Session 12B

Post-harvest Biology and Storage Technology

Chairman & Session Organiser:	Dr Peter Spencer-Phillips *University of the West of England, Bristol, UK*
Platform Papers:	12B-1 to 12B-4
Poster Presentations:	P12B-5 to P12B-10

Recent practical advances in post-harvest storage

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Post-harvest low temperature management remains the fundamental method for maintaining fresh produce quality. However, temperature abuse during the cold chain is common. Delays between harvest and cooling significantly hasten fruit deterioration. Low temperature management is often complemented with selective gaseous atmosphere storage. Controlled atmosphere (CA) and modified atmosphere packaging (MAP) usually imply elevation of CO_2 and/or reduction of O_2 concentration. Proper low temperature storage and management of relative humidity (RH) should always be pre-requisites to CA and/or MAP. In contrast to CA, MAP relies on passive alteration of gaseous concentrations within packaging or within shrouded pallets, and thus is a function of fruit respiration rate versus packaging permeability. Incorrect CA regimes can increase the production of off-flavours and cause discoloration. In addition, it has long been recognised that although CA can increase storage life through decreasing respiration rate of both the fruit product and disease-causing pathogens, it can in fact reduce shelf-life when fruit are removed from a CA environment and transferred to air.

Recently, the effect of the transition between CA and regular atmosphere storage (and *vice versa*) was studied on onion bulbs (Chope *et al.*, 2007a). Removal of bulbs from CA storage (5 kPa CO_2, 3 kPa O_2) resulted in an immediate increase in the respiration rate (measured in air), which then reverted to a lower rate following subsequent storage under air conditions for 21 days. In some cultivars, this could be sufficient to trigger the onset of sprouting and thus account for the detrimental effect of CA storage on shelf-life. Increased respiration was correlated to a decreased concentration of non-structural carbohydrates. Delaying the start of CA storage of onion cv. SS1 bulbs for 21 days was as effective in suppressing sprout growth as CA storage for 42 days. Further investigation into the use of CA storage in this manner, in relation to the optimum time to begin CA conditions, could decrease the cost of CA storage without compromising storage life.

Another important method to extend storage life of fresh produce is centered around the suppression of ethylene. The removal of ethylene and/or inhibition of the effect of ethylene in stored environments is fundamental to maintaining post-harvest quality of climacteric produce. In recent years, however, there has been a paucity of research on developing new and more efficacious ethylene scrubbing materials. In contrast, there has been an exponential increase in research using the ethylene binding inhibitor 1-methylcyclopropene (1-MCP; Watkins, 2006). 1-MCP has been shown to extend the post-harvest life of both climacteric (Watkins, 2006) and non-climacteric (Chope *et al.*, 2007b) fresh produce. Despite various ethylene scrubbing technologies being available (e.g. high temperature catalytic degradation, activated carbon, etc.), most commercial ethylene control systems rely on both adequate ventilation (often periodic) and oxidation of ethylene using potassium permanganate ($KMnO_4$)-based mechanisms. Ventilation, however, is not appropriate in sealed environments (e.g. controlled atmosphere or some packaging formats) or where precise ethylene control is required. $KMnO_4$ supported on activated alumina spheres has limited long-term efficacy in environments with high relative humidity (e.g. cold stores).

Recently, a palladium (Pd)-promoted powdered material that has significant ethylene adsorption capacity (4162 μL g^{-1} material) at 20°C and approximately 100% RH was identified. This was shown to be far superior to $KMnO_4$-based scavengers when used in low amounts and in conditions of high RH (Terry *et al.*, 2007).

Initial screening was carried out in a plug flow reactor with 200 μL L^{-1} ethylene, 10% (v/v) O_2 balanced with He at approximately 100% RH. Further work demonstrated that the Pd-promoted material at either 0.01 or 0.03 g L^{-1} effectively scavenged both exogenously administered (100 μL L^{-1}) and/or endogenously produced ethylene when used for only three days on pre-climacteric banana and avocado fruit, respectively, to sub- μL L^{-1} concentrations within a 24 h period. The efficacy of Pd-promoted material was far superior to $KMnO_4$ when used in low amounts and especially at high RH%. Optimum ethylene adsorption capacity was calculated as approximately 10,000 μL g^{-1}. Accordingly, corresponding inhibition of ethylene-induced ripening was observed. When removed, Pd-material did not disrupt subsequent ripening. The results from this initial work demonstrated that Pd-promoted material has the potential to be used commercially. Future work will elucidate the optimum concentrations, timing of application and format to extend post-harvest life of avocado and other climacteric fresh produce types. If optimised, there remains the possibility of the Pd-promoted material being used to extend shelf-life of climacteric fruit even when the climacteric respiratory rise has been just initiated, as demonstrated for avocado cv. Hass fruit (Terry *et al.*, 2007).

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Chlorophyll fluorescence imaging as a tool to detect abiotic and biotic stresses in plants and to evaluate the physiological state of agricultural and horticultural products

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Several non-destructive optical techniques, such as chlorophyll fluorescence imaging, have been developed in the last decade in order to obtain information about the physiological status of plants and fruit and to study the impact of abiotic and biotic stresses (Valcke, 2003). Chlorophyll fluorescence is emitted predominantly by the photosystem II complex of the photosynthetic apparatus. After illumination of a dark or light-adapted sample, a typical fluorescent transient will appear. The shape of the fluorescent transient will be determined by the physiological status of the sample. A decrease in the variable fluorescence, defined as $Fv = (F_M-F_0)$, or in the photochemical efficiency of photosystem II, defined as $\varphi_{P0} = (F_V/F_M)$, indicates stress induced damage in plants (Strasser *et al.*, 2004).

Recently, camera based systems for chlorophyll fluorescence imaging have been developed. Using very sensitive CCD-cameras, it is now possible to measure fluorescence of samples with lower chlorophyll content, such as ripening fruit (Ciscato, 2000). This technique has already been used in the domain of agriculture and horticulture, to evaluate apple quality at harvest and after storage (Huybrechts & Valcke, 2003; Codrea *et al.*, 2002), for classification of apples according to storage potential which was associated with pre-harvest nitrogen and calcium treatments (Ciscato *et al.*, 2000), for studying the effect of heavy metals on the photosynthetic apparatus (Ciscato *et al.*, 1999) and to follow the endogenous spread of *Erwinia amylovora*, the causal agent of fire blight (Heyens *et al.*, 2002).

The progressive spread of a virus can be followed in asymptomatic leaves using a combination of tissue printing and two-dimensional fluorescence quenching analysis (Pérez-Bueno *et al.*, 2006). Chlorophyll fluorescence imaging has further been used to estimate the effect of ozone on leaf senescence (Gielen *et al.*, 2007). Combining fluorescence imaging with thermal imaging, complementary information obtained from both techniques is exploited to get a better insight of the way a plant responds to pathogen attack (Chaerle *et al.*, 2004).

The fluorescence imaging systems used are custom made. The fixed system is composed of an excitation unit, an imaging unit and a control unit. Chlorophyll fluorescence is detected by a CCD camera fitted with a red cut-off filter. A portable system contains blue LEDs as excitation source, and a filter wheel system in front of the camera. The excitation unit illuminates the surface of the plant material over a larger area with two different light intensities, actinic ($I = 600$ µmol $m^{-2}s^{-1}$) and saturating ($I = 200$ µmol $m^{-2}s^{-1}$). The system takes either 2 images or a sequence of 33, 8-bit images. In the latter case, it selects the maximal image in each sequence after illumination.

The image processing consisted of three steps:

(i) subtraction of the dark signal in order to correct for inherent camera properties;

(ii) correction for differences in excitation light intensity across the camera field and

(iii) elimination of the effect of the geometric features when fruit is measured by applying a masking procedure to minimise the curvature effect of the fruit.

After correction of the images a false colour, X-rain.LUT, can be applied to enhance visualisation, highlighting details of the fluorescence image. The raw images are analysed using ImageJ-tools and the histograms of the pixel intensity distribution are compared, using statistical techniques.

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Sensor system for detection of post-harvest spoilage of stored potato tubers

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A sensor system for the early detection of *Pectobacterium* (previously known as *Erwinia*) soft rot in potato tubers has been developed recently (de Lacy Costello *et al.*, 2002), and assessed in potato store trials to provide 'proof of principle' evidence for its applicability as a disease detection technology. The system operates by the automated collection of air samples from selected areas of the store, and passing the air to an electronic nose (e-nose) containing a set of ceramic sensors selected for their sensitivity to marker volatile organic compounds (VOCs). The resulting data are captured, displayed and recorded by computer. The basic principles of the technology are introduced, and some aspects of the in-store trials presented. Conclusions are drawn about the potential application of this approach for detecting post-harvest spoilage of bulk plant produce, where advance warning of early stages of disease provides the opportunity either to change store conditions to control the problem, or to advise on quality and marketing strategy.

For the in-store detection of *Pectobacterium* soft rot, the e-nose described by de Lacy Costello *et al.* (2000 & 2002) was connected to an automated sampling unit consisting of 16 solenoid valves mounted on a manifold, where each valve inlet could be connected to a selected VOC source (e.g. a 1 tonne box of potato tubers). One of the valves (purge valve) was used to provide a constant flow of clean air through the system whenever samples were not being drawn through one of the other valves. The valves were switched using a relay card mounted in a personal computer (PC), and flow rate, flow temperature and flow humidity sensors within the sampling unit were connected via the parallel (printer) port of the PC. Air was drawn through the system continuously using a modified aquarium air pump. The entire system was protected from electrical power fluctuations by an Uniterruptible Power Supply (UPS, American Power Conversion UK Ltd).

Bespoke software was written to: control the operation of the solenoid valves; gather data from the sensors within the e-nose; gather flow rate, temperature and humidity data from within the sampling unit; and display and log the data. The program was set up to run four six-hour data collection periods in each 24-hour period, and data were collected continuously for 57 and 71 days in two trial periods at the Sutton Bridge Experimental Unit, UK. Data files were written to disc at regular intervals and sent by e-mail for remote analysis. In order to sample air in 1 tonne potato boxes, polyurethane tubes were fitted with Millipore filters (11 µm pore size) to prevent particulate and insect ingress. The filtered ends were placed in the boxes (one in each of 12 boxes of cv. Maris Piper for trial 1, and two in each of 6 boxes of cv. Estima in trial 2) and the other end connected to a valve inlet of the sampling unit. At the end of the trial periods, each box was emptied manually and any rotten tubers were collected for assessment of the cause and amount of infected tissue.

The in-store trials to detect *Pectobacterium* infections proved that the system (e-nose, sampling unit and tubes, software and PC, UPS) worked continuously throughout the trial periods, with 64 samples of air per day taken and analysed remotely with minimal human supervision. The sensing system correctly identified the boxes containing the greatest amount of soft rot when outward signs (visual appearance and odour) of infection were non-existent. The amount of soft-rot detected in these boxes was well below the 1% crisis level, with the results from the 12-box trial being better defined than those for the 6-box trial, implying that the system would work best in large controlled ventilation box stores with sensors monitoring sectors of the store rather than individual boxes. Some temporary and unusually high sensor outputs were explained by other activities in adjacent stores, such as use of the sprouting suppressant chlorpropham (CIPC) in methanol, during the day. Thus in future the sampling protocol should be modified to: a) acquire only one cycle of samples per 24-hour period, preferably overnight so that interference from human activities would be minimised; b) extend the purging (sensor recovery) period between each sampling.

The system has not been tested in a store where tubers are stored in bulk rather than in boxes, there is no controlled ventilation and a significant outbreak of soft rot is in progress. Such trials are desirable. The prototype device has proved itself to be effective and reliable, but more investment is needed if a robust, user-friendly sensor system is required for general use in potato stores by unskilled staff. There is also much potential for variants to be used for other bulk plant produce, as already demonstrated for wheat grain (de Lacy Costello *et al.*, 2003).

Recent work has focused on methods for distinguishing the metabolomic profiles of the most abundant VOCs generated by brown rot and ring rot infections of potato tubers. This technology would be applicable to the detection of other statutory organisms, with a portable device having further uses such as detecting organisms causing biodeterioration of construction materials (Ewen *et al.*, 2004) and in the diagnosis of human disease (Guernion *et al.*, 2001).

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Effect of nitrogen on bulb rot incidence in onion during storage

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Bulb rot is the common cause of losses in stored onion in all onion growing areas in Luzon. Though losses often appear in storage, infection usually begins in the field after heavy rains before harvest, contributing to as much as 30-40% in loss. At present, it appears that there are a number of different species of fungi and other secondary invaders that can cause this disease even in storage, resulting in overlapping of symptoms that are sometimes difficult to categorise. When onions are still in the field, growers normally apply excessive amounts of fertiliser, especially nitrogen, to produce large, heavy and succulent bulbs without considering the vulnerability of onion to infection by rot-causing pathogens. Observations in the field indicated a very high incidence of bulb rots, normally occurring after two months of storage, on those onions heavily fertilised with nitrogen, suggesting that high N application plays a role in the occurrence of bulb rot in storage. This study was conducted to (a) determine the influence of field applied nitrogen on bulb rot incidence during storage, and (b) to determine the different species of bulb rotting pathogens during storage of two varieties of onion.

The study consisted of two phases, the pre-harvest and the post-harvest phase. The former was conducted in the field in Talavera, Nueva Ecija, while the latter was in a cold storage house in Cabanatuan City. Two varieties of onion, Red Creole and Yellow Granex, were used as main treatments, and four nitrogen levels, 0 N (control), 50 kg N/ha, 100 kg N/ha, and 200 kg N/ha, were applied as sub-treatments in a 2 x 4 factorial in a Randomised Complete Block Design with three replications. Half of the N was broadcasted and incorporated in the soil after final harrowing, and the second half was applied at bulb initiation. Seeds of Yellow Granex were sown in elevated seedbeds while seedlings of Red Creole were transplanted in the prepared plots 43 days after emergence.

The timing of harvest was determined using the combination of the following maturity indices recorded at 120 days after sowing of seeds, namely: softening of the neck, topping of the leaves and change of leaf colour. After harvesting, the leaves were trimmed to 2 cm above the bulb. Fifteen bulbs from each treatment were evaluated for texture, hardness, total soluble solid and titritable acidity (% TA). The bulbs were placed in net bags (20 kg capacity) and stored at either room temperature (27°C) or in cold storage at 0°C. Seven replicate bags were used per N treatment per storage condition for Yellow Granex, and three for Red Creole. Treatments were arranged in a 2 x 4 x 2 factorial in a Complete Randomised Design in the storage rooms.

Bulb rot incidence was recorded weekly for six months. Diseased bulbs detected were collected and studied for the presence of rot causing pathogens. Koch's postulates were followed to test the pathogenicity of the isolated microorganisms.

The onion variety Yellow Granex proved to have a higher yield than Red Creole. However, Yellow Granex was not responsive to N treatment, with the highest yield obtained from the treatment with no N added to the soil. Application of 50 to 100 kg N/ha in Red Creole increased yield over the control, but increasing N level to 200 kg/ha resulted in a marked decrease in yield. Bulbs of Yellow Granex were firmer and harder, but higher total soluble solids were noted in Red Creole, whilst both varieties showed comparable titritable acidity. N levels had no influence on these parameters.

At room temperature storage, the incidence of bulb rot was higher and the progress was faster in Yellow Granex than in Red Creole, with 100% of Yellow Granex rotting within four weeks of storage at all levels of N. Red Creole, on the otherhand, lasted up to the 11th week of storage with very low incidence of bulb rot except on bulbs from fertilised treatments, especially at 200 kg/h which had a higher bulb rot incidence than control bulbs.

Cold temperature storage effectively retarded the incidence of bulb rot in both varieties, although Yellow Granex still showed vulnerability at all levels of N application, especially during the second and third week but at a much lower level (0.03% to 0.08%). It was also at this stage that bulbs treated with 200 kg/ha N showed increased susceptibility to the disease. Red Creole was more resistant to rot-causing pathogens during cold storage, with incidence of bulb rot only noticed during the second week of storage but not subsequently.

Aspergillus flavus was the most predominant bulb-rot causing pathogen in Red Creole at room temperature storage. It was detected in 75-85 % of onion bulbs in all the treatments, especially on the controls and bulbs applied with 50 to 100 kg N/ha. Other pathogens detected at lower levels were *Aspergillus niger, Burkholderia cepacia* and *Fusarium* spp. They all occurred in bulbs applied with 200 kg N/ha. The same pathogens were also observed in Yellow Granex in all levels of N application but more frequently than in Red Creole. *A. niger* was the most prominent, especially in bulbs applied with 50 kg N/ha, followed by controls and bulbs applied with 100 kg N/ha. The three other rot pathogens were at their most frequent in the bulbs applied with 200 kg N/ha.

In cold storage, only *Fusarium* spp. and *B. cepacia* were associated with rotting of Yellow Granex, and no rot pathogens was observed in Red Creole.

Control of post-harvest fruit rot in strawberry and apricot by *Metschnikowia pulcherrima*

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Significant progress has been made in developing potential biological alternatives to synthetic fungicides for the control of post-harvest decay and toxin producing fungi of fruits and vegetables. Biological control of post-harvest diseases of fruits with antagonistic bacteria and yeasts has emerged as the most effective alternative to fungicides during the past few years. The initial products BioSave and Aspire have been registered by the United States Environmental Protection Agency (EPA). BioSave 110 (EcoScience Corp., Orlando, FL, USA), containing a saprotrophic strain of *Pseudomonas syringae* van Hall, has been used commercially on pome fruit in the United States since 1996. Aspire (Ecogen, Langhorne, PA, USA) contains the yeast *Candida oleophila* Montrocher, and has been used for control of diseases of apple, pear and citrus fruit. Although these pioneering products are effective, they have limitations with regard to spectrum of activity and efficacy under various environmental conditions and with different fruits.

The ascomycete yeast *Metschnikowia pulcherrima* Pitt & Miller was reported to control *Botrytis cinerea* on apple, and microbial decay of peach and table grapes after harvest (Janisiewicz & Jeffers, 1997; Karabulut *et al.*, 2003). *M. pulcherrima* has been recognized as one of the resident species on cider apple trees (Janisiewicz *et al.*, 2001). It has been isolated frequently from all floral parts and from buds of apple, and has been one of the most frequently isolated microorganisms from wounded apple tissue in an orchard (Anghel *et al.*, 1984). It is also a dominant species on mature grapes and on grapevine flowers (Anghel *et al.*, 1984).

The objective of the work presented here was to evaluate post-harvest applications of the biocontrol agent *M. pulcherrima*, strains L_{28} and L_{29}, to control rot caused by *Botrytis cinerea* on strawberry and *Monilinia* spp. on apricot fruit. This approach has the potential to control strawberry and apricot post-harvest diseases without the need for additional harvest and post-harvest handling procedures, and also to eliminate the need for chemical treatment.

In order to prevent the infection of strawberries with *B. cinerea*, three pre-harvest treatments with *M. pulcherrima* strains L_{28} and L_{29}, alone and in combination (L_{28} + L_{29}) were performed. These treatments significantly reduced the number of decayed fruits of strawberry cvs Favette, Hood, Cardinal and Pandora, and were comparable in efficacy to

chemical control with the fungicide tiofanat metil (0.07% Topsin M-70).

Some reports have demonstrated that a direct relationship exists between the population density of an antagonist and the efficacy of a post-harvest biological control treatment (Sesan *et al.*, 1999). Results presented here showed that the antagonistic activity of *M. pulcherrima* was dependent on its population size. A density of 2 x 10^6 colony forming units (CFU)/ml did not provide a satisfactory level of control, while 6 x 10^6 CFU/ml limited rot development by about 86.8% on Favette, 90.2% on Hood, 89.1% on Cardinal and 86.2% on Pandora strawberry fruits. Application of the two yeast strains in combination allowed the conservation of the fruits in very good condition for more than 10 days at 4°C. This could be due to the ability of the yeast strains used in these experiments to grow at low temperatures.

In order to prevent the infection of apricot fruits by *Monilinia* spp., three treatments with the selected *M. pulcherrima* strains were performed as with strawberry fruits. Post-harvest treatments with strains L_{28}, L_{29}, and $L_{28} + L_{29}$ significantly reduced the number of decayed apricot fruit of cv Dacia even 20 days after treatment, compared to untreated controls. The yeast also suppressed post-harvest incidence of fruit rot significantly better than the fungicide Topsin.

These preliminary tests indicate the new strains of *M. pulcherrima* have biocontrol activity against *Botrytis* rot and *Monilinia* rot of stored strawberry and apricot. *M. pulcherrima* reduced the incidence of fruit rot by 95% and 100%, respectively, in a commercial storehouse at 4°C. The population density of *M. pulcherrima* was approximately 2×10^6 CFU/fruit at 20 days after treatment.

This study has demonstrated that pre-harvest applications of the biological agent *M. pulcherrima* significantly controlled post-harvest diseases of strawberry and apricot fruits. Its effectiveness could be attributed to the persistence of yeast cells on fruit surfaces under post-harvest storage conditions.

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Post-harvest tuber treatment with fenugreek seed and lufenuron as protectants against the potato tuber moth (Lepidoptera: Gelechiidae)

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The potato tuber moth (PTM) *Phthorimaea operculella* (Zeller) is a major pest of stored potatoes worldwide. *Trigonella foenum-graecum* (L.), commonly known as fenugreek, is widely cultivated as an annual legume in Mediterranean countries. Previous investigations have shown that fenugreek seed powder was effective as a feeding deterrent or repellent against insect pests of stored products. No research information is available on the use of fenugreek seed powder against PTM. Lufenuron is a chitin synthesis inhibitor and acts after ingestion against immature stages of Lepidoptera. Edomwande *et al.* (2000) reported that tubers newly treated with lufenuron were highly toxic to PTM first instars larvae, but lufenuron residual activity during potato storage period has not been determined. The ability of powdered *Trigonella* seed, its methanolic extract, or lufenuron to control PTM infestation in an unrefrigerated potato storage building is reported here.

Freshly harvested healthy potato tubers were: (1) sprinkled with powdered *Trigonella* seed at a dose of 10% (w/w); (2) sprayed to runoff with fenugreek methanolic seed extract aqueous solution, emulsified with Tween 20 (0.5%), at either 0.1 or 0.2% (vol:vol); or (3) sprayed with lufenuron at 0.12 g active ingredient (AI)/l water. Fertile PTM moths (4 pairs) were released in wooden cages (45x25x25 cm, covered with 1 mm mesh nylon gauze) over the treated or untreated tubers. Within 72 h after moth release, the tubers were withdrawn from the cages and the number of F_1 emerged adults was recorded.

The residual activities of *Trigonella* seed extract and lufenuron were also assessed. Potato tubers were sprayed thoroughly with (1) *Trigonella* seed extract aqueous solution, (2) lufenuron insecticide, or (3) water (control). After drying, the tubers were stored in the dark at room temperature. Four pairs of fertile moths were introduced to the cages at 0, 7, 14, 21, 28, 35, 45, or 60 days after *Trigonella* and lufenuron applications, and the number of F_1 adults recorded as before.

Results indicated that fenugreek seed powder and its methanol extracts were significant oviposition deterrents for *P. operculella*, and that almost no adult emergence was detected from lufenuron-treated tubers (Table 1). Rizk *et al.* (2001) examined the antifeeding activity of several plants on PTM larval mortality and found that the lowest percentage of mortality was obtained for potato tubers spread with fenugreek seed powder.

This indicates that fenugreek seed powder or its methanol extract possess repellent properties or oviposition deterrence/inhibitor activity (compounds detected by moth's ovipositor as a signal to reduce egg laying) against the *P. operculella* adults rather than larval antifeeding properties. Lufenuron insecticide severely affected the emergence of PTM F_1 adults and accordingly provided a high level of protection of tubers from *P. operculella* attack. This finding is consistent with a previous study which showed that lufenuron has excellent activity against newly-hatched PTM larvae in the laboratory (Edomwande *et al.*, 2000).

Table 1. Mean no. of potato tuber moth F1 adults emerged from tubers treated with fenugreek powdered seed, fenugreek methanolic seed extract (0.1 or 0.2% concentrations) or lufenuron and untreated (control) tubers

Treatments	Mean no. of F1-emerged adults	% effectiveness
Fenugreek powdered seed 10% w/w	15.8c	79.4b
Fenugreek methanolic seed extract 0.1 %	23.4b	68.7c
Fenugreek methanolic seed extract 0.2 %	16.4c	78.7b
Lufenuron 0.12 g [a.i.]/l	0.3d	99.6a
Control	76.4a	0.0d

Means in columns followed by the same letter are not significantly different at $P \leq 0.05$ (Fisher PLSD).

The residual activities of lufenuron and fenugreek seed extract reduced significantly PTM attack for up to 45 days (Table 2). Lufenuron at 0.12 g (a.i.) gave almost 100% protection during 45 days of storage and reduced the mean number of F_1-emerged moths from 81.9 (in control) to 15.2 after 60 days. *Trigonella* seed extract at 0.2% remained effective and significantly reduced infestation over the period tested.

Table 2. Mean no. of potato tuber moth F1 adults emerged from tubers treated with fenugreek methanolic seed extract (0.2% concentration) or lufenuron and untreated tubers over 60 days after treatment

Days after treatments	Mean no. of F1-emerged adults		
	Fenugreek methanolic seed extract at 0.2 %	Lufenuron 0.12 g [AI]/ l	Untreated tubers
7	17.6a	0.0a	86.1a
14	18.6a	0.2a	96.1a
21	19.9a	0.4a	89.8a
28	21.4a	0.4a	91.5a
35	20.6a	1.7a	86.6a
45	19.9a	3.2a	79.2a
60	38.2b	15.2b	81.9a

Means in columns followed by the same letter are not significantly different at $P \leq 0.05$ (Fisher PLSD).

A trap plant strategy, that integrates chemical, physical and biological control, using fenugreek seed powder *vs* lufenuron could be an efficient methodology for managing PTM infestation under storage conditions. Moreover, the potential of this approach could be enhanced by using a 'push-pull' strategy. This would employ the application of an antioviposition agent to the crop (pushing component, fenugreek-treated tubers) and then killing the insects on the trap plant by the application of an insecticide (pulling component, lufenuron-treated tubers).

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Protection of apple fruits from post-harvest spoilage by fungi

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Apple occupies the third position, in term of growing areas, after olive and grape vine in Syria. Losses of apple fruits are increasing due to infection by different post-harvest diseases in chilled and non-chilled stores. Fungicides have been used to control post-harvest diseases of some fruits (Al-Rahma, 1993; Kumar *et al.*, 1996; Valdebenito Sanhueza, 1993). Dipping apple fruits with benomyl or prochloraz before storage reduced post-harvest diseases in Morocco (Besri, 1988), and apple fruits also have been treated with diphenylamine and thiabendazole (Sanderson & Bennet, 1999). Iprodione (dicarboximide)-wax/oil mixtures have been used to control post-harvest diseases on apple and pear fruits (Adaskaveg *et al.*, 1993; Ogawa *et al.*, 1993).

Biocontrol agents have also been used to control post-harvest diseases. For example, the yeast *Candida sake* (strain CPA-1) was found to be particularly effective against *Penicillium expansum*, *Botrytis cinerea* and *Rhizopus nigricans* on apples (Vinas *et al.*, 1996). Similarly, strains of the bacterium *Pseudomonas syringae* (L59-66) mixed with L-proline and L-aspargine have been used to control *P. expansum* on apples (Janisiewicz & Peterson, 1993).

The objective of the present research was to find efficient and safe methods to prevent post-harvest disease of apples in Syria, in order to prevent or reduce the losses as much as possible. The apple varieties Golden Delicious and Starking Delicious were used in all experiments, and fruits were stored in chilled and non-chilled stores. All experiments were conducted at both the Al-Swaida and the Surghaia research stations in Syria.

The effect of spraying apple trees with fungicides was assessed by spraying trees one month before harvest with one of the following fungicides: benomyl 50% (375 g a.i./ha), thiophanate methyl 70% (682.5 g a.i./ha) and iprodione 50% (750 g a.i./ha). Controls were either sprayed with water or left unsprayed. All the fungicides significantly reduced post-harvest decay under chilled conditions at the two locations. In non-chilled stores, thiophanate methyl significantly reduced post-harvest decay at the Al-Swaida station, whereas spraying with water significantly reduced decay at Surghaia station.

The effect of dipping fruits in fungicides before storage was assessed by treatment with the following fungicides: benomyl 50% (2.5 g a.i./10 l water), thiophanate methyl 70% (4.55 g a.i./10 l water) and iprodione 50% (5 g a.i./10 l water). Control fruits were either sprayed with water or left dry. Fruits dipped in iprodione, benomyl and in water had significantly reduced post-harvest decay under chilled conditions at both the Surghaia and Al-Swaida stations. However, dipping fruits in water also significantly reduced post-harvest decay under non-chilled conditions at the Surghaia station.

The effect of dipping re-used apple storage boxes in formalin was assessed by treating plastic, polystyrene, wood and cardboard boxes in formalin (1% v/v). Control boxes were dipped in water. This experiment was conducted only in chilled stores, and the formalin treatment significantly reduced post-harvest decay at both the Al-Swaida and Surghaia stations.

The effect of treating apple fruits after harvest with either the bacteria *Shewanella putrefaciens* and *Citrobacter* spp., or a yeast (strain D) was assessed by dipping fruits in a suspension of the organism. Fruits were also dipped in benomyl or wax oil for comparison, and controls were either dipped in water or left dry. Fruits treated with water, benomyl or *Citrobacter* spp. had significantly reduced post-harvest decay under chilled conditions at both locations. In contrast, dipping fruits in water significantly reduced decay compared with the yeast, *Citrobacter* spp. and *Shewanella putrefaciens* under non-chilled conditions at Surghaia station.

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Insecticidal properties of *Eugenia aromatica* against the pulse beetle *Callosobruchus maculatus* on cowpea seed

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The use of plant extracts in pest control has been gaining attention due to the attendant problems associated with the use of synthetic insecticides. There is also an increasing awareness that plants possess chemicals which naturally protect them from pests and pathogens. Current research efforts on product development are being focused more on ecologically tolerable control measures including the use of inert materials, and powders, oils and extracts from plants. Ofuya & Dawodu (2002) reported the insecticidal properties of *Piper guineense* in the control of the pulse beetle *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), and extensive work on oviposition deterrence and ovicidal properties of some plant powders against *C. maculatus* was documented by Ofuya (1990). The use of *Eugenia aromatica* (Baill.) powders has also been found suitable for the control of insect pests.

The demand for cowpea, *Vigna unguiculata* (L.) Walp., in Nigeria is considerable. Its seeds contain high amounts of protein and B vitamins, and help to prevent starvation among low resource farmers and poor urban populations. A substantial part of world cowpea production comes from Nigeria, with about four million hectares and approximately 1.7 million tonnes produced annually. Profitable production of cowpea is limited significantly by the attack of *C. maculatus* during storage (Ofuya, 2001), and over 90% of post-harvest spoilage is caused by this insect. Although the degree of damage in the field is usually as low as 2%, this still leads to problems during the nine month storage period. Since severe damage caused by this pest lowers the quality and quantity of cowpea seeds available for consumption, and because of the problems associated with use of synthetic insecticides, an alternative option of a naturally available, cheap and non-toxic plant extract is sought. The use of *E. aromatica* on different cowpea varieties was studied to determine its efficacy in protecting seeds against *C. maculatus*, and the varietal resistance of different cowpea varieties assessed.

C. maculatus was obtained from infested cowpea seed from the market, and cultured in the laboratory in a Kilner jar at 28ºC ±3 and a relative humidity of 70 ± 5%. Seven common local varieties of cowpea, Ife Brown, Oloyin, Erusu, Ife Bimpe, Drum, Ilo and Kaura, were used as substrate. The identity of the varieties was ascertained at the International Institute of Tropical Agriculture (IITA), Ibadan. Dry flower buds of *E. aromatica* were purchased locally, and then oven dried to a constant weight at 40ºC. The dried buds were pulverised in a kitchen mill, and the resulting powder was sieved to a particle size of ≤300 *µm*. The prepared powder was placed in a plastic container with a tightly fitted lid, and stored at ambient temperature. The powder of *E. aromatica* was tested at 0.4 g of material per 20 g of each seed of the different varieties in separate plastic Petri plates (8.5 cm diameter). Control seeds had no plant powder applied. Ten pairs of males and females (twenty individuals) of *C. maculates*, aged one to two days old, were introduced into each plate.

Adult mortality was monitored over 48 h, and thereafter all insects were removed. The numbers of eggs laid by the female beetles on the seeds were counted. The number of adults that emerged from these eggs was counted from three weeks after introducing the beetle pairs to the seeds. Each treatment including the control was replicated three times. The natural resistance of each cowpea variety was observed in the untreated controls. There were three replicates of all experiments. Egg count data were subjected to square root transformation, and percentage adult mortality and adult emergence data were arcsine transformed, before being subjected to analysis of variance (ANOVA).

The protective capability of *E. aromatica*, which killed all adult beetles within 48 h, as well as the responses of each variety to infestation when no protectant was applied is shown in Table 1. The highest resistance to infestation was observed in varieties Erusu, Ife Bimpe and Ilo, and the data show that a significantly higher number of adults emerged from the least resistant varieties. Varietal resistance of different crops to insect attack has also been reported in the field, as well as in storage for a number of different crops.

Table 1. Response of *Callosobruchus maculatus* introduced to seeds of cowpea, with and without *Eugenia aromatica* treatment.

Cowpea variety	% adult mortality at 48 h after treatment applied	No. of eggs laid when no treatment applied	% adult emergence when no treatment applied
Ife Brown	100 ± 0.0^{a}	4.1 ± 1.2^{b}	15.5 ± 1.3^{b}
Oloyin	100 ± 0.0^{a}	4.4 ± 1.5^{b}	18.2 ± 1.4^{b}
Erusu	100 ± 0.0^{a}	3.0 ± 1.0^{a}	8.2 ± 1.0^{a}
Ife Bimpe	100 ± 0.0^{a}	3.4 ± 1.1^{a}	10.5 ± 1.5^{a}
Drum	100 ± 0.0^{a}	5.6 ± 1.0^{b}	28.5 ± 2.1^{d}
Ilo	100 ± 0.0^{a}	3.0 ± 1.4^{a}	9.2 ± 1.2^{a}
Kaura	100 ± 0.0^{a}	4.7 ± 1.2^{b}	22.5 ± 1.2^{c}

Means in each column bearing the same letter are not significantly different at the 5 % level of probability (Tukey's test)

These results corroborate reports that *E. aromatica* powders are effective against *C. maculatus*, and that this treatment provides an effective alternative to synthetic insecticides. Good packaging and storage have long been known to be vital in maintaining the activity of chemical insecticides, and the longevity of the *E. aromatica* treatment in stored seed should now be studied.

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Fungicide efficacy and residues in control of post-harvest spoilage of garlic

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Garlic is one of the most important seasoning vegetables, and about 32,000 ha are cultivated in Korea. Most of the harvested garlic is stored as bulbs in low or ambient temperature, because it is consumed raw and used for vegetative propagation. During storage 33-55% loss occurs (Lee, 1981), due mainly to microbial disease and insect pests. The fungi *Penicillium hirsutum*, *Fusarium oxysporum* and *Stemphyllium botryosum* alone cause 5-50% loss (Kim *et al.*, 2003). In order to diminish the damage due to these fungi, fungicides known to give excellent control during four months of storage, after applying at harvest, were selected. Here we report the results of experiments designed to assess their relative ability to reduce post-harvest spoilage using the methods of Vernière *et al.* (2003).

For the determination of *in vitro* antimicrobial activity, cell fragments and spores harvested from 5 day-old cultures were mixed with 15 ml of PDA in a Petri dish to give a density of 10^5 cells/ml. Paper discs of 0.8 mm diameter were placed on the seeded agar and immediately 8 µl of the fungicide solution, at its recommended optimal concentration, were applied. The inhibition zone was measured five days after incubation at room temperature (RT, ca. 26^0C). The fungicides diphenylamine, prochloraz and tebuconazole gave 0.3, 2.2, and 1.3 mm inhibition zones, respectively, for *F. oxysporum*. Cyprodinil, diphenylamine, fenbuconazole, hexaconazole, penconazole, prochloraz, propiconazole, pyrimethalin and tebuconazole gave 0.2, 2.4, 0.8, 0.4, 1.2, 1.5, 1.2, 0.4 and 1.5 mm inhibition zones, repectively, with *P. hirsutum*.

To test the *in vivo* control effect, garlic cloves of varieties Danyang and Namdo were wounded with a toothpick. The wounded cloves were dipped in each fungicide solution for 10 min and dried at RT before inoculation of each wound site with 5 µl of 10^5 cells/ml of *F. oxysporum* and *P. hirsutum*. When phenylamine, prochloraz and tebuconazole were applied at optimal concentration, the mycelium of *F. oxysporum* started to grow at five, seven and five days after inoculation, respectively, and 80%, 63.3% and 83.3% of the inoculated cloves were infected at 11 days after inoculation. When diphenylamine, prochloraz and tebuconazole were applied at five times their optimal concentrations, the mycelium started to grow at seven, 11 and seven days post-inoculation, respectively, and 10.4%, 23.3% and 60% of cloves were infected by 11 days.

For *P. hirsutum*, tebuconazole applied at its optimal concentration completely inhibited infection. When diphenylamine, penconazole and propiconazole were applied at optimal concentration, *P. hirsutum* infection was observed seven days after inoculation, and 20-23.3% of the cloves were infected by 11 days. With cyprodinil, prochloraz and pyrimethalin, infection occurred five days after inoculation, with 60-100% of the cloves infected by 11 days. Treatment with five times optimal concentration of propiconazole, pyrimethalin and tebuconazole inhibited growth of *P. hirsutum* completely, with 3.3% of cloves infected following penconazole treatment, and 3.3-66.7% infected after cyprodinil, diphenylamine and prochloraz treatment at 11 days after inoculation.

In a separate experiment, garlic was treated with fungicides before and after harvesting, and then the harvested bulbs were stored in plastic nets in the greenhouse. The amount of infection was assessed every month for four months. When mixtures of either diphenylamine and dimethoate, or tebuconazole and dimethoate, were applied to the leaves of garlic before harvesting, 11.05-23.22% and 9.38-47.87%, respectively, of bulbs of Danyang, and 13.54-19.38% and 15.32-21.77%, respectively, of Namdo became infected. This compared with 27.14-52.82% of Danyang and 24.52-35.18% of Namdo infected in non-treated controls. When either the diphenylamine and dimethoate mixture or the tebuconazole and dimethoate mixture were applied to harvested bulbs by dipping, 25.85-56.86% and 19.01-38.19% of the treated bulbs of Danyang, and 8.82-12.63% and 5.32-12.70% of Namdo, respectively, became infected.

The residues of diphenylamine, tebuconazole and dimethoate within treated bulbs was analysed every month from harvesting time. Diphenylamine was extracted with water/acetonitril (90/10, v/v) and the extracted solution was refined by passage through Extrelut-NT 20 cartridges (diatomaceous earth material of high pore volume). The solution was analysed by HPLC with a fluorescence detector (Saad *et al.*, 2004). Tebuconazole and dimethoate were extracted with water/acetone (70:30, v/v), and the extract partitioned against dichloromethane. It was refined with SPE-florisil cartridges and analysed by gas chromatography. The residual amount of agrochemical in garlic treated before harvesting was different depending on the pesticide. Residual amounts of tebuconazole exceeded the maximum residue level (MRL; 0.1 mg/kg) set for domestic consumption. The residual amounts of dimethoate and diphenylamine were below the MRL for all treatments. The MRL (for apple) of tebuconazole, dimethoate and diphenylamine is 0.1, 1 and 5 mg/kg, respectively.

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Plant secondary metabolites for protection of stored pulse grain from the pest *Callosobruchus chinensis* (L)

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The adzuki bean weevil, *Callosobruchus chinensis* (L.), is the most widespread and destructive primary insect pest of stored grain legumes. This pest often causes extensive quantitative and qualitative losses of stored grain legumes in India, and microbial spoilage causes further loss. As grain legumes are important and economical sources of proteins in Indian vegetarian diets, control of their pests is needed. Minimisation of pest-associated losses is one of the practical means to increase yield and availability of grain legumes. Synthetic pesticides have been found to have hazardous effects on human health, non-target organisms and the environment. They also disrupt biological control by natural enemies and have led to outbreaks of other insect species, and sometimes resulted in the development of resistance to pesticides. These problems have highlighted the need for the development of selective insect-control alternatives of biological origin. Plants are potentially good alternatives in this regard as they are rich in many bioactive chemicals.

Secondary metabolites have been extracted, isolated and characterised from locally available plants of *Annona squamosa, Calotropis procera* and *Acorus calamus*, and investigated for their efficacy to control *C. chinensis*. The isolated secondary metabolites were characterised by chromatographic (TLC, HPTLC) and spectroscopic (IR, NMR and mass spectroscopy) analysis. The secondary metabolites were identified to belong to the flavonoid group of compounds in leaves of *A. squamosa* and *C. procera*, as well as in rhizomes of *A. calamus*. Terpenoids and long chain fatty alcohols were present in *A. squamosa* seeds. These secondary metabolites were found to be toxic to adults of *C. chinensis* depending on dose and exposure period. They also reduced oviposition, and showed ovicidal, larvicidal and development inhibitory effects by affecting the number and weight of emerging adults as a function of concentration. They also showed very promising activity against microbial contaminants of grain legumes during storage. These metabolites showed a synergistic effect when combined together, and affected survival of *C. chinensis*. Pure flavonoid standards gave comparable results to the secondary metabolites extracted from plants.

Studies on the biochemical basis behind efficacy of these metabolites suggested that they can affect various enzymes needed for survival, growth and development of *C. chinensis*. They particularly affected the activities of: (i) dietary enzymes like amylase, protease and lipase; (ii) nervous system enzymes like acetylcholine esterase; (iii) antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase; (iv) development related enzymes like ecdysone-20-monooxygenase. The effect on levels of detoxifying antioxidant enzymes and generation of free radical species suggest irreversible cellular damage. Lipid peroxidation decreases membrane fluidity, increases leakiness of the membrane and inactivates membrane bound proteins. It has also been demonstrated that superoxide anion radicals can cause damage to membrane bound proteins, membrane lysis, injury to many other organelles and lipid peroxidation.

The plant secondary metabolites may also be localised in the alimentary canal of the beetle, causing gastric irritation. The secondary metabolites were found to inhibit esterases, and therefore might be affecting the physiology and nervous systems of the beetle. Ecdysone-20- monoxygenase, an important enzyme in the moulting process, was also inhibited by these secondary metabolites. A reduced chitin content of the emerging insects at sublethal doses suggests that the secondary metabolites might be affecting development processes of the beetle.

We hypothesise that the plant secondary metabolites have multiple modes of action, and the exact mechanism of their activity needs further investigation. The results suggest that plant secondary metabolites have significant effects on metamorphic and reproductive related events in the beetle. There appears to be a relationship between oxidative stress and membrane damage, physiology and nervous system, digestion, ecdysteroid titers and oviposition levels, but further experiments are needed to clarify the mechanisms of action and interaction in these crucial life cycle events.

The end user formulation and a strategy for post-harvest grain protection was successfully tried using the plant secondary metabolites. Efficacy trials of the formulation, carried out on mung beans for a year, showed no adverse effect on nutritional parameters and germination of grains compared to untreated control grains.

These studies suggest that plant secondary metabolites can act as potential grain protectants via contact, oviposition deterrent, ovicidal, larvicidal and development inhibiting modes of action at various stages of growth and behaviour of progeny of *C. chinensis*. They also have antimicrobial activity against microbial spoilage organisms. The mode of action and mechanism of efficacy of the secondary metabolites lies in their chemical structure and their effect on the biochemical function of insect and microbial systems, through inhibition of important enzymes needed for survival of these organisms. As the plant secondary metabolites are able to provide dual effects by controlling insect as well as microbial pests during storage, their application can be a useful and sustainable strategy for the protection of grains from post-harvest spoilage.

Information on mode of action and structure activity relationships would be useful for the design of synthetic analogues of these natural and biorational insecticides. Thus the present work not only provides a simple, reliable, cost-effective and eco-friendly strategy affording protection to pulses during storage, but also secondary metabolites (flavonoids and terpenoids) that have potential to be included in future insecticide development programmes.