Respiration rate of potatoes (Solanum tuberosum L.) as affected by soft rot (Erwinia carotovora) and determined at various storage temperatures

M. A. Fennir[1]
J. A. Landry[2]
G. S. V. Raghavan[3*]

[1]Department of Agricultural and Biosystems Engineering. McGill University, 21 111 Lakeshore Rd, Saint Anne de Bellevue, QC, Canada, H9X 3V9

[2] Département de Génie de la Production Automatisée, École de Technologie Sup rieure (ÉTS), 1100 rue Notre-Dame W. Montreal, QC, H3C 1K3

[3*]Contact author: Phone: (514) 398-8731, Fax: (514) 398-8387
E-mail: vijaya.raghavan@mcgill.ca

Keywords: Potatoes, soft rot, respiration, infestation, progression, respiration quotient

ABSTRACT

This work investigated the effects of soft rot (Erwinia carotovora) on respiration rate of potatoes stored at three storage temperatures (5, 10, and 15 ^{o}C) covering temperature ranges commonly encountered in storage. Five treatments were evaluated: healthy (H), healthy with holes (HW), inoculation with no incubation (I0), inoculation with 24 hr incubation (I1), and inoculated with 48 hr incubation (I2). Respiration rate was measured daily using a gas analysis system and disease progression analysis was periodically performed. At 5 and 10 ^{o}C disease did not develop in the I0 treatment and this showed no significant difference from the control treatments (H and HW), while the I1 and I2 treatments exhibited a significant increase in disease development with a similar increase in respiration rate. At 15 ^{o}C storage temperature, disease developed in the I0 treatment shortly after the start of the experiment and the trend in respiration rate was similar to those of the I1 and I2 treatments.

1. Introduction

Respiration is a vital biochemical process for all living cells. From a postharvest perspective, it will continue after harvesting and during storage but at slower rates [1]. Measuring respiration rates of agricultural products is generally considered as invaluable information for determining storage conditions of regular and modified atmosphere storage systems, and the design of modified atmosphere packaging [2-4].

While a produce is being stored, its respiration process is affected by several factors, generally classified into produce and storage environment related factors [5]. The first group includes: variety, maturity, growing conditions, heat and water stresses, nutrient deficit etc, while storage factors include: temperature, gas composition, and ethylene [2-4, 6].

Over the years, numerous respiration studies have been conducted, leading to significant improvement in produce quality and storability. For potatoes, in particular, respiration rate has been addressed in relation to maturity [7-8], injuries [9], sprouting [10] and storage conditions [11-13]. However, little if any attention has been given to quantifying the respiration rate of potatoes as affected by disease infestation. Such investigations may lead to the development of a methodology of using respiration rate as a disease detection tool by monitoring its rates while produce is being stored. However, prior to initiating such investigations, the need for quantifying the effects of disease infestation on respiration rate was addressed.

This paper reports the experimental investigation of the effect of disease on the respiration rate of potatoes at three storage temperatures (5, 10, and 15 ^{o}C) covering temperature ranges commonly encountered in potato storage. The work is a part of a postharvest management research project designed to address postharvest stresses such as respiration rate and its use as a disease detection tool in potato storage.

2. Material and Methods

2.1 Storage and Gas Analysis Systems

A respiration measurement setup consisted of 24 storage chambers each with storage volume of nearly 25 litres, a sampling sequencer model 402AS-24 microprocessor automatic sequencer (NOVA Analytical Systems Inc., Hamilton, ON) and an infrared gas analyzer model 309BT (NOVA Analytical Systems Inc., Hamilton, ON). Containers were made from a 9.5 mm (3/8") thick PVC pipe sections of 254 mm (10") internal diameter (ID) and 584.2mm (23") long. **Figure 1** shows a schematic view of the storage container. The bottom was glued to the main pipe and the top cover was inserted in the pipe, sat on a rubber gasket and pushing it against a 2.54 mm (1") high internal ring that was attached to the main pipe section at a 63.5mm (2 ") from the top edge of the container. At closure the top cover was pressured at its centre by passing a threaded rod via a metal plate that sat on the two sides of the container. On the top, two connections were made, one was connected to the sequencer via 6.35mm OD (1/4") rigged pipe and on the other a solenoid valve was installed to allow replacing air that is drawn for analysis. The tubing system was connected to the sampling sequencer that facilitated an automatic sampling form the 24 containers. Volume of the tubing system as well as the volume of sampled air were taken into consideration in the respiration rate calculations.

Before the start of the experiment, containers were washed with chlorinated water. Then they were tested for airtightness using a digital pressure indicator model DPI 601 (SPR Control Systems, Mississauga, ON) to detect any leaks. Each container was tightly closed, pressurized to 2 kPa and monitored for 30 minutes. A container was considered airtight if no pressure drop was observed within the test period. Containers were arranged inside a walk-in cold room equipped with an on-off temperature control system that facilitates the maintenance of storage temperatures between 0 and 17 ^{o}C.

2.2 Potatoes

Potato tubers cv. Chieftain were obtained from a local grower in mid-December, 2000. They were received in 22.73 kg (50 lb) bags, suberized and treated with an unknown sprout inhibitor. Tubers were kept in a walk-in cold room adjusted to 5 ^{o}C and 90% relative humidity. Before every disease experiment, several bags were opened and medium to large size tubers were selected. At every storage temperature, about 75 kilograms of potatoes were used.

2.3 Treatments

Five treatments were used and defined as: healthy (H), healthy with hole (HW), inoculated with no incubation (I0), inoculated & incubated for 24 hrs (I1), and inoculated & incubated for 48 hrs (I2). Four replicates were used for the H treatments, while five replicates were used for the remaining treatments, the first four were used for respiration measurement and the fifth was designated for disease progression analysis.

2.4 Preparing Tubers and Storage Containers

Tubers of each treatment and its replicates were weighed and identified prior to

further experimentation. For each treatment, tubers were washed by soaking in 0.1% chlorinated water. Tubers were then individually wiped with a soft sponge, dried on paper towels, and kept overnight in a dark place. Tubers of the healthy treatments H and HW were placed in the containers prior to inoculation of the tubers for the diseased treatments. For the I0, tubers were placed in the containers soon after their inoculation, while the I1 and I2 tubers were placed in the storage containers after incubation for 24 and 48 hours, respectively. After placing the tubers in the tightly closed containers, gas analysis was started.

2.5 Inoculums and Inoculation Process

The soft rot bacterial agent Erwinia carotovora subsp. carotovora was obtained from the Agriculture Canada Research Station, St-Jean-sur-Richelieu, QC, Canada, and grown in petri-plates following standard microbiological procedures. A bacterial suspension with colony forming unit of 107 CFU/ml was obtained following procedures reported by **Ranganna** [14]. For each inoculated treatment about 100 ml of the bacterial suspension was prepared and divided equally among the five replicates of each inoculated treatment (20 ml per replicate) and applied to the tubers.

No holes were made on tubers of the H treatment, while for tubers of the HW treatment they were drilled similar to those of the inoculated treatments; but no inoculum was applied. For every inoculated treatment, the process began by drilling 2 to 3 holes in each tuber, followed by filling the holes with the inoculum. A low speed hand drill equipped with a 5 mm diameter bit modified to make a 25 mm deep hole, was used for making the inoculation holes. The drill bit was kept disinfected throughout the preparation process by keeping it in ethanol. After drilling and to facilitate the application of a similar inoculum volume for all treatments, holes were cleaned with a sterilized wire hook, then filled with the bacterial suspension using a sterile syringe. Again, to assure perfect filling, the inoculum was applied gradually and tubers were tapped several times. While loading the container, tubers were placed in the holes-up position, to avoid spilling of the inoculum. For the incubated treatments, tubers were placed holes-up in a plastic bag which was closed for the entire incubation period and kept in a room equipped with a heater and thermostat adjusted to 25 ^{o}C. Afterwards, potatoes were transferred to the storage containers and these were tightly closed and placed in the cold room.

2.6 Gas Analysis

Gas analysis was performed daily over the entire experimental period. At every analysis, air was withdrawn continuously for 30 seconds on two occasions in each run. CO2 content inside the container was kept below 3% and once values near this threshold was recorded, containers were opened, and aerated for one minute using pressurized air.

2.7 Evaluation of Disease Progression

Starting from the first day of incubation and throughout the experiment, a sample was taken periodically from the storage containers designated for sampling. A

cross-sectional cut was made through the middle of every hole on the tuber, and the infected area, mainly the softened tissue developed around the infected hole was carefully removed, and diameters and depths of infected area were measured and recorded.

For most treatments, at advanced stages of disease progression, the determination of the volume of the infected area was not possible because of the way disease grew. Although large size tubers were used, infected holes became larger and joined together, and at more advanced stages, most of the inspected tuber was decayed. At such a stage, the uninfected part was weighed and its percentage was determined. At every disease progression evaluation day, the progression of the infected area was compared with the calculated respiration rate within their respective time interval.

2.8 Experimental Design

For the respiration rate investigation, a factorial design of 3 5 was used and the analyses followed the procedure suggested by **Littell *et al.*** [15]. Statistical analyses were performed using the Statistical Analysis System (SAS) software version 12, and significancy was declared at the 0.05 probability level.

3. Results and Discussion

3.1 Influence of Different Treatments and Temperature on the Respiration Rates

The respiration rates of stored potatoes as CO2 produced and O2 consumed for the five treatments at 5, 10 and 15 C are presented in Figures 2-4. Visual analysis showed that the respiration rates using either CO2 produced or O2 consumed exhibit similar trends for all the treatments at different temperatures. Therefore, only respiration rates as CO2 produced are used in describing and discussing the influence of different treatments and temperature on the respiration rates.

Of all temperatures studied, H and HW treatments have the lowest respiration rates among all the treatments. Furthermore, the respiration rates were kept constant throughout the storage period at lower temperature (5 and 10 ^{o}C). At 15 ^{o}C, the respiration rate was kept constant until about 20 days of storage and it increased slightly after that period. Inspection of the potatoes showed that sprouting of the H and HW tubers started at the beginning of the third week in storage. Sprouting was expected because of the sudden exposure on the mature tubers to higher temperatures.

Comparison of the respiration rates of the H and HW treatments showed no significant difference at 5 ^{o}C, and slightly higher for HW that H at higher temperatures. This suggests that physical injury of mature tubers may cause a minor increase in respiration rates. The result is different from the report by **Peterson et al.** [8] on the wounded immature tubers.

I1 and I2 treatments have the highest respiration rates among the five treatments at all temperatures. Compared to the H treatments at the same temperature, a 3.7 to 4.8

and 3.9 to 4.8 times increase in the respiration rate were observed in I1 and I2 respectively. This suggests that disease infection can greatly increase the respiration rates of the stored tubers. **Figures 2, 3 and 4** also revealed the progression of the disease infection of the tubers as indicated by the respiration rates. At 5 and 10 ^{o}C, the respiration rates increase gradually over the whole storage period indicating a steady development of the disease infection. For the 15 ^{o}C storage, the respiration rates of I1 and I2 treatments first increase up to about 2 weeks then drop gradually. Inspection of the tubers revealed that the drop in respiration rates is due to the decay of the tubers.

Compared to the I1 treatment, I2 treatment showed significantly higher respiration rate at 5 ^{o}C, not significantly different value at 10 ^{o}C, and significantly lower at the decaying stage of 15 ^{o}C storage. This demonstrates that longer incubation time prior to the storage leads to a more advanced stage of disease infection on the tubers; this more advanced stage of infection significantly affects the respiration rate at lower temperature and is less significant at higher temperatures.

The behaviour of the I0 treatment depends on the temperature. At 5 ^{o}C, throughout the 41 days of storage, the respiration rate is not significantly different from H or HW treatments suggesting the low temperature could prevent the disease infection from developing. At 10 ^{o}C, the respiration rate of the I0 treatment is slightly higher than H and HW at the beginning of the experiment; it gradually declines over the following week and is stabilized after that to a value that is not significantly different from H and HW treatments. This suggests that at 10 ^{o}C, although no physical infection symptoms were observed, the disease still resulted in higher respiration rates. At 15 ^{o}C, the respiration rate increases steadily to a maximum value at about three weeks storage then drops gradually. This suggests that at 15 ^{o}C, no incubation is needed for the disease to develop. And the drop in respiration rate after 3 weeks is revealed by inspection of the tubers for both I1 and I2 treatments, resulting from the decay of the tubers.

The influence of temperature on the mean respiration rate throughout the storage period of each treatment is presented in **Table 1**. Slight increase was observed for all treatments when the temperature rose from 5 to 10 ^{o}C. Another 5 ^{o}C increase in the temperature caused much greater increase in the mean respiration rate than the first 5 ^{o}C. The greatest increase was observed on the I0 treatment which is due to the initiation of the disease infection by the higher temperature. The much lower increase in the I2 treatment is mainly due to decaying of the infected tubers in the late stage of storage. The magnitude of the increase for all the other treatments is similar to the increase from H treatment to either I1 or I2 treatments.

The above discussion demonstrates that both disease infection and temperature affect the respiration rates of the stored potatoes. In practice, low temperature not only slows down the respiration rate but also prevents the disease development of the spore

contaminated tubers. On the other hand, high temperature not only increases the respiration rate by itself, but also helps the development of the disease for the contaminated or disease-developed tubers. For those tubers with the disease present, disinfection is suggested because low temperature alone could not prevent the development of the disease.

3.2 Disease Progression Analysis

The disease progression in terms of percentage of infected tissue mass for the three inoculated treatments I0, I1 and I2 at 5, 10 and 15 ^{o}C are presented in **Figures 5, 6, and 7**. No infection was observed during the whole storage period for I0 at 5 and 10 ^{o}C. The inoculated sites of the tubers with I0 treatment at 5 ^{o}C were visually normal; and no changes in colour and physical appearance were observed. The inoculated sites (holes) developed dry surface without any signs of disease suggesting that low storage temperature generally suppresses disease progression, and early infected tubers may not develop disease. However at 15 ^{o}C, the disease developed slowly in the first few days and then suddenly spread to more than 70% by 17 days; and the progress slowed down after that and ended up with more than 90% at the end of the storage period of 32 days.

For the I1 and I2 treatments, the percentage of infected tubers increased steadily throughout the 41 days of storage period for the 5 ^{o}C storage; and at the end, about 70% of the tubers were infected with disease. At 10 ^{o}C, the disease developed steadily up to about 24 days and levelled off after that. More than 80% of the tubers got infected by the end of the 32 days storage period. For the 15 ^{o}C storage, disease developed gradually at the beginning and the percentage infected increased rapidly after about a week to about 70%. At the end of the storage period of 32 days, more that 90% of the tubers were infected.

The results obtained with the disease progression showed very similar trend to the ones obtained with the respiration rate measurement for all three treatments at all temperatures. This suggests that by analyzing the respiration rate of the stored potatoes, the degree of damage caused by the disease infection could be detected with a result comparable to a visual disease detection. However, the respiration rate measurement could provide more information on the physiological condition of the stored tubers than obtained with a visual analysis alone.

4. Conclusions

In potato storage, respiration rate can be used to monitor the possible disease infestation of the tubers and the degree of infestation. The result is comparable with the one obtained with disease progression analysis using visual inspection. The respiration data helps provide more information regarding the physiological status of the stored tubers whereas the disease progression data is limited in scope.

During the storage of potatoes, temperature and disease infestation are two most important factors that influence the respiration rate, consequently the shelf life of the tubers. Temperature itself affects the respiration rate but more importantly it can affect the development of disease. The development of disease on the spore-contaminated tubers could be slowed by storage at low temperatures. Even for the tubers with already developed disease, the progress of the disease can be slowed down by the low temperature environment. On the other hand, high temperature facilitates the development of either spore-contaminated or disease-developed tubers. Therefore, low temperature is important in potato storage in any circumstances and exposure to high temperature should be avoided for potatoes with possible spore contamination, to prevent the early development of the disease.

ACKNOWLEDGMENTS

The authors acknowledge the Conseil des Recherches en Pêche et en Agroalimentaire du Québec (CORPAQ), Natural Science and Engineering Research Council of Canada (NSERC) for funding the project, and Fonds la Formation de Chercheurs et dl'Aide à la Recherche (FCAR) for giving the first author a scholarship.

REFERENCES

[1] Burton W G (1982). Post-harvest physiology of food crops. Longman Group Ltd. New York.

[2] Wills R. H H.; Lee T H; Granham D; McGlasson W B; Hall E G (1981). Postharvest, an Introduction to the Physiology and Handling of Fruits and Vegetables. The AVI Publishing Company Inc, Westport, CT.

[3] Kader A (1992). Postharvest Biology and Technology of Agricultural crops. Publication No. 3311.Division of Agricultural and Natural Resources, University of California, Davis, CA.

[4] Raghavan G S V; Alvo P; Gari py Y; Vigneault C (1996). Refrigerated and controlled modified atmosphere storage. In Biology principles, and applications, ed. Somogyi L P, Ramaswamy H S & Hui Y H (eds.), pp. 135-167. Lancaster: Technomic Publ. Co. USA.

[5] Kays S. J (1991). Post-harvest Physiology of Perishable Plant Products. The AVI publication company, Inc. Westport CT.

[6] Hardenburg R E; Watada A E; Wang C Y (1990). The Commercial Storage of Fruits, Vegetables and Florist and Nursery Stocks. US Department of Agricultural.

[7] Kumar G N M; Knowles, N R (1996). Nature of enhanced respiration during sprouting of aged potato seed-tubers. Physiologia Plantarum. Vol.97: 228-236.

[8] Peterson C; Wyse L R; Neuber H (1981). Evaluation of respiration as a tool in

predicting internal quality and storability of potatoes. American Potato Journal. Vol.58:246-256.

[9] Kader A (1987). Respiration and gas exchange of vegetables. In Postharvest Physiology of Vegetables, Weichmann J (ed), pp. 113-170. Marcel Dekker, New York.

[10] van Es A; Hartmans K J (1987). Respiration, In Storage of Potato, Rastovski A & Van Es et al, (eds), pp. 133- 140. Wageningen: Pudoc Wageningen.

[11] Hunter J (1986). Heat of respiration and weight loss from potatoes in storage. In: Engineering for Potatoes, Cargill B F (ed), pp. 511-550. Michigan State University & ASAE Publication.

[12] Schaper L A; Varns J L (1978). Carbon dioxide accumulation and flushing in potato storage bins. American Potato Journal. Vol.55:1-14.

[13] Schippers P A (1977). The rate of respiration of potato tubers during storage. 2. Results of experiments in 1972 and 1973. Potato Research. Vol.20:189-206.

[14] Ranganna B (1996). Thermal treatments for short term storage of potatoes (Solanum tuberosum L.). PhD Thesis. McGill University, Canada.

[15] Littell R. C; Herny P R; Ammerman C B (1998). Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science. Vol.76:1216-1231.

Figure 1. A schematic of the storage container

Figure 2. Respiration rates for the five treatments at 5 ^{o}C, as CO2 produced (top) and as O2 consumed (bottom).

Figure 3. Respiration rates for the five treatments at 10 ^{o}C, as CO2 produced (top) and as O2 consumed (bottom).

Figure 4. Respiration rates for the five treatments at 15 ^{o}C, as CO2 produced (top) and as O2 consumed (bottom).

Figure 5. Disease progression in the inoculated treatments stored at 5 ^{o}C.

Figure 6. Disease progression in the inoculated treatments stored at 10 ^{o}C.

Figure 7. Disease progression in the inoculated treatments stored at 15 ^{o}C.

Table 1. The influence of temperature on the mean respiration rate for each treatment

	H	HW	I0	I1	I2
R_{10}/R_5	1.15	1.25	1.57	1.42	1.10
R_{15}/R_5	3.66	4.14	11.92	3.68	2.82

Note: R_{15}, R_{10}, and R_5 are the mean respiration rates throughout the entire storage period for each treatment at 15 ^oC, 10 ^oC and 5 ^oC, respectively.

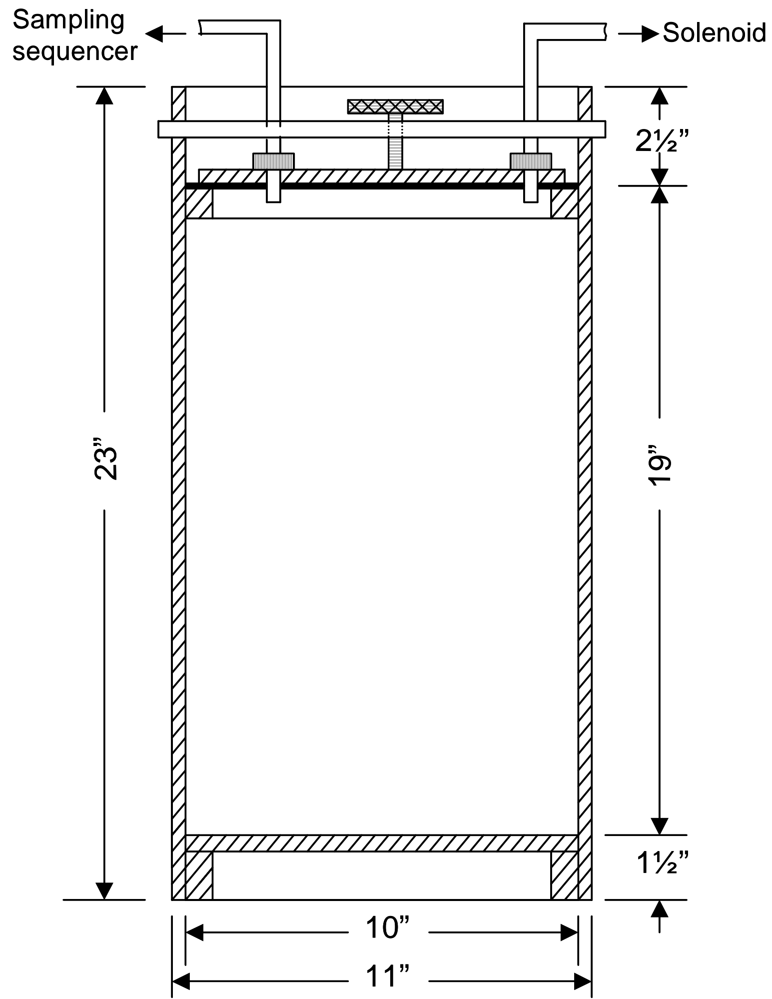

FIGURE 1

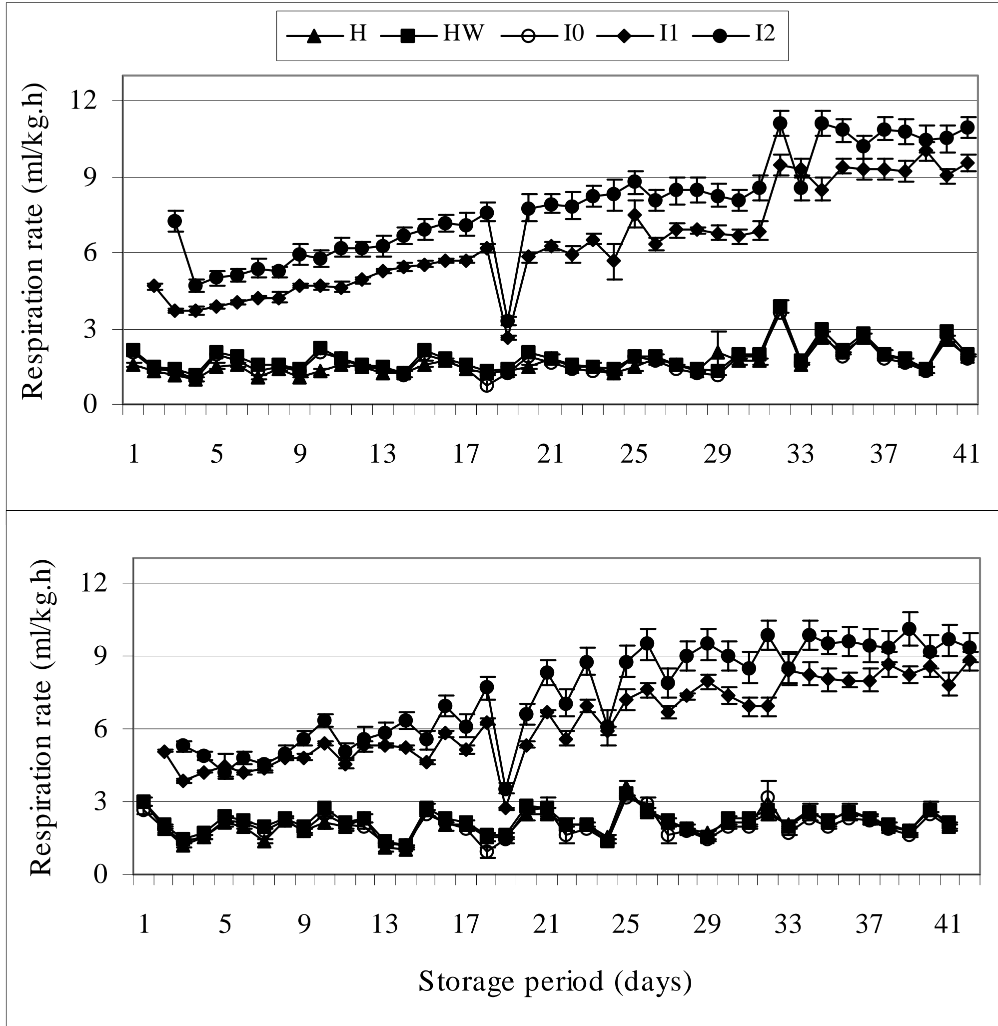

FIGURE 2

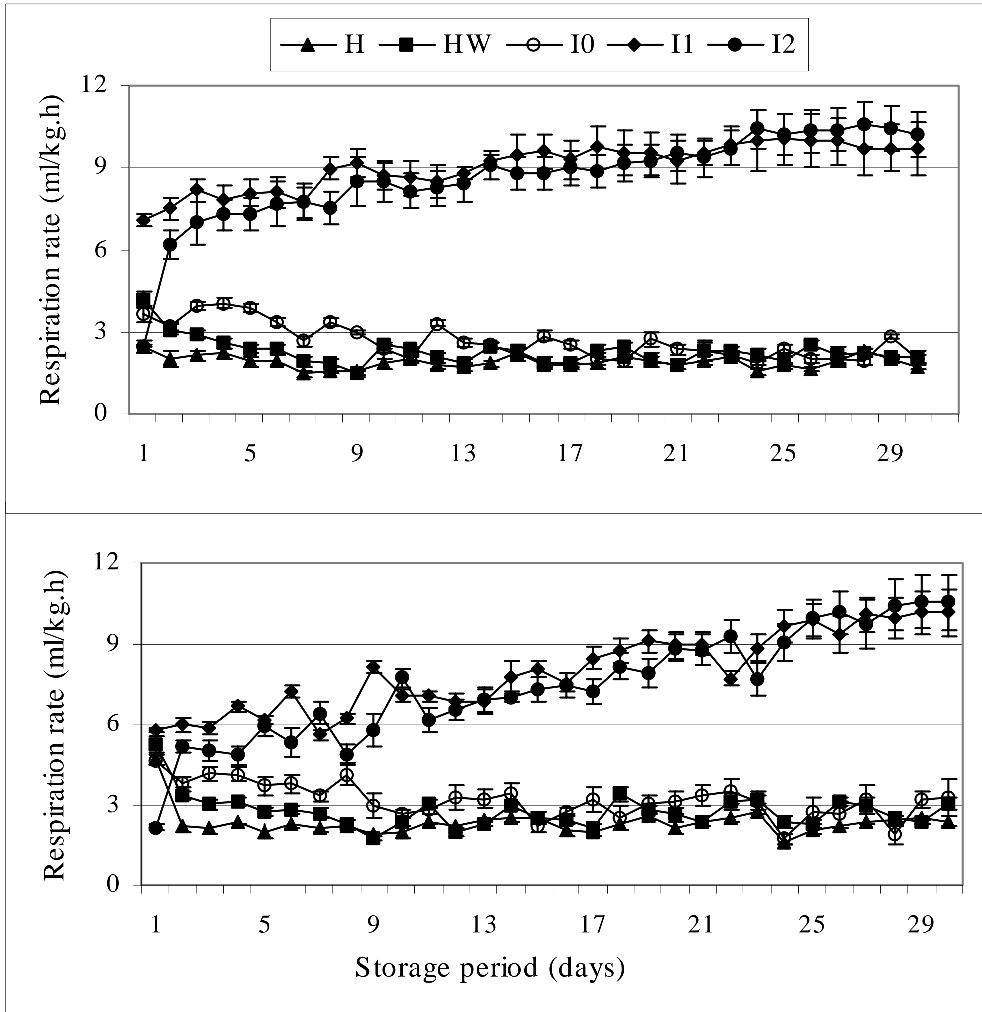

FIGURE 3

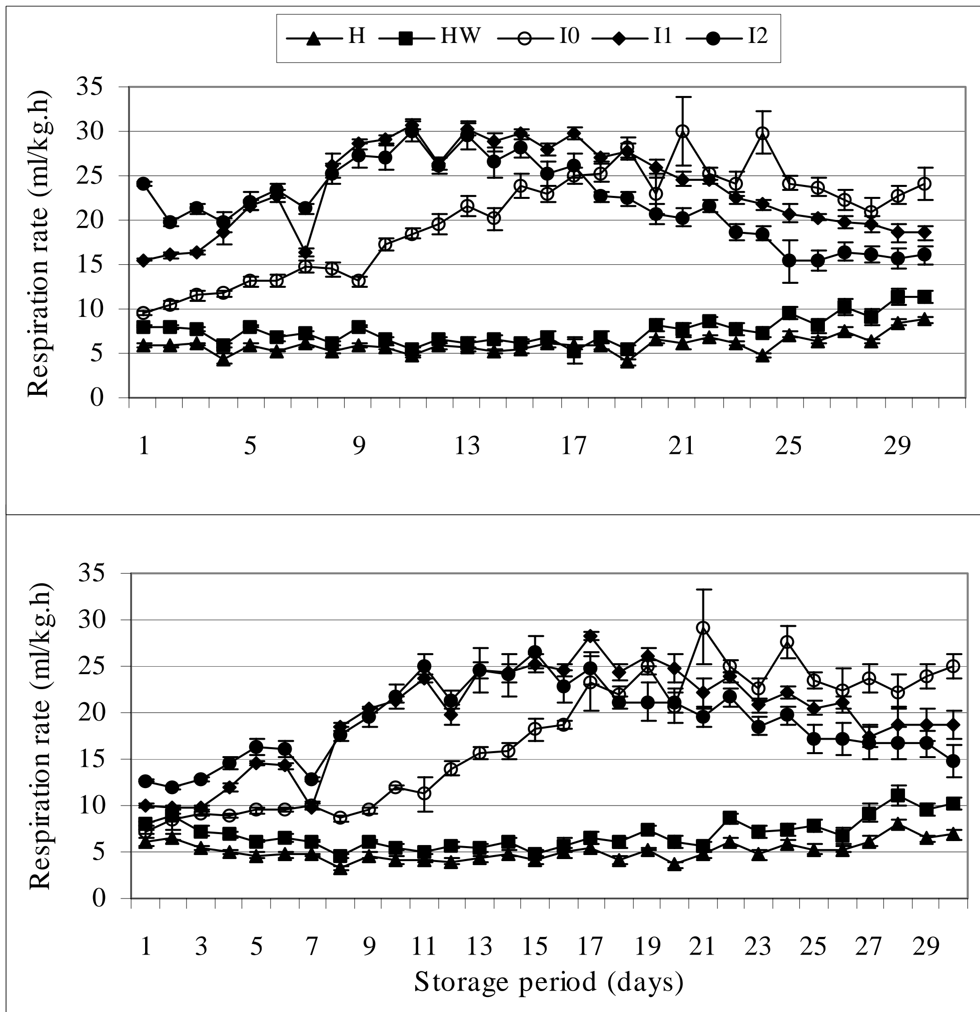

FIGURE 4

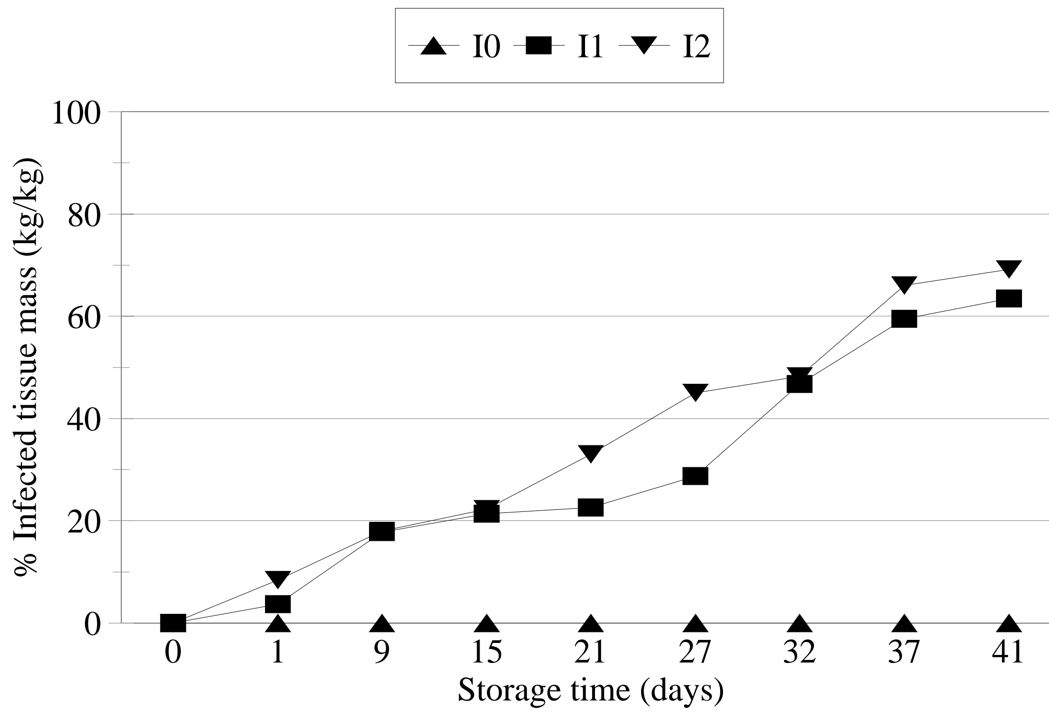

FIGURE 5

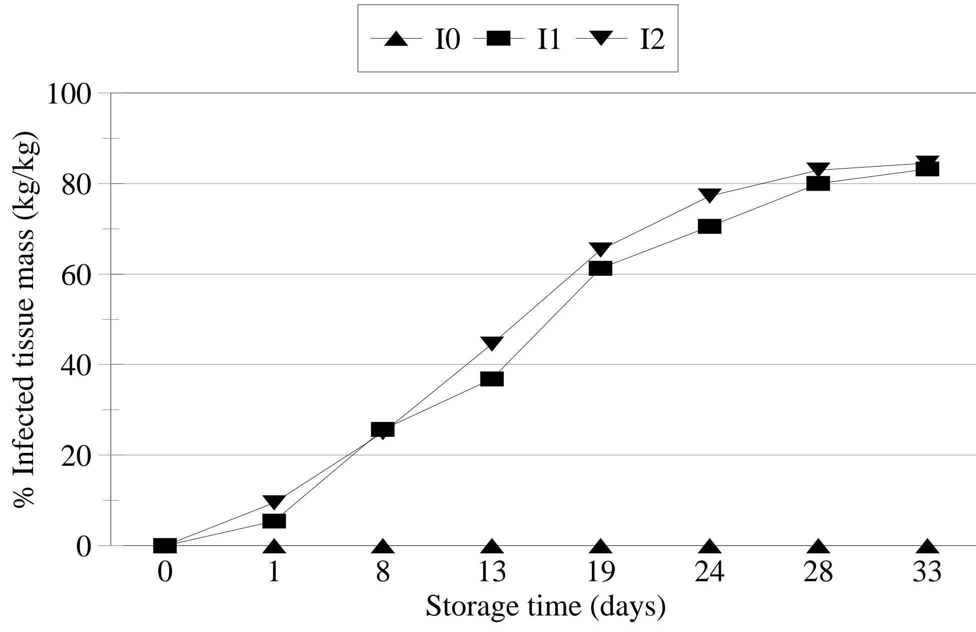

FIGURE 6

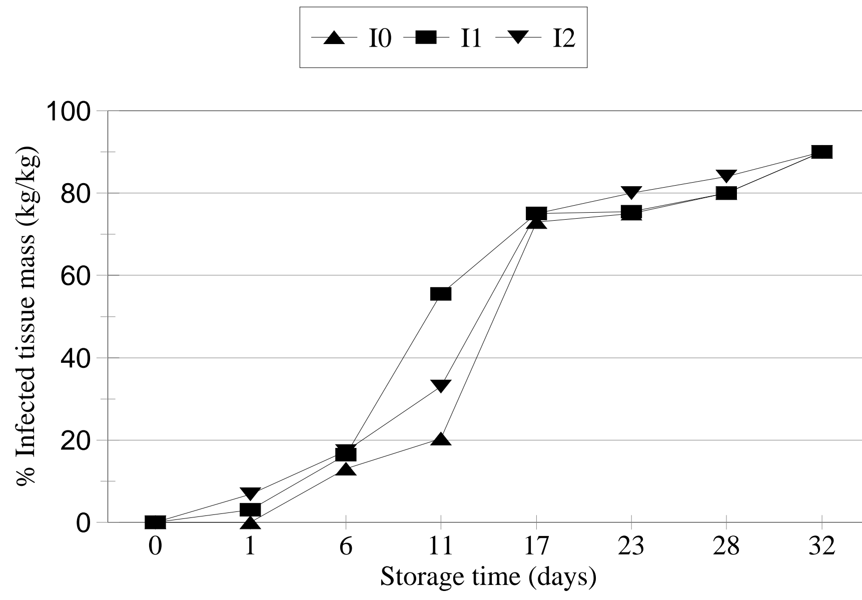

FIGURE 7