Project Report

Evaluating the efficacy of a screen humidity cell in filtering pathogens and other particulates out of air in potato stores

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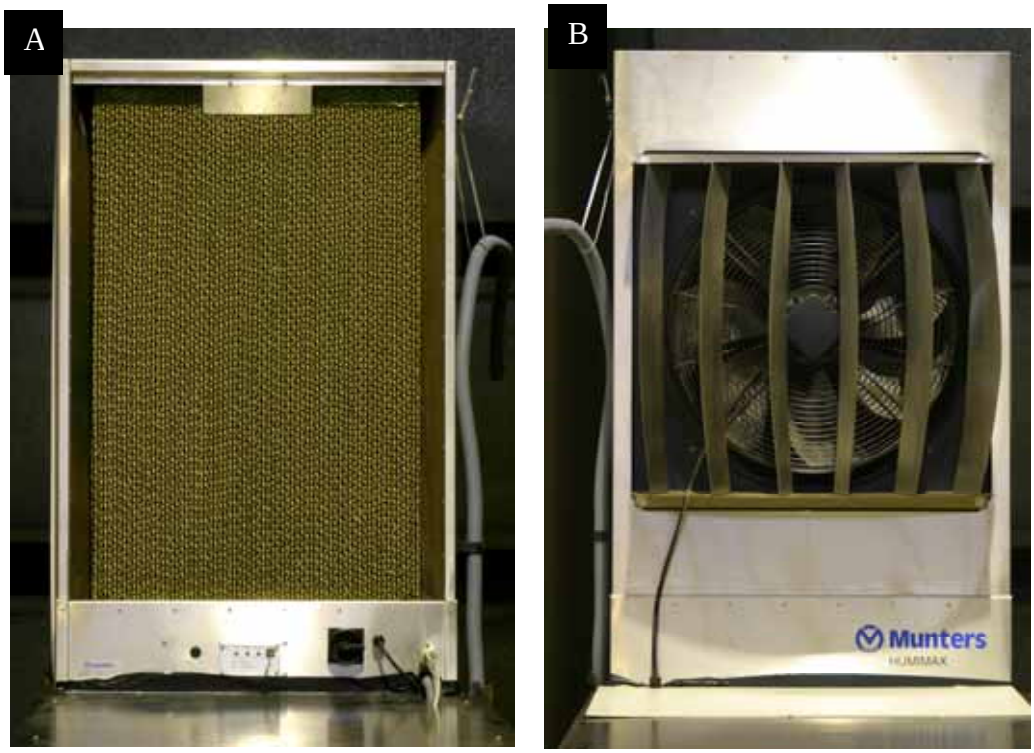
Summary

- An evaporative humidifier (the Munters HM2 4000) and an ultrasonic nozzle humidification system were equally effective in maintaining the atmosphere in 12-tonne experimental stores at the desired 95% RH.
- A series of experiments were conducted to test the efficiency of a Munters HM2 4000 evaporative humidifier at removing fungal spores and other microorganisms from the atmosphere in 12-tonne experimental potato stores.
- The Munters humidity cell was able to trap 99% of fungal spores that had been sprayed directly into the airflow of the humidity cell.
- In trials using 12-tonne experimental stores where dust or spores had been introduced, stores with the Munters HM2 4000 and ultrasonic nozzle humidification (i.e. atomised water) systems installed had similar levels of fungal spores present in the air throughout 11 and 17-day experiments.
- However, bacterial contamination was higher in sampled store air when humidified using an atomised system than with an evaporative humidification screen. It is likely that the atomised water was itself the source of contamination.



Munters HM2 4000 evaporative humidifier showing A) the evaporative media at the front (or intake side); and B) the fan blowing humidified air out into the store.

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Introduction

Potato storage has become extremely sophisticated. Environmentally controlled store systems are able to maintain crop temperatures within 1°C of accuracy. This has enabled potatoes to be stored for up to 11 months. However, successful long-term storage of potatoes remains a challenge for growers whether it is for seed, consumption or other processes (Eltawil *et al*. 2006). Because, even with sophisticated means of potato storage, losses during storage can be as high as 150 million tonnes per annum in the United States (Varns *et al.*, 1985 cited in Eltawil *et al*., 2006).

Cold storage (below 4°C) can increase the storage life of the crop by reducing the metabolism of the tuber and slowing or preventing sprouting. At such low temperature, the cool air has a drying effect, causing water loss from the potato. To counteract the drying effect systems that increase the humidity of storage atmospheres can be useful. Sparks (1973) demonstrated how a 10% difference in RH can affect weight loss in stored potatoes. The cultivar, Russet Burbank, was stored at 85% and 95% RH. After a period of 330 days, the weight loss in the crop stored at 85% RH was 1.3% greater than in the crop stored at 95% RH. More recently, in refrigerated small-scale experimental stores, Wiltshire *et al* (2004) demonstrated that after a 35-week storage duration, weight loss in cv Estima held at 98% RH was 4.4% compared with 5.1% in crop held at 90% RH. This difference was significant.

Currently there are 3 types of humidification system in operation: high-pressure nozzles, centrifugal spinning disks and evaporative media (Small and Pahl, 2003). High-pressure nozzles work by passing high-pressure water through an aperture. This results in the production of a mist containing tiny droplets of water. The centrifugal spinning disc allows water to hit a spinning plate, which causes the water to fly off at a high speed and dispersed as minute droplets. Evaporative media operates by passing air through a water-laden membrane that allows the air to become saturated with water vapour. This differs from atomised systems where water droplets are used to saturate the air.

High-pressure nozzles and centrifugal spinning discs are less popular now than they were previously. This is partly due to the difficulty in regulating the water dispersal when using those systems (Small and Pahl, 2003). Also, with atomisation systems, there is a possibility of water droplets landing on the crop increasing the risk of rots and blemish diseases. The decline in humidifiers that produce mists has lead to an increase in interest in evaporative media humidifiers.

The purpose of this trial was to evaluate the ability of the Munters humidifier to filter out airborne fungal spores and bacteria. With this in mind, a series of experiments were set up to a) determine the trapping efficiency of fungal spores that were directly sprayed into the airflow produced by the Munters humidity cell; and b) compare the level of fungal spores and bacteria from air in stores where humidity was controlled either by atomised water or by humidity cell.

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Materials and Methods

Method development

Air brush optimisation

An airbrush (Badger Products, USA) was used throughout the duration of the trial to inoculate the experimental stores with known quantities of fungal spores. The method of dispersing the spores by airbrush was optimised by varying the pressure of the air to produce the finest droplet size. To determine the optimum conditions for producing the smallest droplet size, the airbrush nozzle height was set at a standard 9.53mm and the air pressure adjusted from 20 psi (1.4 bar) to 40 psi (2.8 bar) in 5 psi (0.35 bar) increments. Droplets produced by the airbrush were directed over water sensitive paper (Syngenta, UK) for approximately 5 seconds. The paper was fixed in methanol, and the droplets measured under microscopy. The finest droplet size was a combination of the nozzle height at 9.53mm and air pressure of 30 psi (2.1 bar).

Optimising the impinger

The glass wash bottle (impinger) was connected to a universal pump and to an outlet sample piper. Natural inoculum of dry *Penicillium* spp spores were obtained from mouldy oranges and the spores harvested in 100mL of distilled water with 0.05 µL of the surfactant, $Tween_{20}$, added. The spore suspension was atomised into a controlled atmosphere 560 L chamber and sampled using the impinger. In order to determine the optimum air flow, sampled air was bubbled through distilled water containing 0.1% $Tween_{20}$ at air flow volumes ranging from 800 mL/min to 20 L/min. The sample water from the impinger was examined using a microscope and Neubauer haemocytometer, and spores counted. If there was not sufficient spores for a count, the sample water (a known volume) was centrifuged and the supernatant drawn off, providing a 10-fold concentration.

Evaporative humidifier

The *Humimax*™ Evaporative Humidifier HM2 4000 (manufactured by Munters, Sweden; supplied by Crop Systems Ltd, North Walsham, UK) is designed to provide a maximum humidified airflow of 4,000 m^3 h^{-1}. The unit dimensions are: 650 mm (depth with air deflector) by 1,175 mm (height) by 730 mm (width). A humidification pad is mounted in the air inlet. The air to be humidified is drawn through the pad by an axial fan positioned in the outlet of the unit. The base of the unit forms a water reservoir. Water is supplied to the reservoir through a water filter and valves that regulate the flow of water. The system humidifies stores by passing air through a wetted pad or cell, where the air picks up moisture and is circulated through the crop.

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Evaluating the efficiency of spore trapping using wet and dry screens

The effect of humidifier hydration was evaluated in three formats: wet, dry and with no screen fitted.

The open treatment was carried out first. The humidifier screen was dried using the equipment's internal drying program. After 3 hours the evaporative media was removed. The plumbing of the humidifier was then short-circuited, so the water was only re-circulated in the sump. Natural inoculum, by encouraging store air to circulate through the humidifier (Section 2.2.1) or a conidial suspension of *F. sulphureum* (Section 2.2.2) were introduced directly into the flow of air entering the humidifier. In order to detect conidia in the airstream leaving the humidifier, eight Petri dishes containing quarter strength potato dextrose agar (PDA) were attached to the backboard positioned in the airstream from the humidifier (Appendix 1). During inoculations, the humidifier fan was turned on and the Petri plates were uncovered for 1 or 10 minutes. After exposure, the plates were sealed and incubated at 18°C.

The experiment was then repeated with a dry screen and wet screen (normal operating condition after hydration for a minimum of 3 hours). Petri plates were set out and the air sampled as described above.

Evaluating the spore trapping efficiency using natural inoculum

The air in the store was used as the inoculum source. Eight Petri dishes were placed on a backboard placed where air exited the humidifier. Petri dishes were exposed to the airflow by removing the lids and replacing after 10 minutes. The plates were examined for evidence of fungal and bacterial colonies after 5 days' incubation at 19°C. The effect of having no screen, wet screen or dry screen on the number of colony forming units depositing on the Petri dishes was analysed by ANOVA using Genstat release 8.1.

Evaluating the spore trapping efficiency using *Fusarium sulphureum* conidia

A conidial suspension containing approximately 3.1×10^6 conidia/mL was sprayed for 1 minute directly into a Munters HM2 4000 evaporative humidifier (when open, dry or wet as described above) using an airbrush (at 2.1 bar air pressure). Eight Petri dishes containing PDA were placed on a frame where the air exited the humidifier. The Petri dishes were exposed to the airflow by removing the lids and replacing after 1 minute. Colonies resembling *F. sulphureum* after 5 days' incubation at 19°C were counted. The effect of having no screen, wet screen or dry screen on the number of colony forming units depositing on the Petri dishes was analysed by ANOVA using Genstat release 8.1.

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Comparison of the deposition of artificially inoculated **Polyscytalum pustulans** *conidia in stores with atomiser and screen humidification systems***.**

Polyscytalum pustulans was identified as a suitable test pathogen as it is typically associated with aerial store contamination. The conidia are small (approximately 10 x 2.5 µm), relative to other fungal conidia such as *Helminthosporium solani* (approximately 40 x 7.5 µm) and *Fusarium sulphureum* (macroconidia approximately 35 x 4 µm). Therefore, the small spores of *P. pustulans* would provide a suitable material for testing the ability of the screen cell in filtering out spores from air.

Two clean 12-tonne experimental stores were used. One had a Munters humidifier screen installed in the centre of the store at a height of approximately 1 m. The other store had an ultrasonic nozzle humidifier in place (control store). To keep the environmental conditions in the stores as similar as possible, each was set to operate at 10°C, 95% RH. Approximately 20 mL of suspension containing 2.3 x 10^6 conidia/mL in distilled water (with 0.1% $tween_{20}$) was sprayed into the plenum air recirculation duct (Appendix 2). Petri dishes (90 cm diameter) containing PDA were used to sample air for conidia. The plates were placed on a platform 30 cm off of the floor. Two replicate plates were exposed for 60 minutes before spraying and for 60 minutes daily over a period of 17 days. At each sampling occasion, the lids of each Petri dish was removed for 60 minutes, after which time the lids were replaced, then plates were incubated at 18°C. After approximately 3 days, any colonies that grew were identified as fungal or bacterial and each counted. Water from the humidifier sump was transferred to 2 L measuring cylinders and the contents allowed to settle overnight. The sediment was examined under microscopy for microorganisms. Swabs were taken of the evaporative media to determine if there were any spores from the previous experiments.

Comparison of the deposition of microorganisms from natural inoculum in stores with atomiser and screen humidification systems.

Two clean 12-tonne experimental stores used. One store had a Munters humidifier screen installed adjacent to the plenum at a height of approximately 1 m, The other store had an ultrasonic nozzle humidifier in place (control store). To keep both stores as similar as possible, the humidity of the control store was set to run at 10°C, 95% RH. At each sampling occasion, six Petri dishes containing PDA were placed at predetermined positions throughout each store (Appendix 2). Background samples were taken prior to running the fans. At each sampling occasion, the lids of each Petri dish was removed for 30 minutes, after which time the lids were replaced, then plates incubated at 18°C. After approximately 3 days, any colonies that grew were identified as fungal or bacterial and each counted. At the start of the experiment, 200g of store dust was collected from the experimental unit and was used to represent natural inoculum. The dust was divided into two, 100g aliquot. Each aliquote of dust was

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distributed into each experimental store via a pipe, using compressed air to push the inoculum into the plenum airflow into the store. After inoculum delivery into the air, plates were put out and the air sampled for 30 minutes. The air was sampled at least daily, over a period of 17 days.

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Results

Method development

Air brush optimisation

The relationship between spray volume and droplet size is presented in Figure 1. The finest spray was a combination of 30 PSI using a distance of 9.5mm between air nozzle suspension delivery pipe. This pressure was used for dispersing spores for the duration of the trial.

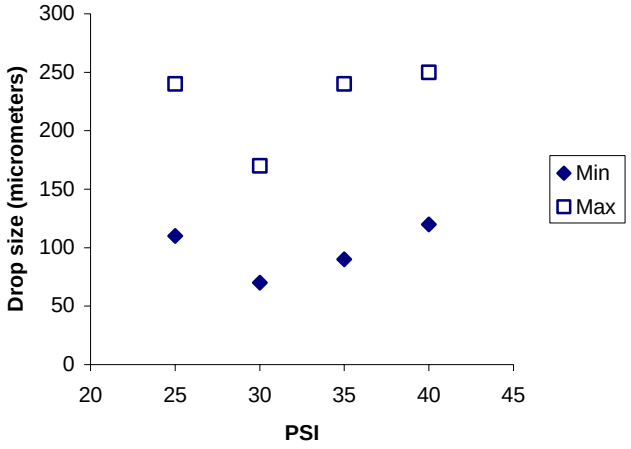

FIGURE 1 THE RELATIONSHIP BETWEEN THE SIZE OF WATER DROPLET PRODUCED BY AIRBRUSH AND AIR PRESSURE.

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Optimising the impinger

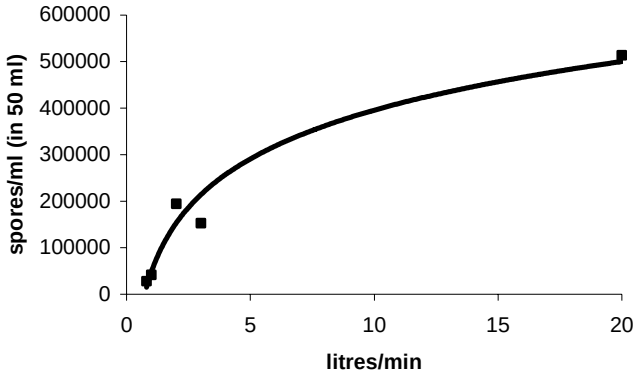

FIGURE 2 THE RELATIONSHIP BETWEEN THE NUMBER OF SPORES RECOVERED FROM THE IMPINGER AT VARYING FLOW RATES.

The relationship between the number of spores collected and the impinger pump volume approximated a negative exponential (i.e. the increase in spores collected decreases with flow volume) (Figure 2). From this it was decided to use the maximum flow rate for trapping spores using the impinger. However, recovery of spores using the method described above was unsuccessful in the 12-tonne experimental stores. Therefore, in an attempt to improve detection, the water volume was reduced so the air blew directly onto the surface of the water. This increased the capture efficiency by 50% compared to bubbling air through the water. Never-the-less, detection of spores using the impinger remained poor and it was decided to use 90 cm diameter Petri dishes containing PDA to trap airborne spores and bacteria in all further experiments.

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Munters screen humidifier: evaluating the efficiency of spore trapping using wet and dry screens

Evaluation using natural inoculum

Figure 3 shows the effectiveness of the humidification screen in removing spores from air circulating through the unit. The wet and dry screens removed 85% and 55% of spores, respectively, compared with the open humidifier (i.e. screen removed).

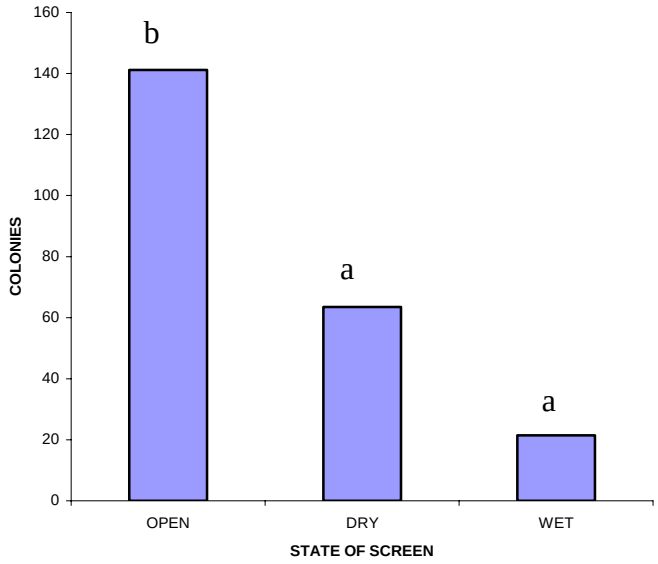

FIGURE 3 THE NUMBER OF COLONY FORMING UNITS RECOVERED ON PETRI DISHES POSITIONED BEHIND THE HUMIDIFIER UNIT WITH HUMIDIFIER SCREEN REMOVED (OPEN); SCREEN INSTALLED DRY; AND SCREEN INSTALLED WET (I.E. NORMAL OPERATION). $LSD_{(P=0.05)}$ = 49 (14 df). Different letters above the columns indicate a significant difference at $P<0.05$.

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Evaluation using *Fusarium sulphureum*

Figure 4 shows the effectiveness of the humidification screen in removing *Fusarium sulphureum* micro- and macro- conidia from air circulating through the unit. The wet and dry screens each removed 99% of conidia compared with the open humidifier (i.e. screen removed).

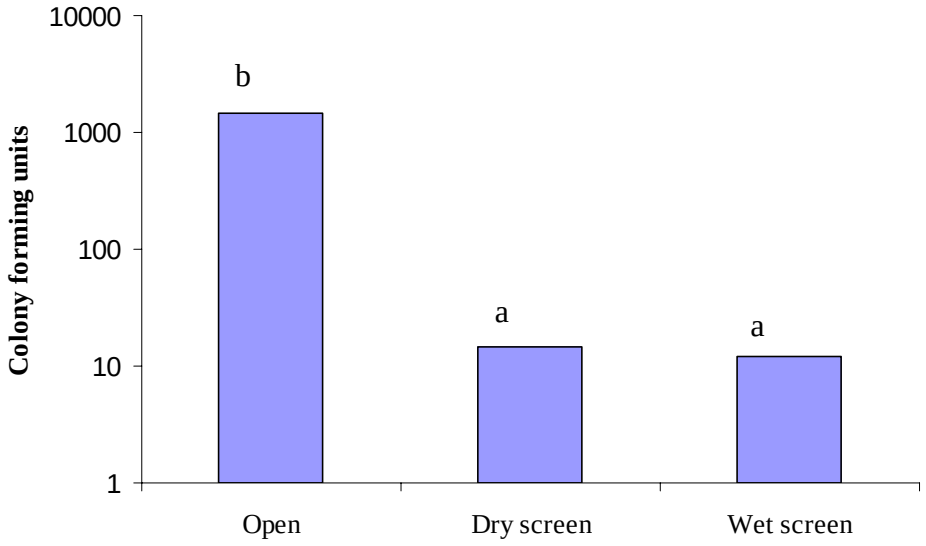

FIGURE 4 THE LOG NUMBER OF COLONY FORMING UNITS OF *FUSARIUM SULPHUREUM* RECOVERED ON PETRI DISHES POSITIONED BEHIND THE HUMIDIFIER UNIT WITH HUMIDIFIER SCREEN REMOVED (OPEN); SCREEN INSTALLED DRY; AND SCREEN INSTALLED WET (I.E. NORMAL OPERATION). $LSD_{(P=0.05)}$ = 911 (14 df). Different letters above the columns indicate a significant difference at $P<0.05$.

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Comparison of the deposition of artificially inoculated Polyscytalum pustulans *conidia in stores with atomiser and screen humidification systems.*

Following inoculation of store air by *P. pustulans* conidia, there was a sudden decrease in conidia numbers from the initial inoculation of the stores within the first 24 hours (Figure 5). The spore deposition remained at a low level for the duration of the sampling period. The overall mean number of *P. pustulans* cfu recovered from the screen humidified store was slightly lower than in the atomised store (P=0.067). However, this was due to the large difference in initial *P. pustulans* detected within minutes of the spores being introduced (P=0.004).

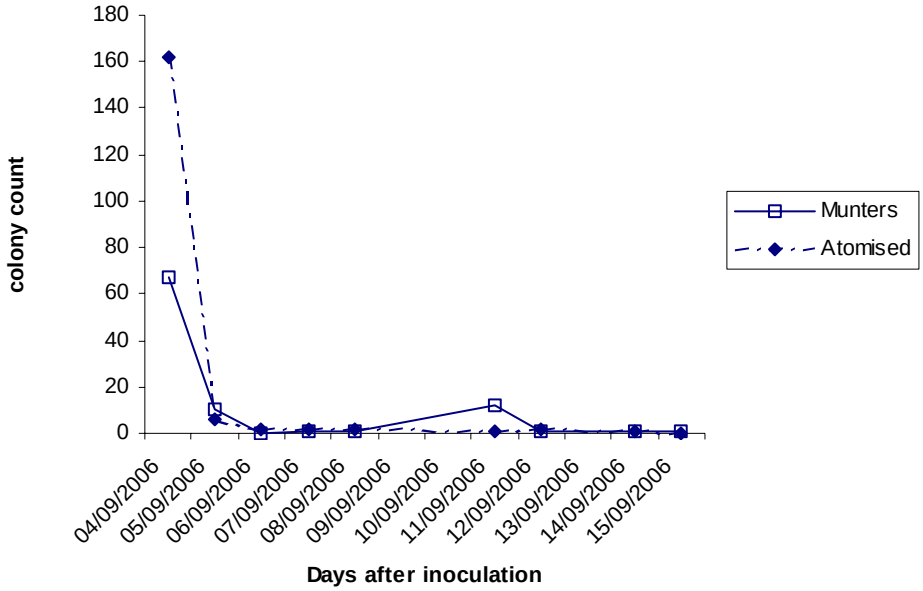

FIGURE 5 A COMPARISON OF THE HUMIDIFIER STORE AND THE CONTROL STORE WITH THE NUMBER OF COLONIES GROWING ON P1/4+ OVER TIME.

Comparison of the deposition of micro-organisms from natural inoculum in stores with atomiser and screen humidification systems.

The RH in Store 28 (with the Munters HM2 4000 evaporative humidifier) and Store 29 (with ultrasonic nozzle humidification) was maintained at approximately 95% throughout the duration of the 17-day trial (Figure 6). This was the target RH for each store.

Figure 7 shows a sharp decrease in fungal colony forming units (cfu) recovered from air in experimental potato stores within 24 hours of natural contamination being introduced. Hours after introducing dust into two stores (one with an atomiser humidification system, the other with a screen humidification system), the levels of fungal cfu for both stores remained low. The overall mean number of fungal cfu

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recovered from both stores were the same (P=0.515). However, there was a slight interaction between spore deposition and time after inoculation (P=0.073) where spore deposition was slightly higher in the store with atomised humidification, compared with that in the store with the humidity screen installed, just after the introduction of dust, and at one sampling point 15 days after inoculation. Note, there were no systematic differences in spore deposition between locations of Petri dishes placed around the stores (P=895).

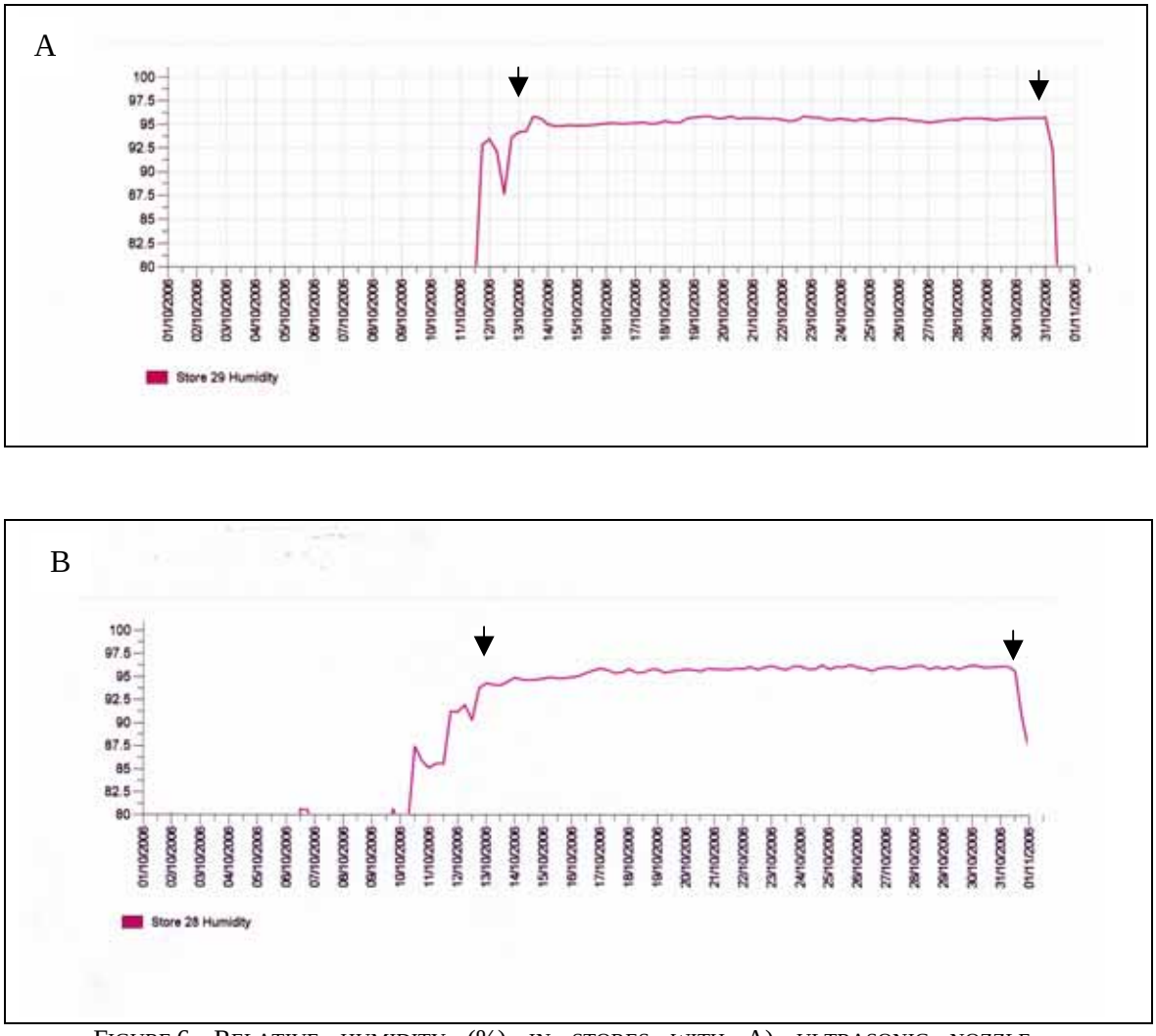

FIGURE 6 RELATIVE HUMIDITY (%) IN STORES WITH A) ULTRASONIC NOZZLE HUMIDIFIER; AND B) MUNTERS HM2 4000 EVAPORATIVE HUMIDIFIER.

The arrows indicate the start and end of the monitoring exercise, when the store doors were closed.

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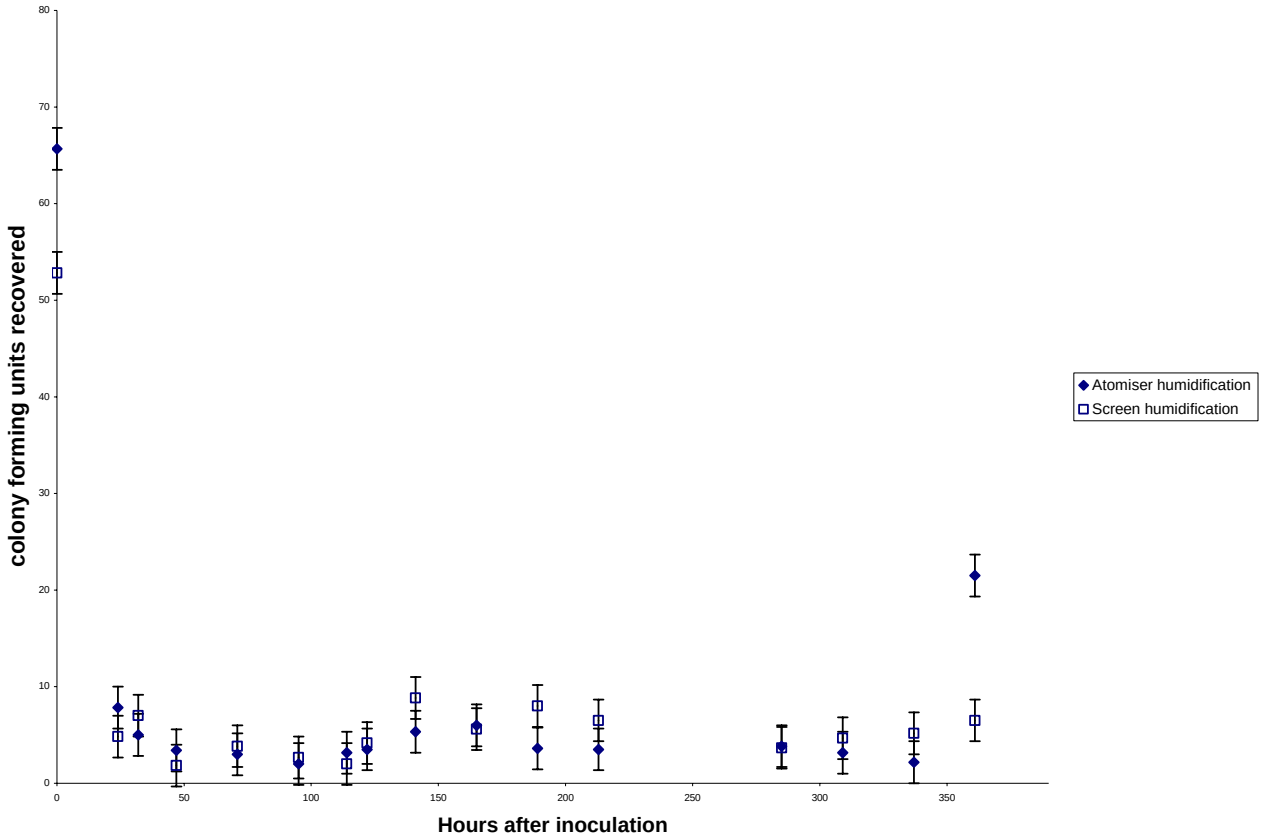

FIGURE 7 NUMBER OF FUNGAL COLONY FORMING UNITS RECOVERED OVER A 15 DAY PERIOD FROM TWO EXPERIMENTAL STORES (ONE, WITH ATOMISER HUMIDIFICATION; THE OTHER WITH SCREEN HUMIDIFICATION).

Stores were initially inoculated with 100g of dust collected from experimental potato stores. Error bars show the standard error of the mean data values.

Immediately after introducing dust into the two experimental stores (one with an atomiser humidification system, the other with a screen humidification system), the levels of bacterial cfu were initial low (16 and 10 cfu in the atomised and screen humidified stores respectively) (Figure 8). Subsequently, the number of bacteria recovered from air in the store with screen humidification remained low for the duration of the trial. However, the number of bacteria recovered from air in the store with atomised humidification was considerably higher that that in the atomised store ($P<0.001$) but showed a general decline over the duration of the trial. Note, there were no systematic differences in bacterial deposition between locations of Petri dishes placed around the stores ($P=0.564$).

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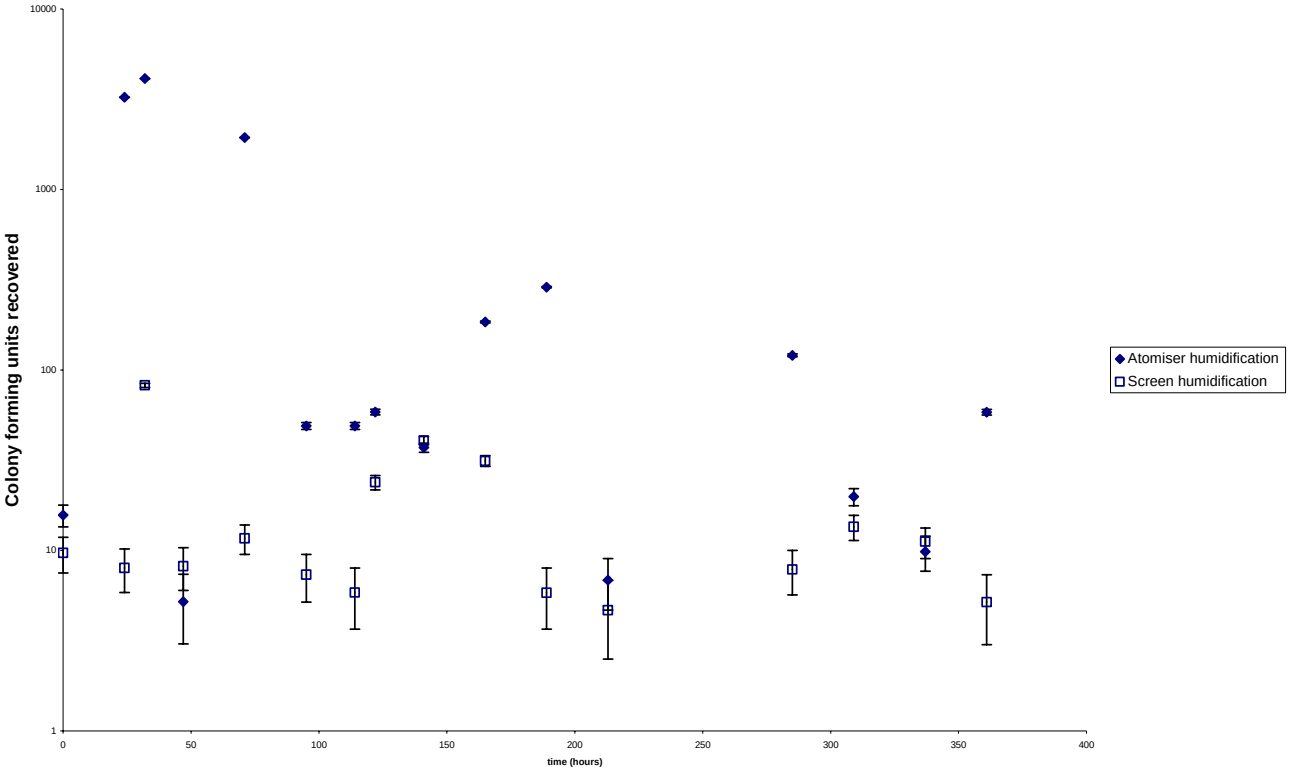

FIGURE 8 NUMBER OF BACTERIAL COLONY FORMING UNITS RECOVERED OVER A 15 DAY PERIOD FROM TWO EXPERIMENTAL STORES (ONE WITH ATOMISER HUMIDIFICATION; THE OTHER WITH SCREEN HUMIDIFICATION).

Stores were initially inoculated with 100g of dust collected from experimental potato stores. Error bars show the standard error of the mean data values.

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Discussion

Both the Munters HM2 4000 evaporative humidifier and the ultrasonic nozzle humidifier were able to maintain empty 12-tonne experimental stores at the target humidity of 95% RH for the duration of a 17-day monitoring trial. Of course, the primary purpose of humidification systems is to maintain crops at the desired humidity in order to prevent tuber weight loss and pressure bruising. Both the evaporative and ultrasonic humidifiers performed equally well in terms of their RH control under the test conditions used in the trial presented in this report.

The elucidation of fungal spores and bacteria in store air proved to be difficult. One method, using an air sampler or impinger, was not sensitive enough to detect microorganisms at the level found in potato store atmospheres. Typically, spores of airborne pathogens, for example, *Helminthosporium solani* (silver scurf) was found at approximately 0 to 24,000 conidia per day (Rodriguez *et al*, 1996). The method tested during the work reported here, using an impinger, was able to detect spores at around 0.3 to 5.1% efficiency using hydrostatic pump flow rates of 0.8 to 20 L/min respectively. In practice, the impinger running at 20 L/min was not able to satisfactorily detect fungal spores in air whilst the Munters humidifier was in operation. This would be due to the overwhelming effect of the integrated fan on the Munters unit that has a far higher flow rate that the impinger pump. Consequently, the impinger was unable to sample air quickly enough to draw in spores into the collection vessel. It was therefore decided that leaving Petri dishes containing PDA open for a standard duration offered a more reliable measure of spore concentration than sampling air using an impinger.

It was demonstrated that the Munters humidity cell was able to trap conidia that were present naturally in store air, as well as *Fusarium sulphureum* conidia that had been sprayed directly into the airflow of the humidity cell. The trapping efficiency of the cell, relative to having no cell in place, was independent on the cell wetness: a fully wet cell was 99% efficient in trapping spores of *Fusarium sulphureum*; a completely dry cell was similarly 99% efficient. However, in normal operation, continuous water irrigation of the humidification cell is likely to flush fungal spores and dust particles into the water outlet reservoir thus aiding the filtration of particulates from the air (R. Andrews, Crop Systems Ltd, personal communication). Comparing wet and dry cells could be regarded as academic because evaporative humidification systems will only work for their intended purpose if the cells are kept wet. However, the data show that the physical presence of the cell – wet or dry – has a major filtering effect rather than the presence of water *per se* within the cell.

Conidia of *P. pustulans* disappeared from store air within a few hours of being introduced into the plenum air recirculation duct of 12-tonne experimental controlled environment stores at SBEU. It is the opinion of the authors that the store infrastructure (fan coil, plenum surface etc) attracts and traps spores, either through electrostatic or hydrostatic attraction. Natural inoculum in store dust also disappeared within hours of being introduced, probably because the relatively large particles settle out of air quickly. The Munters humidity screen and a conventional ultrasonic atomiser humidification system were compared for their ability to remove airborne

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fungi and bacteria in 12-tonne experimental stores. The pattern of spore deposition in stores with the Munters and atomiser systems were similar: the overall mean number of fungal colony forming units (in dust) or as conidia introduced via atomised suspension were the same in both stores irrespective of the humidification system used. However, bacterial contamination was higher in sampled store air when humidified using an atomised system that with an evaporative humidification screen. It is likely that the atomised water was itself the source of contamination. No attempt was made to identify the bacteria although it is improbable that these microorganisms were pathogenic to potato. There is, however, a chance that weak human pathogens could enter the water system. It is therefore advisable to undertake risk assessments when operating potato stores fitted with humidification and, on the basis of these findings, atomised systems in particular.

In summary:

1) An evaporative humidifier and ultrasonic nozzle humidifier maintained 12-tonne experimental stores at the target humidity of 95% RH.
2) The number of fungal colony forming units detected in recirculating air in stores was the same irrespective of whether an evaporative humidifier or ultrasonic nozzle humidifier had been in operation.
3) The number of bacterial colony forming units detected in recirculating air in stores was much higher in the store where an ultrasonic nozzle humidifier had been in operation compared with a store with an evaporative humidifier.

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References

Eltawil M, Samuel D, and Singhal O, 2006. Potato Storage Technology and Store Design Aspects. Agricultural Engineering International: the CIGR Ejournal. Invited Overview No. 11. Vol. VIII. April, 2006.

Lightheart B, Prier K, Bromenshenk J, 2005. Detection of aerosolized bacterial spores (*Bacillus atrophaeus*) using free-flying honey bees (Hymenoptera: Apidae) as collectors.

Rodriguez DA, Secor GA, Gudmestad NC, Francl LJ, 1996. Sporulation of *Helminthosporium solani* and infection of potato tubers in seed and commercial storages. *Plant Disease* **80**: 1063-1070.

Small D, Pahl K, 2003. Potato Storage management. In. *Guide to Commercial Potato Production on the Canadian Prairies* published by the Western Potato Council, Canada.

Sparks WC, 1973. Influence of ventilation and humidity during storage on weight and quality changes of Russet Burbank potatoes. *Potato Research* **16**: 213-223

Tyler M.E, and Shipe E.L, 1959. Bacterial Aerosol Samplers. I. Development and Evaluation of the All-Glass Impinger. *Applied Microbiology* **7**: 337-349

Varns, J.L, Schaper L.A, and Preston D.A, 1985. Potatoes losses during the first three months of storage for processing. American Potato Journal **62**: 91-99.

Wiltshire J, Milne F, Peters J, 2004. *Improving the understanding of skin set and bloom in potatoes.* Final project report to the BPC, Oxford.

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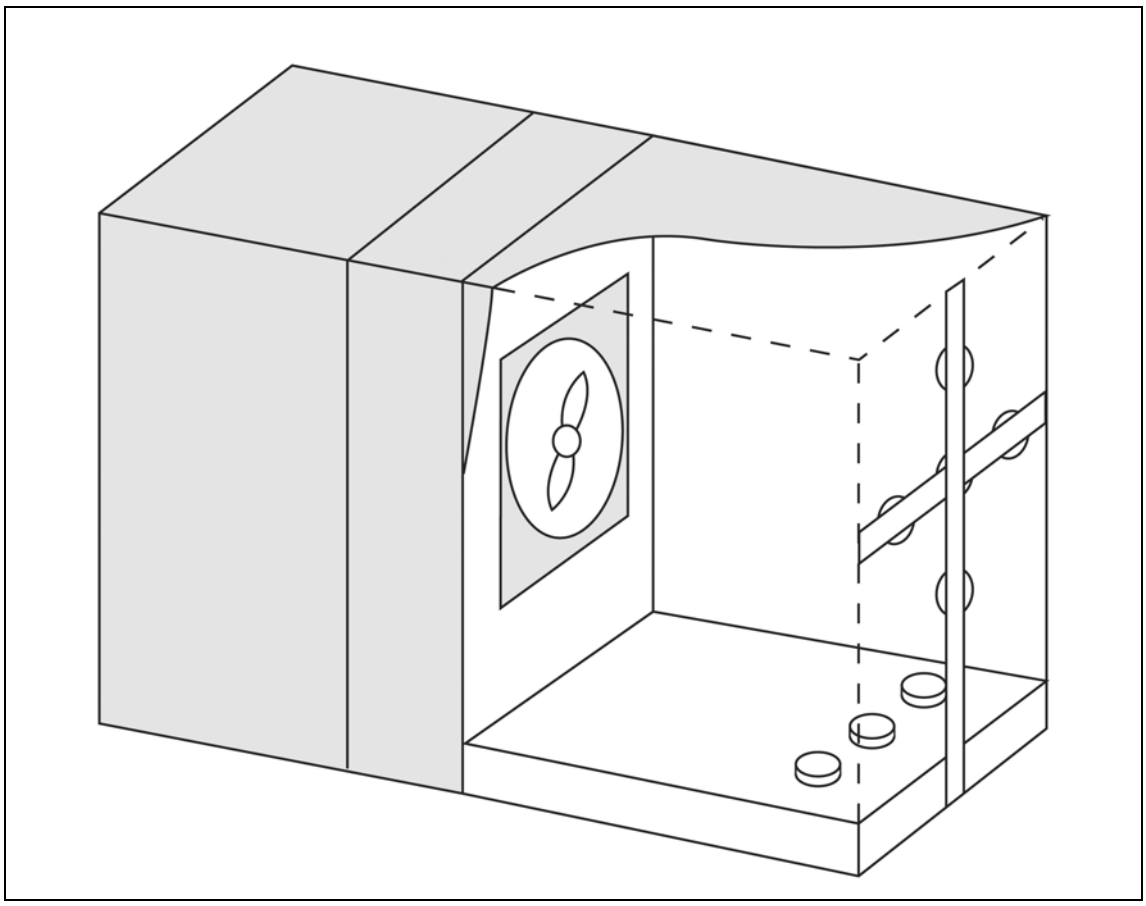
Appendix 1

Image of Munters HM2 4000 evaporative humidifier with frame holding Petri dishes at the rear of the unit.



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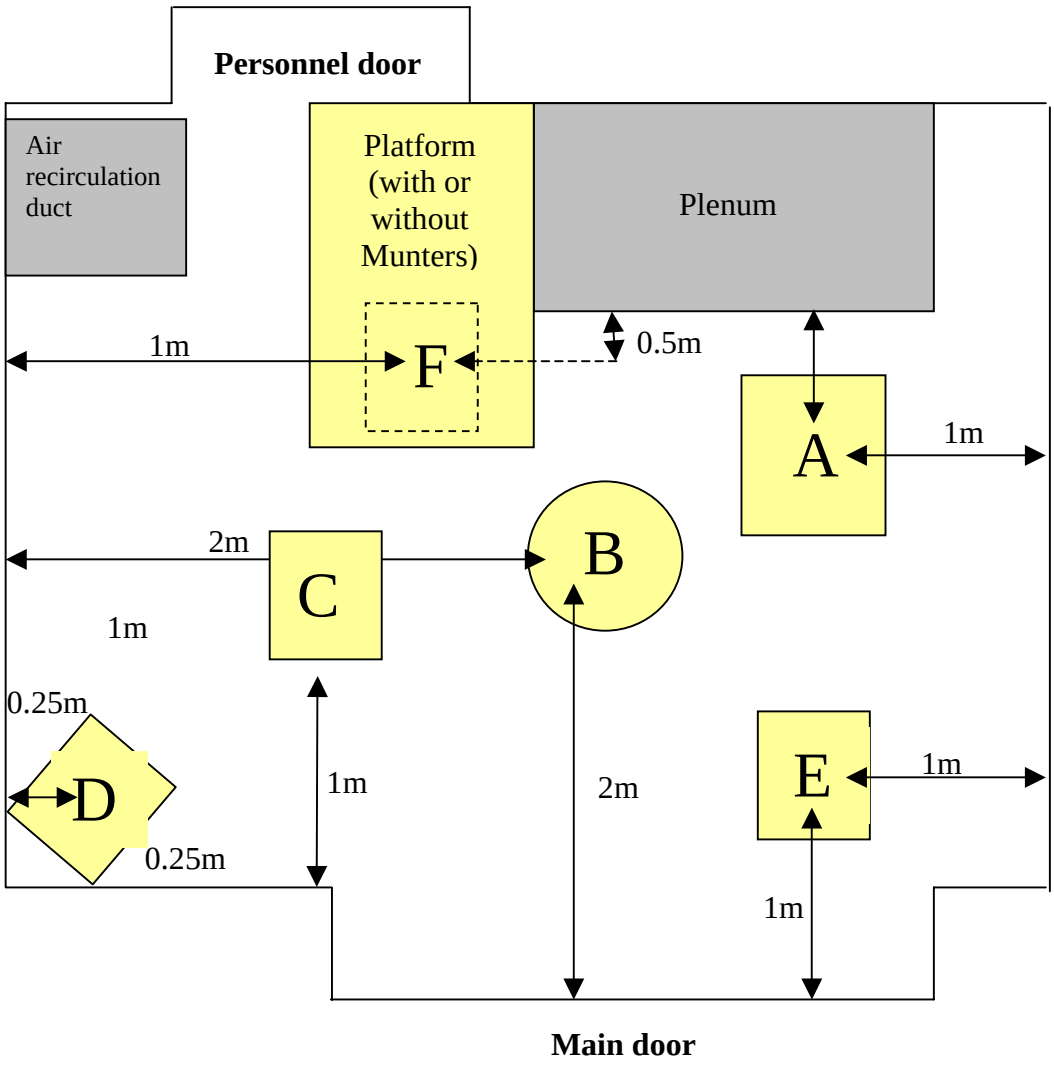
Appendix 2

Plan of store showing location of Petri dishes (A to F).



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