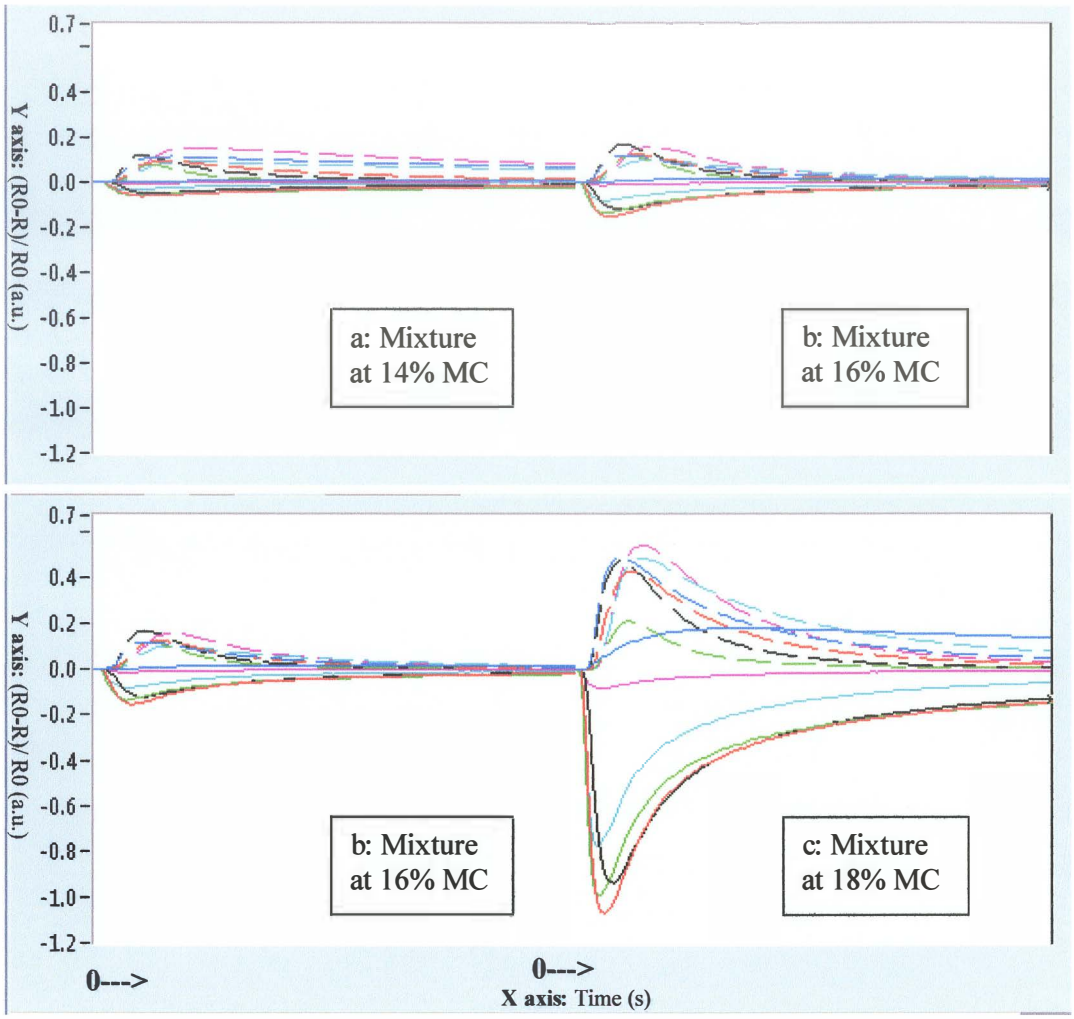

Figure D.5. Comparison of sensor reaction curves to mixture of two insects/kg RFB and RGB infested-wheat at 14, 16 %, and 18% moisture content wheat, showing the sensor array responses to mixture of RFB and RGB insect-infestation wheat at three moisture contents.

Appendix E

Sensor responses to the volatiles from different moisture contents of wheat by PCA and DFA analysis (data from up to 72 h holding time in Tedlar bag)

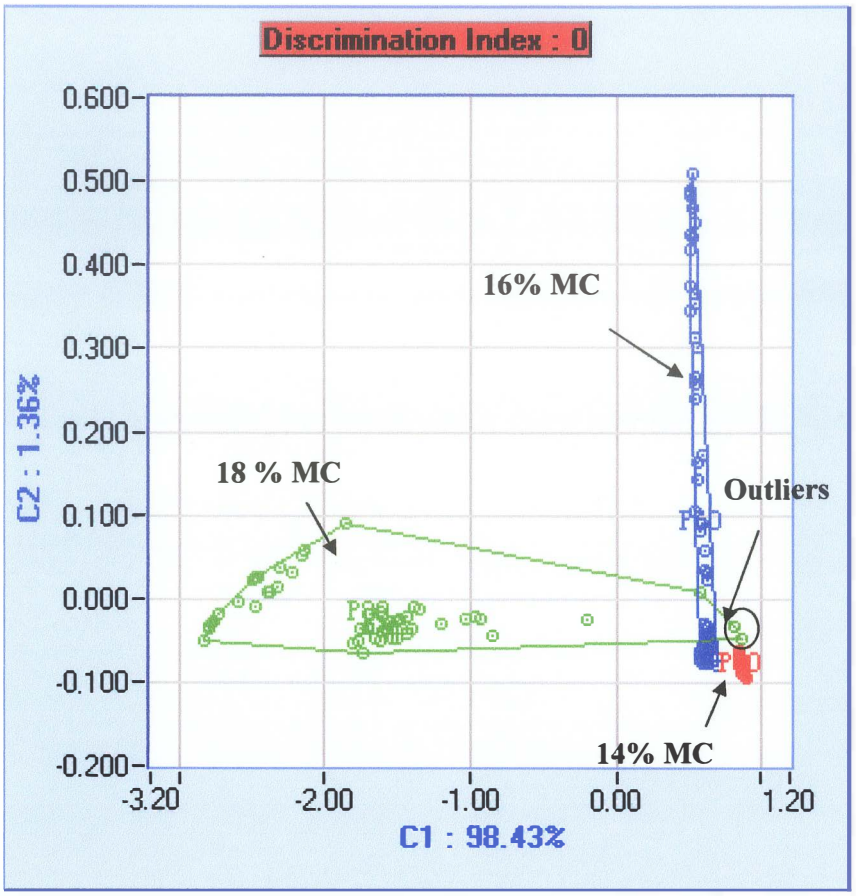

Figure E.1. PCA plot of clean wheat at 14%, 16% and 18% MC, and data are from up to 72 h holding in Tedlar bags.

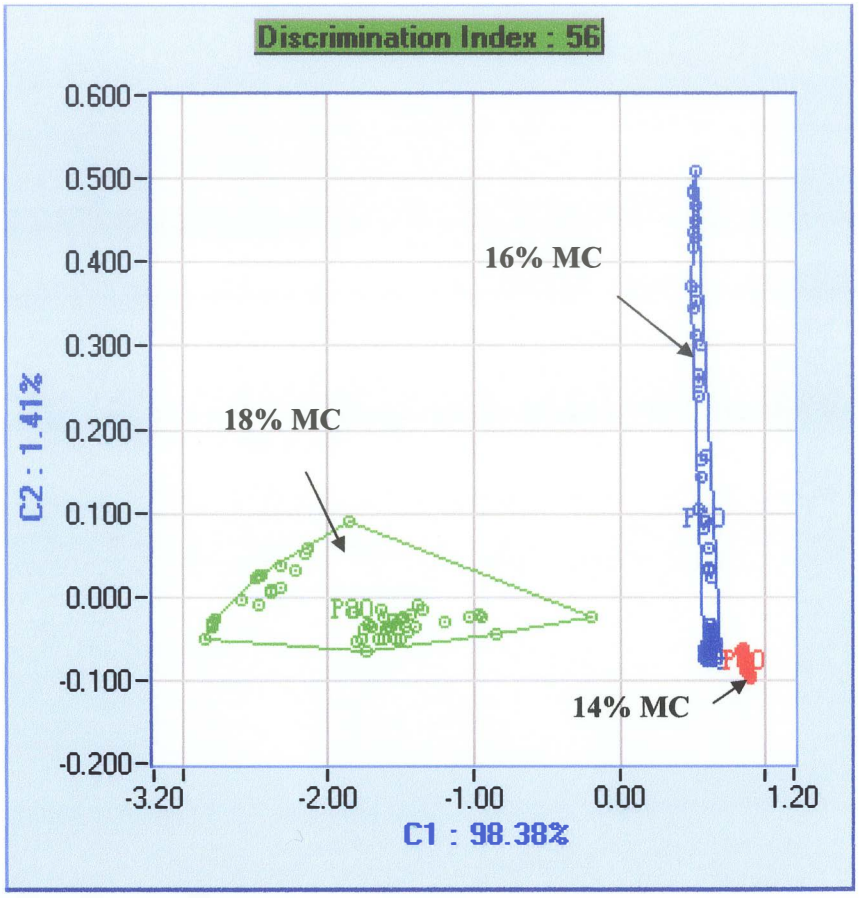

Figure E.2. PCA plot of clean wheat at 14%, 16% and 18% moisture content after the removal of outliers, and data are from up to 72 h holding in Tedlar bags.

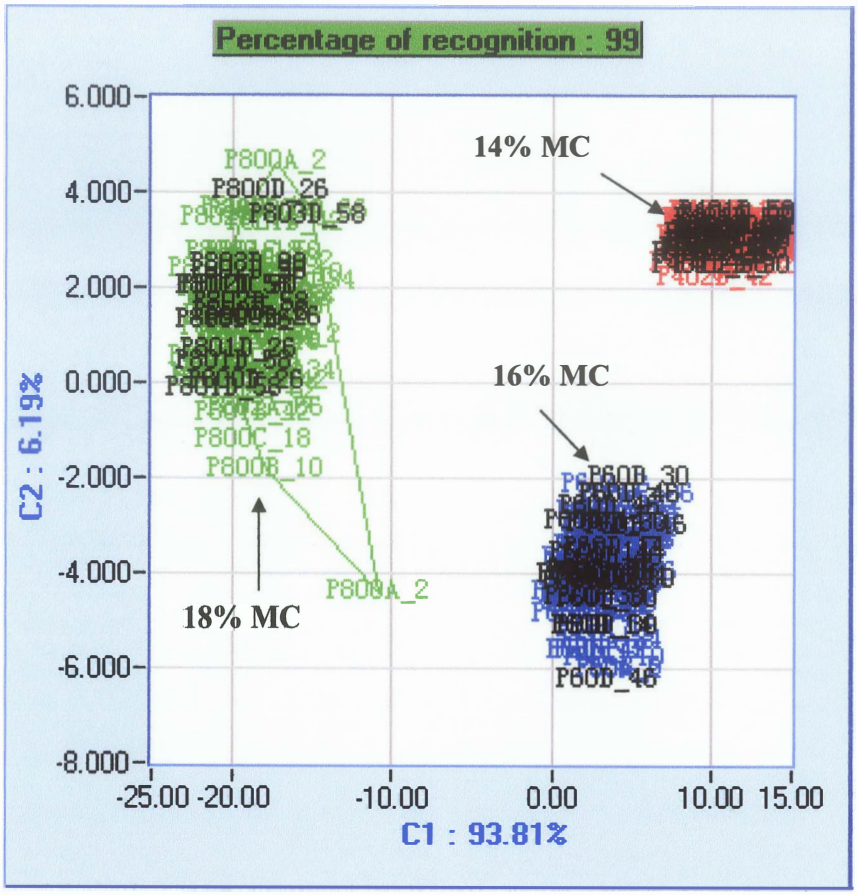

Figure E.3. DFA plot of non infested-wheat at 14%, 16% and 18% moisture contents.

Note: Data in black are unknown data for validation and prediction.

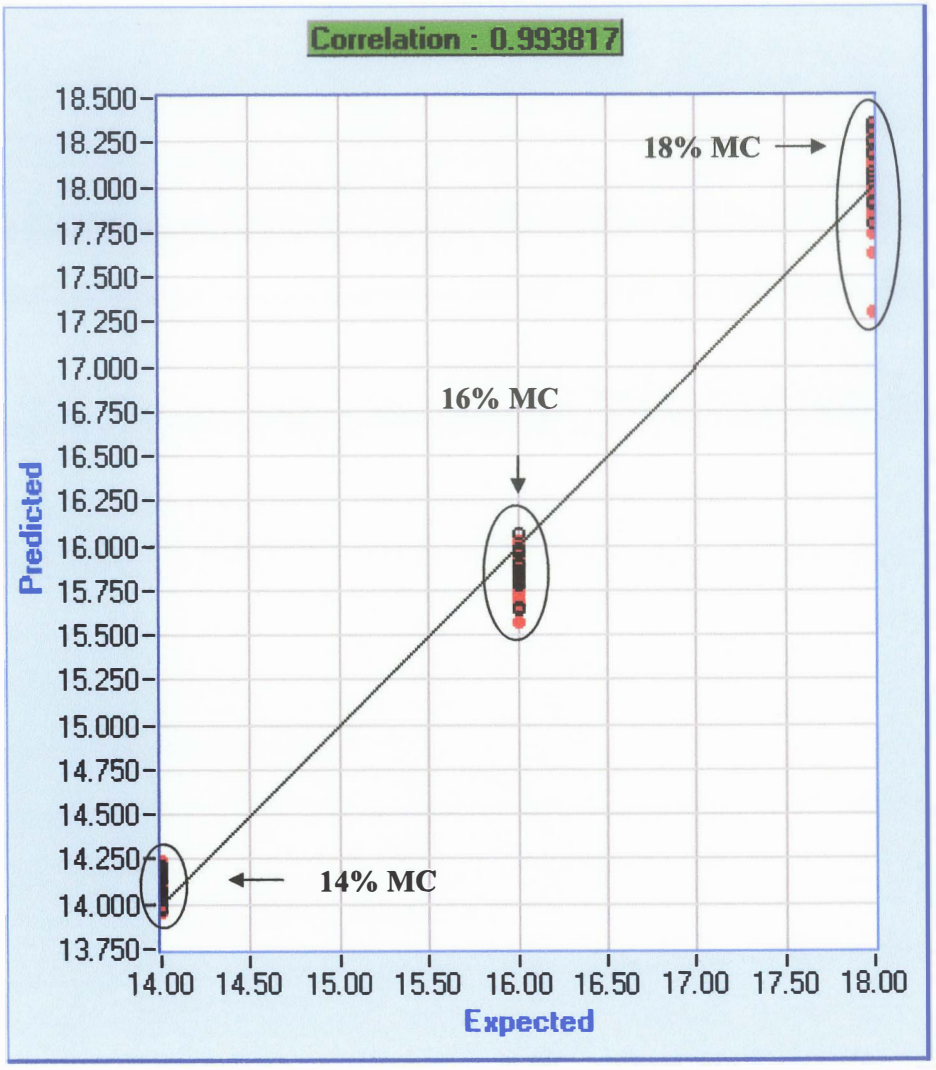

Figure E.4. PLS plot of non infested-wheat at 14%, 16% and 18% moisture contents.

Note: The graph plots the training values (X-axis) versus the predicted (Y-axis). Data in black are unknown data for validation and prediction.

Appendix F

Comparison of sensor reaction curves to headspace volatiles

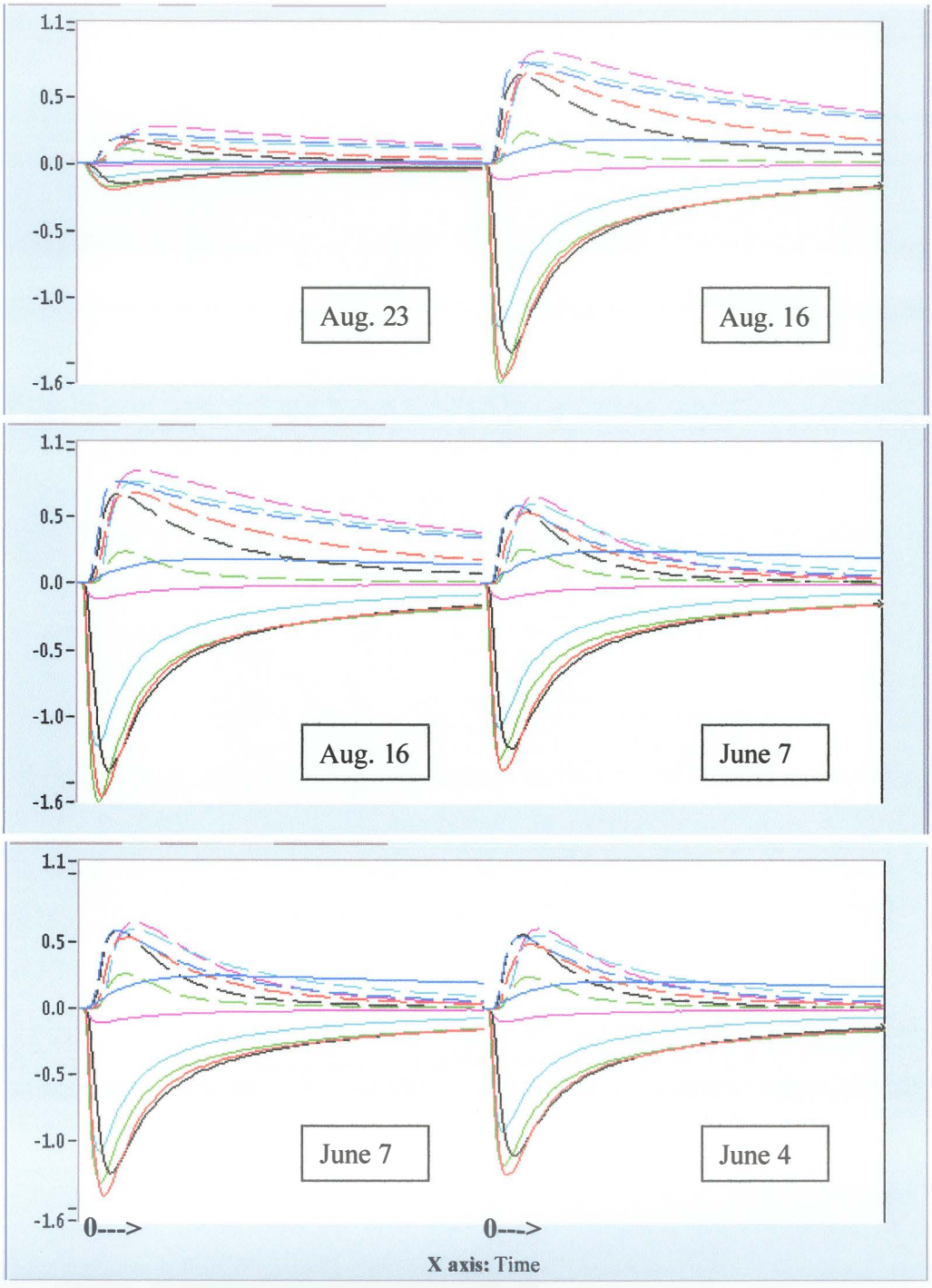

Figure F.1. Comparison of sensor reaction curves to P801A_34 at 18% moisture.

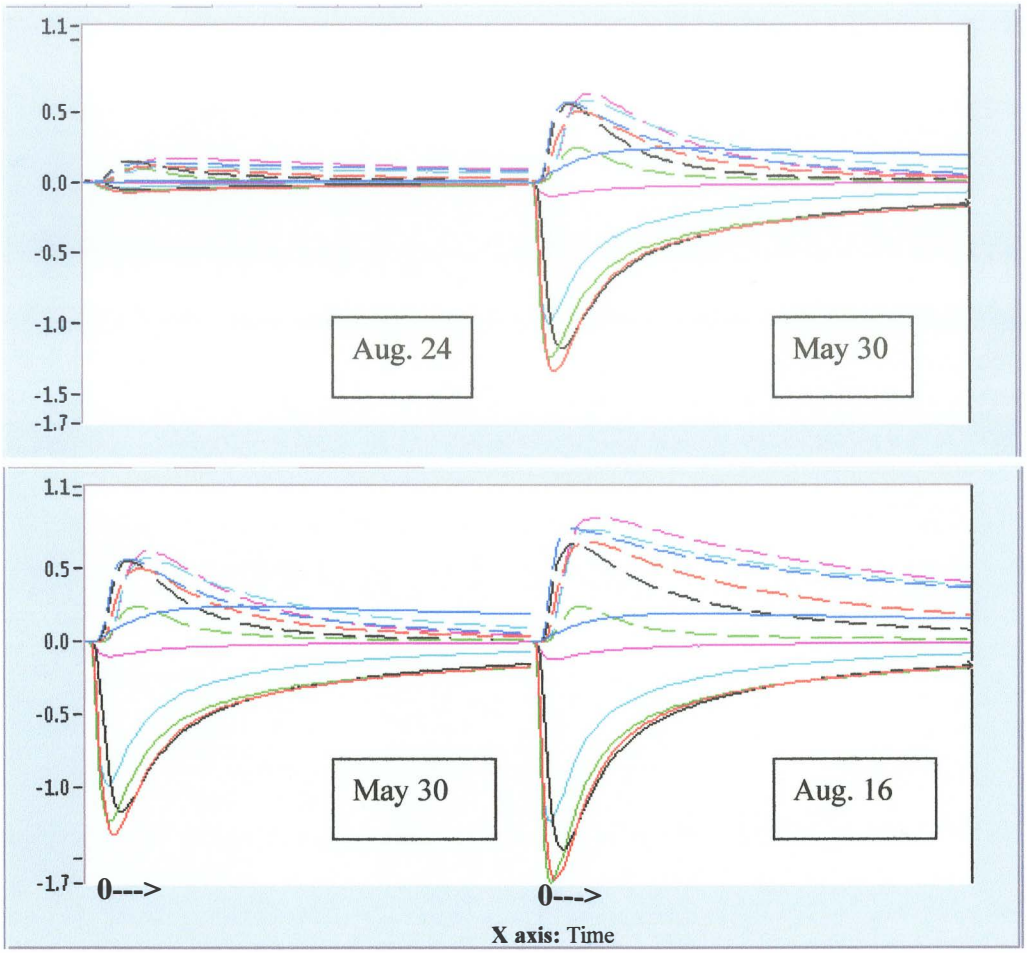

Figure F.2. Comparison of sensor reaction curves to P802B_42 at 18% moisture.

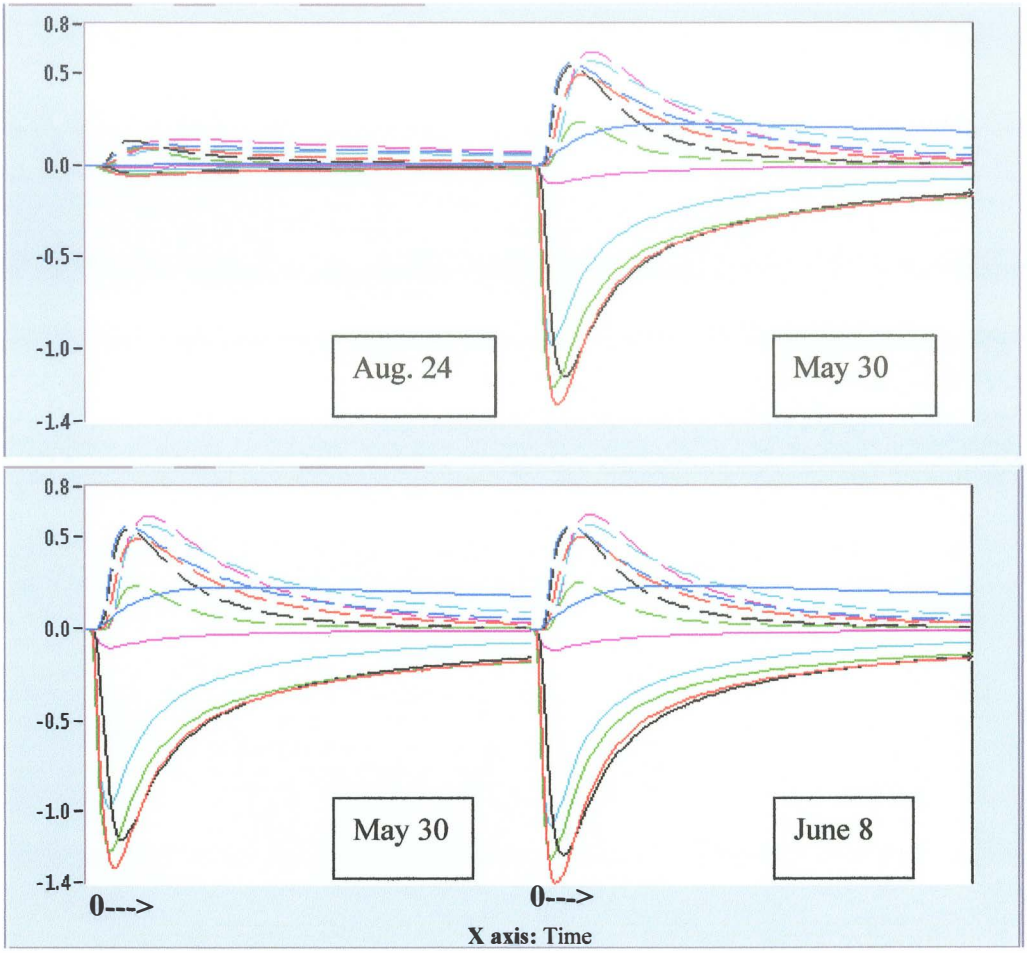

Figure F.3. Comparison of sensor reaction curves to P802C_50 at 18% moisture.

Appendix G

The training and testing scores for the PLS model

Table G.1. Recognition scores for training data on DFA model

Training Data	Expected (%)	Predicted (%)
P400A_2	14	14.14
P400A_2	14	14.20
P400A_2	14	14.05
P400A_2	14	14.19
P400A_2	14	14.02
P400A_2	14	14.03
P400A_2	14	14.08
P402A_34	14	14.08
P402A_34	14	14.08
P402A_34	14	14.13
P401A_34	14	14.13
P401A_34	14	14.00
P401A_34	14	14.01
P402A_34	14	14.18
P402A_66	14	14.22
P401A_66	14	14.03
P400B_10	14	14.14
P400B_10	14	14.08
P400B_10	14	14.08
P400B_10	14	14.18
P400B_10	14	14.09
P400B_10	14	14.04
P400B_10	14	14.06
P401B_42	14	14.12
P401B_42	14	14.15
P402B_42	14	14.09
P401B_42	14	14.17
P401B_42	14	14.01
P401B_42	14	14.03
P402B_42	14	14.16
P402B_74	14	14.13
P402B_74	14	14.17
P401B_74	14	14.05
P400C_18	14	14.15
P400C_18	14	14.04
P400C_18	14	14.19
P400C_18	14	14.16
P400C_18	14	13.96
P400C_18	14	14.04
P400C_18	14	14.23

Training Data	Expected (%)	Predicted (%)
P401C_50	14	14.15
P401C_50	14	14.17
P401C_50	14	14.07
P401C_50	14	14.06
P401C_50	14	14.17
P401C_82	14	14.13
P402C_82	14	14.25
P402C_82	14	14.20
P402C_82	14	13.96
P401C_82	14	14.05
P402C_82	14	14.16
P60A_18	16	15.81
P60A_18	16	15.85
P60A_18	16	15.97
P60A_18	16	15.87
P60A_18	16	16.02
P60A_18	16	16.04
P60A_18	16	15.91
P60A_2	16	15.66
P60A_2	16	15.57
P60A_2	16	15.77
P60A_2	16	15.87
P60A_2	16	15.83
P60A_2	16	15.93
P60A_2	16	15.87
P60A_34	16	15.65
P60A_34	16	15.81
P60A_34	16	15.86
P60A_34	16	15.80
P60A_34	16	15.99
P60A_34	16	16.01
P60A_34	16	15.87
P60B_22	16	15.72
P60B_22	16	15.83
P60B_22	16	15.91
P60B_22	16	15.82
P60B_22	16	15.97
P60B_22	16	16.02
P60B_22	16	15.93
P60B_38	16	15.66
P60B_38	16	15.82
P60B_38	16	15.84

Training Data	Expected (%)	Predicted (%)
P60B_38	16	16.03
P60B_38	16	15.90
P60B_6	16	15.70
P60B_6	16	15.64
P60B_6	16	15.85
P60B_6	16	15.90
P60B_6	16	15.90
P60B_6	16	15.96
P60B_6	16	15.92
P60C_10	16	15.72
P60C_10	16	15.70
P60C_10	16	15.89
P60C_10	16	15.92
P60C_10	16	15.94
P60C_10	16	16.03
P60C_10	16	15.93
P60C_26	16	15.65
P60C_26	16	15.80
P60C_26	16	15.85
P60C_26	16	15.77
P60C_26	16	15.91
P60C_26	16	15.92
P60C_26	16	15.81
P60C_42	16	15.67
P60C_42	16	15.81
P60C_42	16	15.84
P60C_42	16	15.84
P60C_42	16	16.02
P60C_42	16	16.03
P60C_42	16	15.95
P800A_2	18	17.31
P800A_2	18	18.20
P800A_2	18	18.11
P800A_2	18	18.33
P800A_2	18	17.64
P800A_2	18	17.95
P800A_2	18	17.96
P802A_34	18	18.00
P800A_34	18	18.28
P801A_34	18	18.20
P801A_34	18	18.35
P801A_34	18	17.87

Training Data	Expected (%)	Predicted (%)
P802A_66	18	17.83
P802A_66	18	18.08
P800B_10	18	17.89
P800B_10	18	18.22
P800B_10	18	18.14
P800B_10	18	18.30
P800B_10	18	17.76
P800B_10	18	17.89
P800B_10	18	17.97
P801B_42	18	17.99
P802B_42	18	18.28
P801B_42	18	18.21
P801B_42	18	18.34
P801B_42	18	17.88
P801B_42	18	18.04
P802B_74	18	18.25
P802B_74	18	18.08
P800C_18	18	17.93
P800C_18	18	18.24
P800C_18	18	18.18
P800C_18	18	18.30
P800C_18	18	17.82
P800C_18	18	17.82
P800C_18	18	17.98
P801C_50	18	18.08
P802C_50	18	18.26
P801C_50	18	18.21
P802C_50	18	18.36
P801C_50	18	17.92
P801C_50	18	18.07
P802C_82	18	18.23
P802C_82	18	18.07

Table G.2. The prediction scores in PLS

Testing Data	Predicted (%)
P400D_26	14.19
P400D_26	14.07
P400D_26	14.17
P400D_26	14.16
P400D_26	14.03
P400D_26	13.99
P400D_26	14.22
P402D_58	14.18
P400D_58	14.20
P401D_58	14.15
P401D_58	14.16
P401D_58	14.09
P401D_58	14.08
P401D_58	14.21
P401D_90	14.06
P402D_90	14.15
P401D_90	14.22
P402D_90	14.00
P402D_90	14.04
P402D_90	14.21
P60D_14	15.81
P60D_14	15.78
P60D_14	15.95
P60D_14	15.91
P60D_14	15.99
P60D_14	16.07
P60D_14	15.97
P60D_30	15.65
P60D_30	15.81
P60D_30	15.87
P60D_30	15.81
P60D_30	15.98
P60D_30	15.99
P60D_30	15.85
P60D_46	15.63
P60D_46	15.79
P60D_46	15.79
P60D_46	15.82
P60D_46	15.98
P60D_46	16.00

Testing Data	Predicted (%)
P800D_26	17.91
P800D_26	18.26
P800D_26	18.20
P800D_26	18.36
P800D_26	17.81
P801D_26	18.03
P802D_58	18.09
P802D_58	18.25
P801D_58	18.25
P801D_58	17.93
P801D_58	18.07
P802D_90	18.32

Appendix H

Detection of the presence of insects and insect species differentiation by PCA and DFA analysis (data from up to 72 h holding time in Tedlar bags)

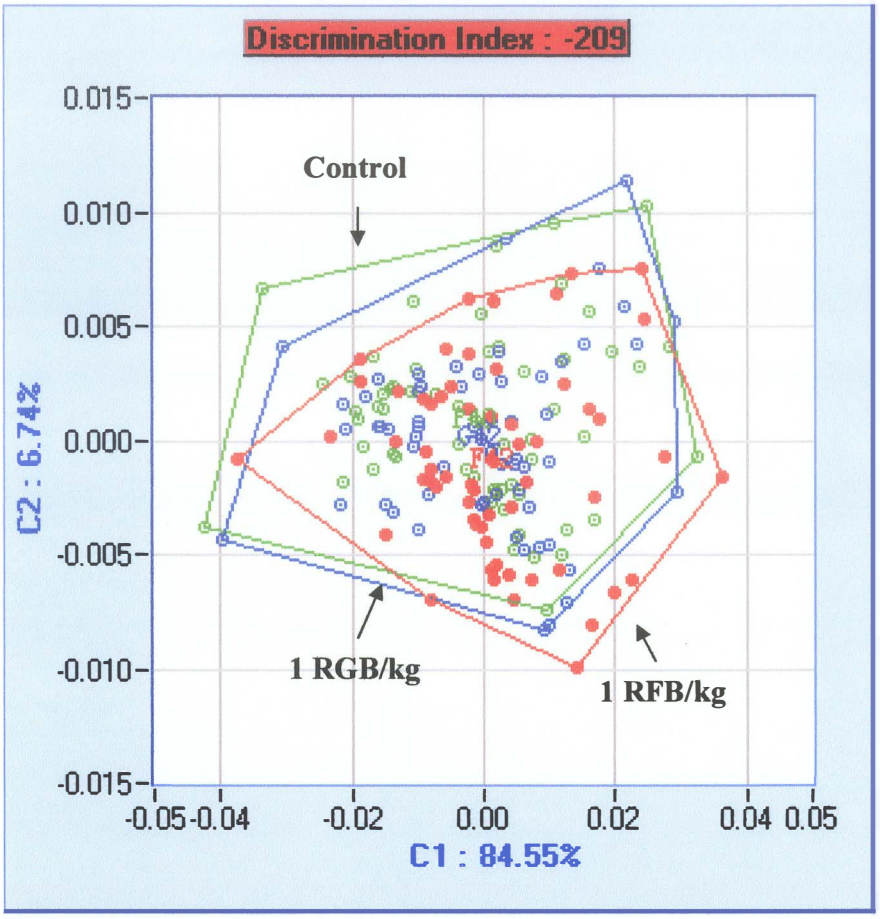

Figure H.1. PCA plot of control and low infested-wheat by RFB and RGB at 14% moisture content.

Note: Three groups overlap with each other showing no differentiation among the groups.

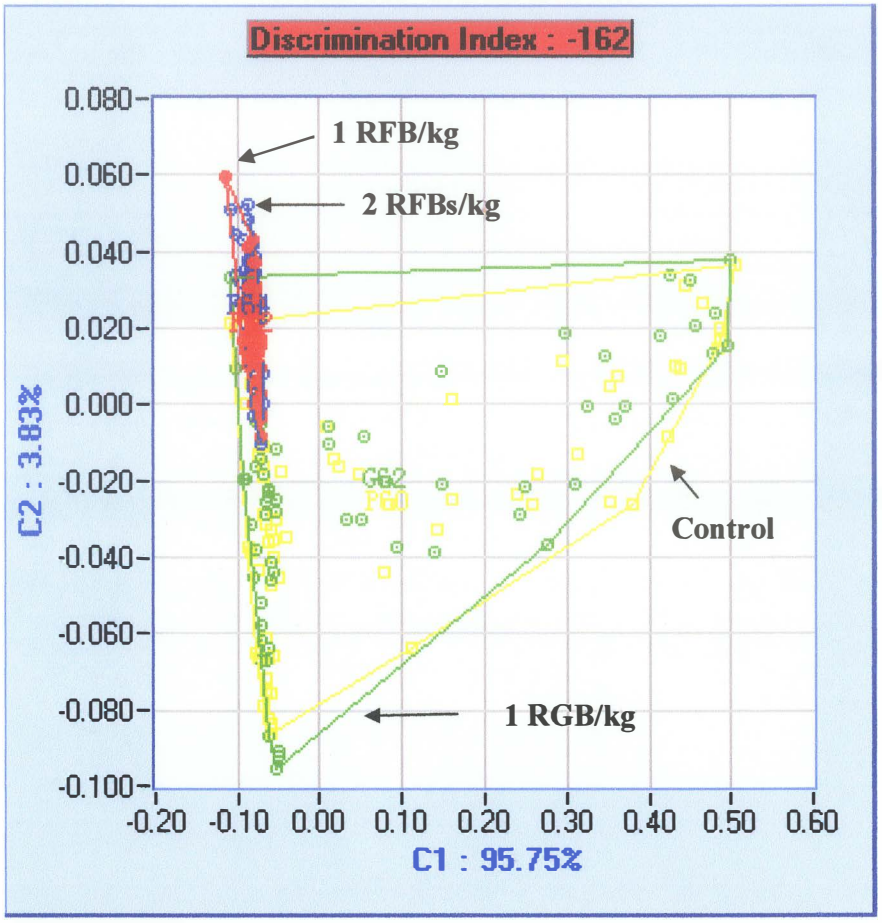

Figure H.2. PCA plot of control and low infested-wheat by RFB and RGB at 16% moisture content.

Note: Four groups overlap with each other showing no differentiation among the groups.

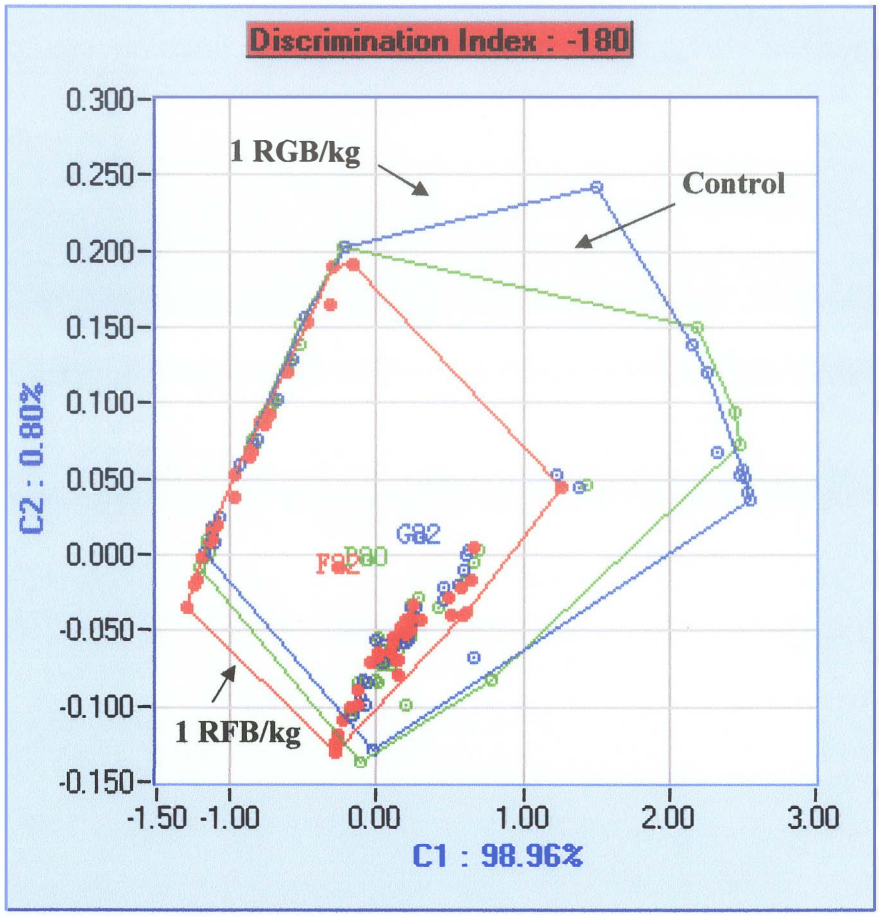

Figure H.3. PCA plot of control and low infested-wheat by RFB and RGB at 18% moisture content.

Note: Three groups overlap with each other showing no differentiation among the groups.

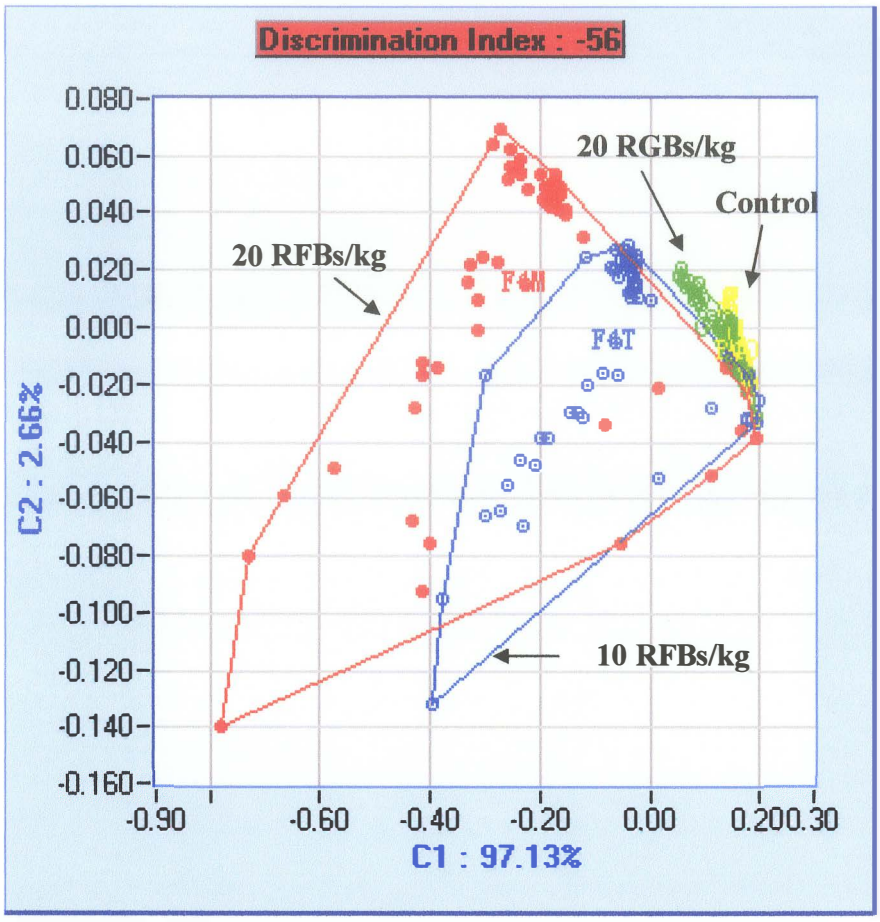

Figure H.4. PCA plot of control and high infested-wheat by RFB and RGB at 14% moisture content.

Note: 20 RFBs/kg is separated from Control.

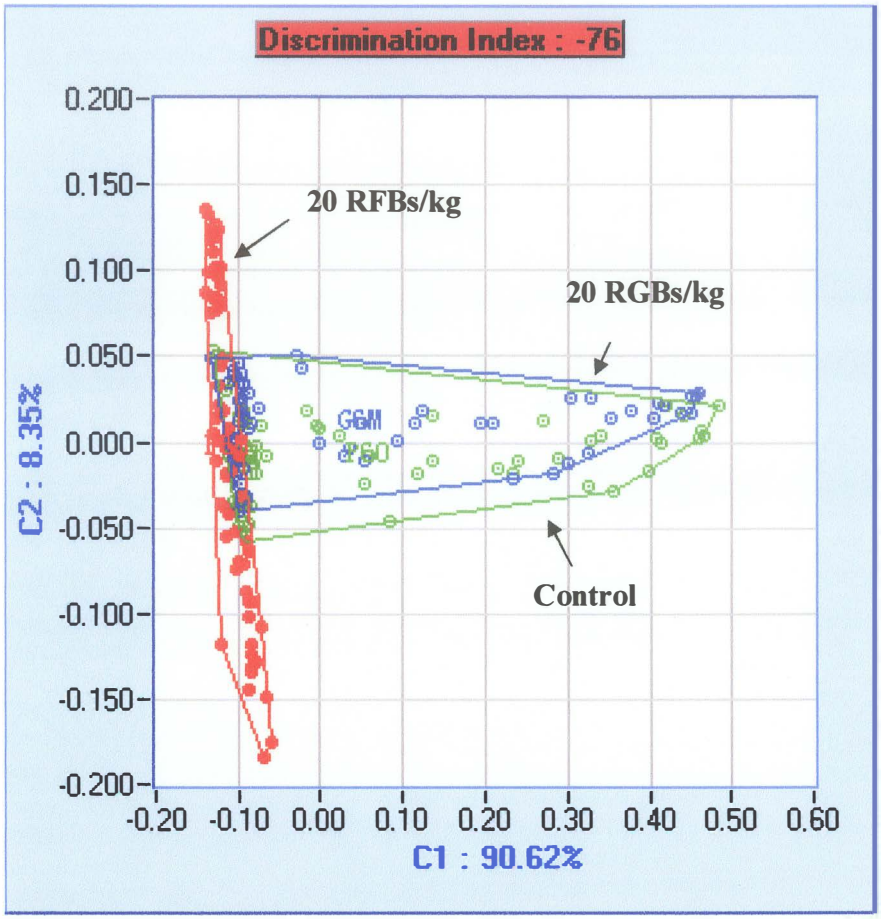

Figure H.5. PCA plot of control and high infested-wheat by RFB and RGB at 16% moisture content.

Note: 20 RGBs/kg and Control are overlapped with each other.

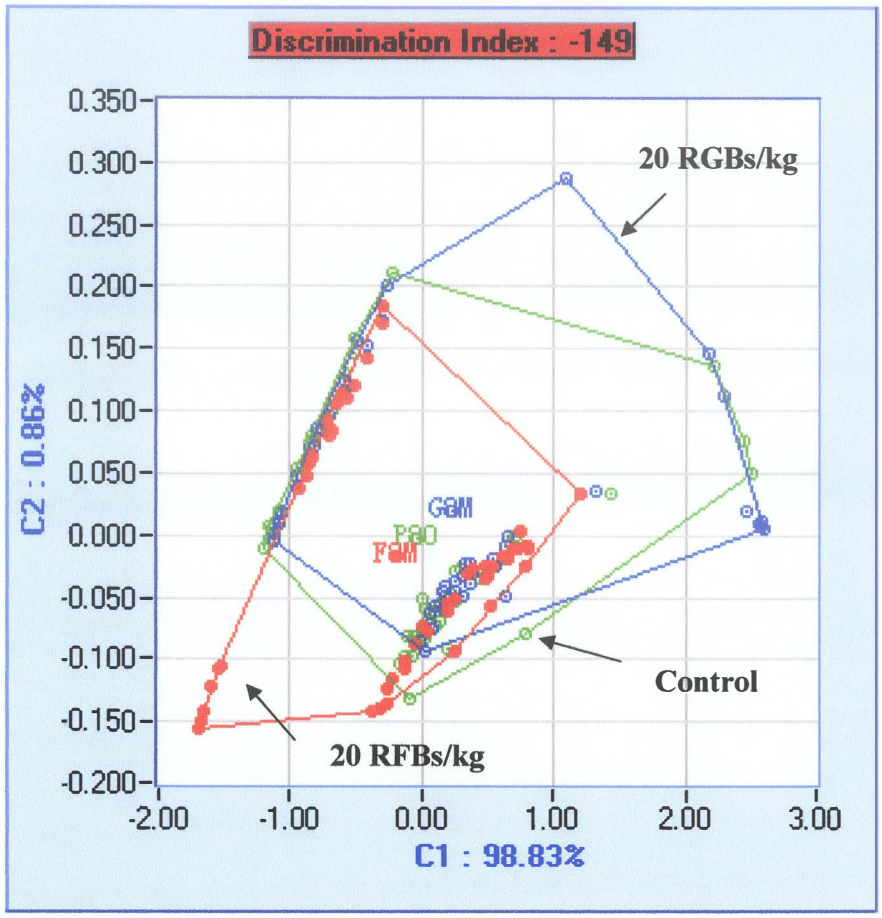

Figure H.6. PCA plot of control and high infested-wheat by RFB and RGB at 18% moisture content.

Note: Three groups overlap with each other showing no differentiation among the groups.

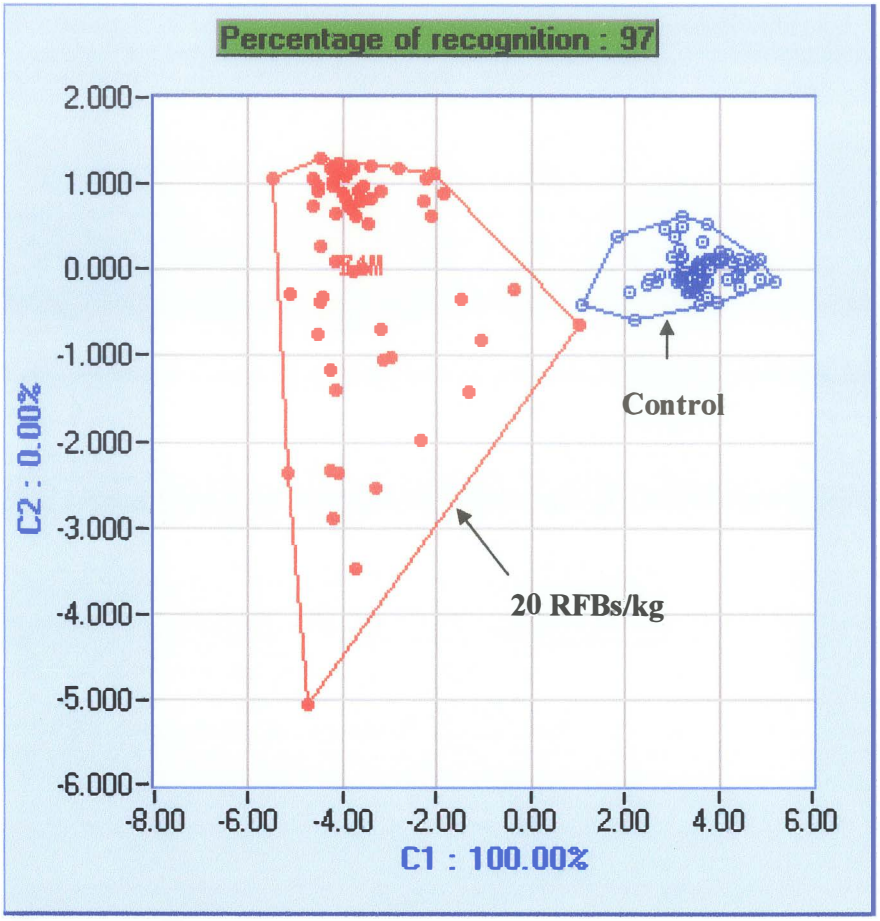

Figure H.7. DFA plot of control and infested-wheat by RFB at 14% moisture content.

Note: Two groups are separated showing the difference between the two groups.

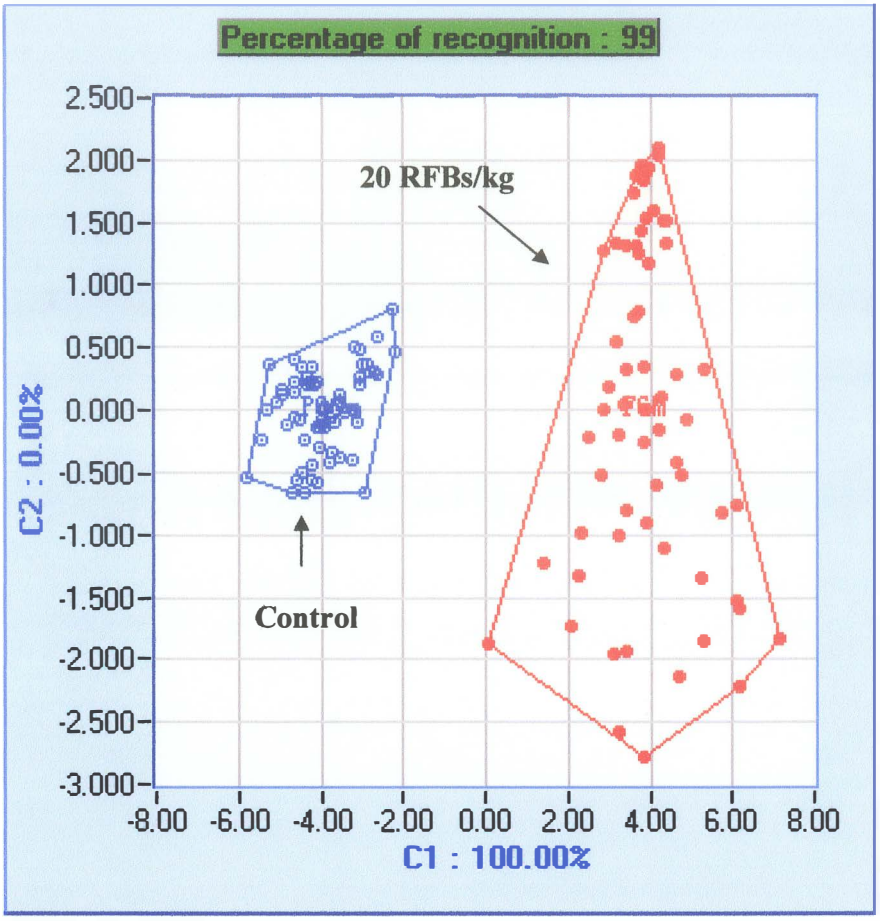

Figure H.8. DFA plot of control and infested-wheat by RFB at 16% moisture content.

Note: Two groups are separated showing the difference between the two groups.

Appendix I

Insect infestation level identification by PCA and DFA analysis (data from up to 72 h holding time in Tedlar bags)

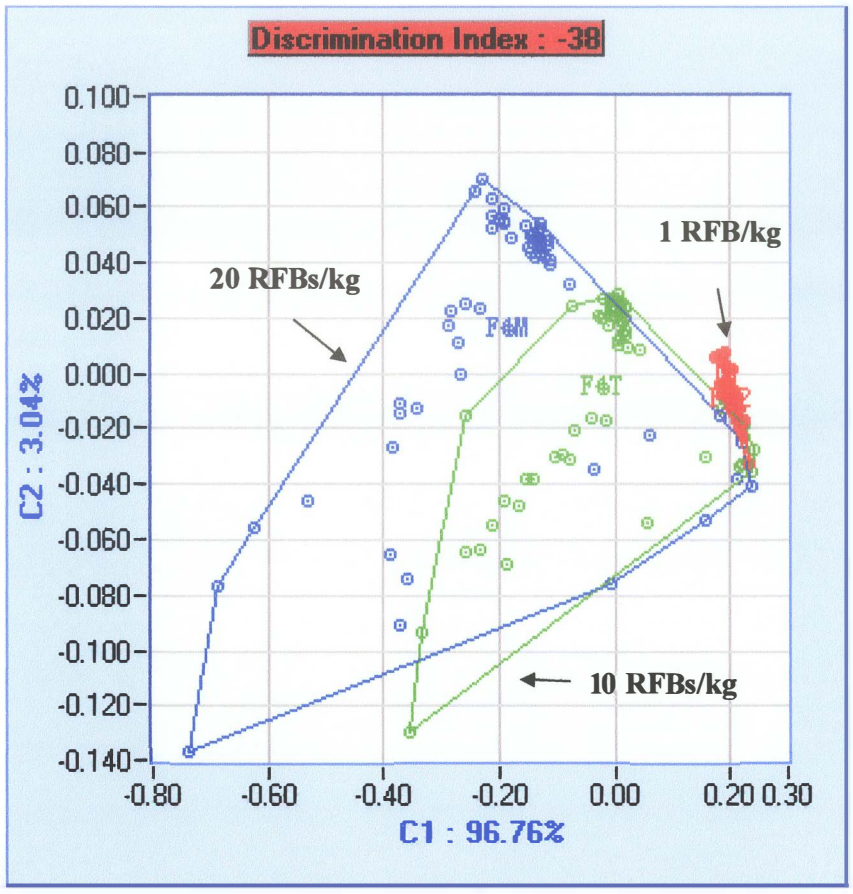

Figure I.1. PCA plot of 14% moisture content wheat infested by RFB at three infestation levels.

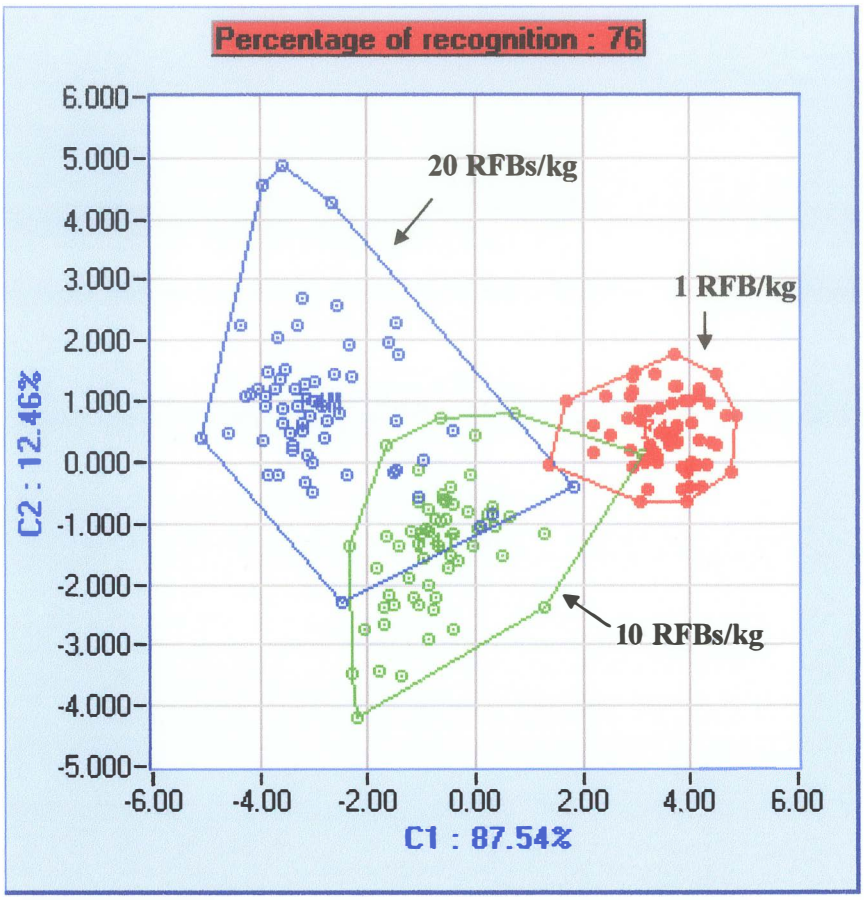

Figure I.2. DFA plot of 14% moisture content wheat infested by RFB at three infestation levels.

Note: 20 RFBs/kg and 10 RFBs/kg are overlapped with each other.