Newsletter 2015
Special Issue

VolcaniVoice

Agricultural Research Organization - Volcani Center Annual E-Newsletter

FACING CHALLENGES IN POSTHARVEST FOOD LOSSES

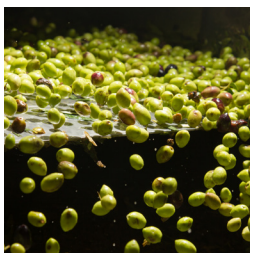
April 28-30, 2015

LOSSES • FRUITS • VEGETABLES • TRANSPORT • STORAGE • AGRO INDUSTRY
SPOILAGE • INNOVATION • HANDLING • SUPERMARKET • CONSUMERS • PACKAGING

israel agritech אגריטק 15

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Inside This Issue




Volcani Voice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Message from the Editors

There is now wide recognition that traditional approaches to improving agriculture are not adequate to meet the needs of a world in the face of climate changes and where key inputs for agriculture are rapidly being diminished. New forms of agriculture are needed that will support a wide range of environmental services, are resilient to climatic and other external shocks, and are ecologically efficient in use of resources.

To meet these challenges new sciences, new partnerships and modes of collaboration, as well as improved innovation systems will be required. Ecological efficiency must be a major consideration in future agriculture[1]. Agriculture and forestry must become net sinks of carbon and not sources[2]. Agriculture must prosper with fewer non-renewable inputs. The role of low-carbon agriculture in alleviating global climate change will require major technical innovations. The potential of biotechnology to go beyond a small number of specific traits in commodity crops, to a broader range of crops tailored for specific conditions must be studied. All of these innovations must also sustain continued increases in crop and livestock production to meet demands. No doubt, science is faced by the challenge in making a "quantum leap" to reach a level of increased agricultural productivity that will supply 9 billion with food security by 2050.

Improving the productivity and yield of crops will contribute to the increasing food demand but this avenue must be parallel to the intensive efforts at developing technologies for reduction in food losses before, during and after harvest and concomitant downstream activities including public consumption**.** Recent studies commissioned by the FAO[3] estimated yearly global quantitative food losses and waste at roughly 30% for cereals, 40-50% for root crops, fruits and vegetables, 20% for oilseeds, meat and dairy, and 30% for fish. Similar amounts are lost in developed and developing countries however, whereas in developed countries the losses are due to retailer and consumer waste, in the developing countries the losses occur during the production, harvest, postharvest and processing phases[4]. This would require managing the supply chain including: losses due to pest and diseases, post-harvest handling, storage, transportation, processing, product quality control, packaging, marketing, distributing as well as at the consumer level. To attain such an integrated management, research is required for breeding new cultivars with better resistance to extreme environments, pests and diseases, and favorable postharvest traits. In addition, research technologies are needed to reform the supply chains so as to minimize physical damages; extend commodity storage life and control postharvest pests and diseases[5].

On recognizing the crucial role of minimizing post-harvest losses, the ARO is organizing the International Conference on ***"Facing challenges in Postharvest Food Losses"*** hosted through The 19th International Agricultural Exhibition & Conference – Agritech, during April 28-30, 2015. Moreover, this special issue of Volcani Voice is dedicated to the abovementioned important challenge and represents a compilation of manuscripts on research, pursued by Volcani scientists, aimed at minimizing food losses.

References:

[1] Keating, B.A., Carberry, P.S., Bindraban, P.S., Asseng, S., Meinke, H. and Dixon, J. (2010) Eco-efficient agriculture: concepts, challenges and opportunities. *Crop Science* 50, S-109–119.

[2] Houghton R. A. (1996) Converting Terrestrial Ecosystems from Sources to Sinks of Carbon. *Ambio* 25, 267-272.

[3] Gustavsson J., Cederberg C., Sonesson U., et al. (2011) Global food losses and food waste. Extend, causes and prevention. Food and Agriculture Organization of the United Nations, Rome http://www.fao.org/fileadmin/user_upload/ags/publications/GFL

[4] Prusky, D. (2011) Reduction of the incidence of postharvest quality losses, and future prospects. Food Security 3, 463–474

[5] ARO 90 International workshop: Increasing future food production: Challenges and opportunities for the new era http://www.agri.gov.il/en/pages/929.aspx

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Estimation of postharvest losses of fruit and vegetables in Israel

By Ron Porat[1*], Tzipi Freidkin[2] and Zafrir Grinhut[3]

*[1]Department of Postharvest Science of Fresh Produce, *Email:* rporat@volcani.agri.gov.il*

[2]Research, Economy and Strategy Division, Israeli Ministry of Agriculture, Bet Dagan, Israel;

[3]Agro Ecology Department, Israeli Agriculture Extension Services, Bet Dagan, Israel.

In order to deal with the expected world food crisis, in which there will not be enough supply of food to feed the growing world population, it is necessary on the one hand to increase agricultural production and on the other hand reduce postharvest losses. Recent estimations of postharvest food losses by various world leading organizations, such as FAO, UNEP, USDA and OECD suggest that about one third of the total global amount of food production, estimated at $1 trillion per year, is lost during the postharvest food supply chain. Furthermore, postharvest losses and food waste of fruit and vegetables, which are particularly perishable commodities, are estimated to be around 45%! This study provides first estimations of postharvest food losses of major fruit and vegetable crops in Israel.

The problem of postharvest food losses has major economic, social and environmental impacts. Therefore, in order to evaluate the degree of postharvest food losses in Israel, we initiated in collaboration with the Research, Economy and Strategy Division of the Israeli Ministry of Agriculture and the Agro Ecology Department of the Israeli Agriculture Extension Services, a preliminary research aimed to estimate the amounts of postharvest food losses of 4 main fruit crops in Israel (apple, citrus, grapes and banana) and 4 main vegetables crops (tomato, cucumber, pepper and potatoes) during the postharvest food supply chain in Israel. Besides providing the necessary information concerning the situation of postharvest food losses in Israel, the achieved data may further assist in developing appropriate policies and actions needed to reduce and minimize the problem in the future.

Postharvest food losses refer to agricultural produce that for various reasons was lost at some time point during the postharvest food supply chain and thus not used for human consumption (Fig. 1). According to previous studies conducted by the FAO[1] and others, postharvest food losses may occur at five main stages: 1) at harvest, 2) in the packinghouse, 3) at the wholesale market or distribution centers, 4) in supermarkets and retail shops, and 5) at the consumers home. The latter food loss at the consumer's homes is also referred to as "food waste", and is one of the major causes for postharvest food losses in developed countries[2]. In the present study, we estimated the amounts of postharvest food losses of apple, citrus, grapes, banana, tomato, cucumber, pepper and potatoes, by interviewing the main growers, extension service officers, packinghouse operators, wholesale market and managers of logistics centers, and heads of fruit and vegetables sales departments in supermarket chains.

***Figure 1**: Postharvest food losses. The photograph was taken at the Zrifin wholesale market, 2014.*

We observed that about 3 to 11% of the fruit tested are lost at harvest due to various causes, such as rots or damage in the field or due to low quality below the required commercial standards in terms of size, shape and color (Fig. 2).

In contrast, we observed much larger amounts, 10 to 25% losses, in vegetables from the field, mostly due to economic considerations such as low prices or lack of market demand making the harvest unprofitable (Fig. 2).

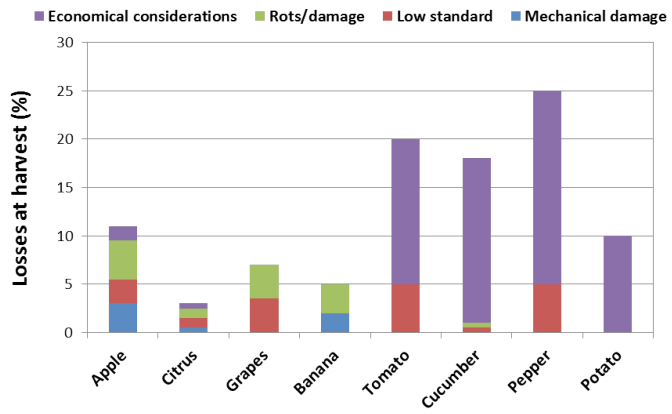


***Figure 2**: Estimates and causes of fruit and vegetable losses at harvest.*

The total estimations of postharvest food losses in Israel are provided in Fig. 3.
It can be seen that between 20 to 28% of fruit, and 28 to 46% of the vegetables were lost at some stage of the food supply chain. Noteworthy are the large amounts of losses observed for banana and fruity vegetables that occurred at the retail stage in the supermarkets (Fig. 3).

…….the total amounts of postharvest food losses and waste for some perishable commodities may even exceed 50%.

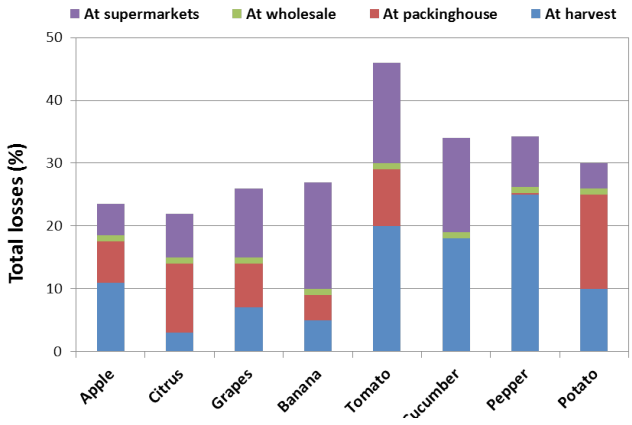


***Figure 3**: Overall estimations of postharvest losses of various fruit and vegetables during the food supply chain in Israel.*

Overall, we observed that between 20 to 46% of the total produced fruit and vegetables are lost during the first four stages of the food supply chain. In addition, it should be noted that previous studies conducted in the US and OECD countries indicated that the amounts of postharvest food waste at the consumer's homes may reach up to 20%[2]. Thus, the total amounts of postharvest food losses and waste for some perishable commodities may even exceed 50%.

These estimations indicate that as found in other countries, postharvest food losses provide a major problem also in Israel, and that it will be of value to conduct a more detailed evaluation of this issue in the future. Furthermore, together with the Research, Economy and Strategy Division of the Israeli Ministry of Agriculture it will be necessary to develop- appropriate policies and actions in order to minimize as much as possible the amounts of postharvest food losses in Israel.

References

[1] FAO. (2011). Global food losses and food waste – extent, causes and prevention, by J. Gustavsson, C. Cederberg, U. Sonesson, R. van Otterdijk & A. Meybeck. Rome (http://www.fao.org/docrep/014/mb060e/mb060e00.pdf).

[2] Buzby, J.C., Wells, H.F. & Hyman, J. (2014). The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. EIB-121, US Department of Agriculture, Economic Research Service (www.ers.usda.gov/publications/eib-economic-information-bulletin/eib-xxx.aspx).

About the first Author

Dr. Ron Porat is Head of the Department of Postharvest Science of Fresh Produce of the Institute of Postharvest and Food Sciences, ARO, The Volcani Center. His research focuses on keeping quality and improving the postharvest storage performances of various fruit crops, especially citrus, pomegranates and guava. Dr. Porat's laboratory expertizes in sensory analysis and evaluation of fruit flavor and aroma. Other research interests include evaluation of waxes, modified atmosphere packaging, plant growth regulators, and chilling tolerance. Finally, Dr. Ron Porat also deals with the key issue of food losses and waste along the postharvest marketing chain and ways to eliminate them.

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Reduction of postharvest losses of grapes depends on prevention of cracking during development and proper disinfection after harvest

Lichter Amnon*, Kaplunov Tatiana, Zuthay Yohanan, Daus Avinoam and Lurie Susan
Department of Postharvest Science of Fresh Produce,
**Email: vtlicht@agri.gov.il*

Table grapes are highly susceptible to postharvest losses due to infection by the gray mold fungus *Botrytis cinerea*. Protection of table grapes against decay during storage relies on a 'multiple hurdle approach', which entails using a combination of methods to reduce decay at critical control points. *Botrytis*, is a typical wound pathogen and the presence of cracks in the peel will enhance its potential to cause decay of table grapes. An important pre-harvest strategy to maintain table grapes during storage is to reduce the level of peel cracking before harvest. By applying the growth regulator gibberellic acid at late stages of fruit set, a significant reduction of cracking from over 40% to less than 10% was achieved. Consequently, postharvest losses were significantly reduced. Another major strategy to reduce losses after storage is to remove and disinfect the pathogens that may be present on the peel after harvest. The current commercial practice is to use SO_2 sheets which release the gas upon absorption of water during storage. However, until sufficient levels of SO_2 are established, the pathogen can enter the berries and thereby avoid exposure to the gas. We showed that application of ethanol solution after harvest reduced the level of postharvest decay. These reduced levels were comparable to application of SO_2 during storage. Our results demonstrate the importance in understanding the mode of infection, and by using a whole system approach, a significant impact in prevention of postharvest losses in table grapes can be attained.

Introduction

Grapes as a fleshy soft fruit have an inherent vulnerability to cracking. As in tomato, cracking in grapes can have a genetic propensity and physiological or horticultural triggers can induce interruptions in the peel. These form direct entry pathways for pathogens[3]. Cracks can either be long and deep and then they are termed 'macro-cracks', or small and shallow and then they are termed 'micro-cracks'. The microcracks can either transverse the cuticle, or remain superficial at the cuticle level. When cracks occur, there is a high risk of contamination by microorganisms residing on the surface. Given the presence of pathogens and the right environmental conditions the pathogens will start developing thus causing decay in the vineyard. If however, the fruit is protected by fungicides, or cracks have enough time to cure, the fruit may be saved. But there are situations where pathogens will penetrate or remain on the cracks and their growth will be arrested by natural antifungal compounds. The development of the pathogens will resume during storage when natural

defenses of the fruit gradually decline, resulting in significant postharvest losses. There are multiple ways in which we can intervene in the process one of which is to reduce the level of cracking using growth regulators that can modulate the structure of the peel during the early stages of fruit development. Gibberellic acid (GA) is widely used in seedless table grapes to complement for the lack of GA produced by the seeds and to enlarge the berries. The berry size of seeded table grape varieties is not affected by external GA[1], making them a good model to study the effect of GA on the peel. 'Zainy' is a local late ripening table grape variety with large berries, loose clusters, and high yield but with a propensity to cracking.

Another method to reduce the damage of cracking and the negative effects of opportunistic pathogens, is to disinfect the fruit after harvest. The postharvest disinfection approach by ethanol dips was developed by us together with American colleagues more than 10 years ago[2] but it has not been used commercially to-date partly because producers favor dry postharvest treatments rather than dipping, which requires an additional treatment of drying.

The objective of this paper is to bring to the attention of the audience of Volcani Voice the significance of reducing cracking in fruit and the importance of proper disinfection of the fruit surface prior to storage. These principles are true for many fruit types but the details are likely to change from system to system.

Results and Discussion

Morphological aspects of cracking

In sensitive grape varieties, cracks can often be observed on the peel (skin, exocarp) after veraison – the time point when the berries start to change color or soften and accumulate sugars. Macro-cracks are elongated cracks that can be readily observed without magnification (Fig. 1A). They can be linear and appear along the cheek, circular around the pedicel, or they can cross the bottom end of the berry. Opportunistic pathogens of the genus Alternaria or Cladosporium often use macro-cracks to initiate an infection in the vineyard (Fig. 1B) while *Botrytis cinerea* will often be the dominant pathogen after cold storage. Micro-cracks are expressed in several ways but often they are accompanied by browning due to oxidation of the injured tissue. In some cases, the cuticle along the crack can be sunken (Fig. 1C), likely because of dehydration. The same image can be observed by projecting light on it and observing the emitted light (auto-fluorescence). In the case of Fig. 1D the red background is the auto-fluorescence of the chlorophyll in the peel of the green berry and the cracks are decorated by green lining of phenolic compounds which accumulate around the crack. Clusters affected by cracking often develop browning and decay after cold storage (Fig. 1E).

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

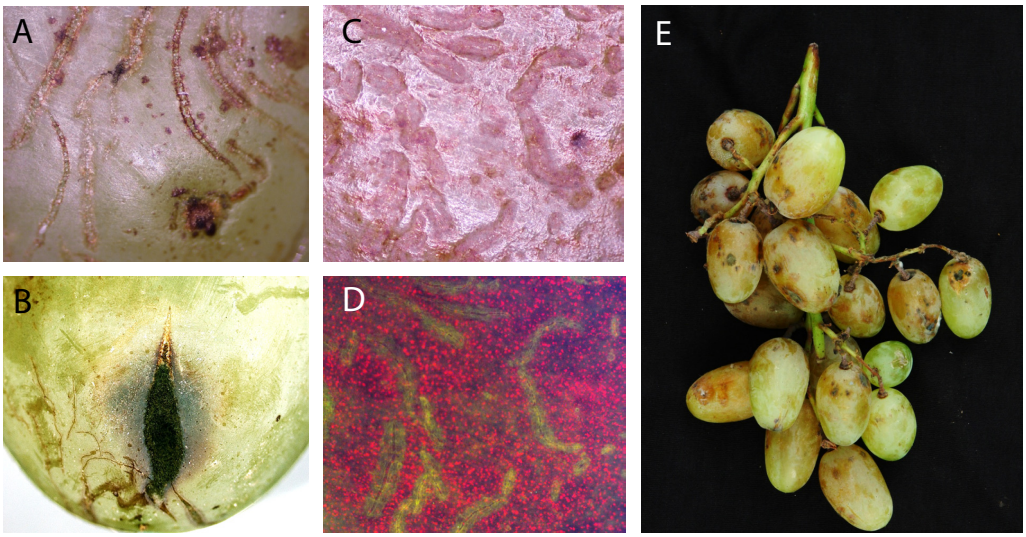


***Figure 1**: Macro and micro-cracks in 'Zainy' table grapes. A. Macro-cracks. B. Development of decay in the vineyard on a deep macro-crack. C. Surface of a berry with micro-cracks and sunken tissue along the crack. D. The same image as in C viewed by auto-fluorescence with excitation at 470 nm and emission above 500 nm. E. The appearance of 'Zainy' after storage without protection against decay.*

Reducing cracking with gibberellic acid and the consequences for decay after storage

In our studies we found that reproducible and statistically significant results for the reduction of cracking in 'Zainy' grapes were obtained through the application of gibberellic acid (Fig. 2A). GA was applied either at early fruit development stage, when berries were at diameter of 6 mm or at berry size of 8 to 10 mm. It should be emphasized that application of GA at 6 mm is the preferable stage for berry enlargement in seedless varieties. The control, untreated grapes, showed about 50% cracking, which is considered high but according to our experience with 'Zainy' not exceptional. While early treatment with GA had no significant effect, late treatment reduced decay to just above 10%, which translates to about a 4-fold reduction in cracking (Fig.2A). Dual treatment at both the early and late stages did not reduce cracking further, suggesting that the application stage is important. Application of the cytokinin, CPPU[4], did not reduce cracking significantly. We found that calcium, which is thought to reduce cracking, had no significant effect on berry cracking.

When cracks occur, there is a high risk of contamination by microorganisms residing on the surface

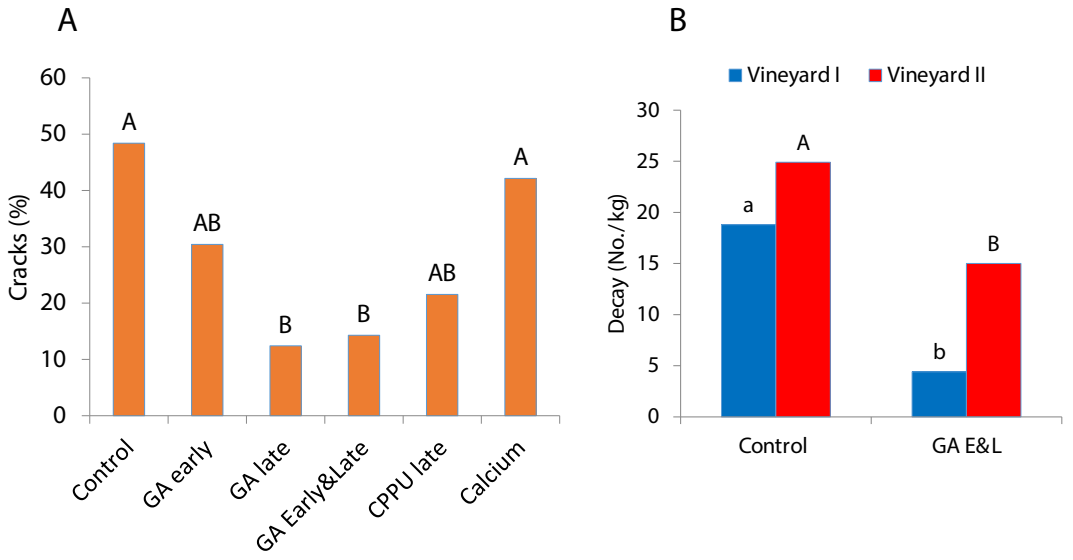


Figure 2: *Reduction of cracking and postharvest decay by gibberellic acid (GA).*
A. Application of GA (20 ppm with 0.025% triton-X100) at berry diameter of 6 mm (early), or 10 mm (late), or at both stages. The cytokinin CPPU (2 ppm with triton) was applied at 10 mm berry diameter. Calcium was applied 3 times at a rate of 0.4% as calnit.
B. Dual GA treatment at early and late fruit set (E&L) and control untreated fruit were stored at 0°C for 5 weeks and 3 d at 20°C. The experiment included fruit from two vineyards in Moshav Lachish and statistical analysis was performed separately for each vineyard shown as lowercase and uppercase letters above the bars.

In a subsequent experiment two vineyards were treated with a dual spray of GA aimed at ensuring treatment at a responsive stage of development. The first application was at berry diameter of 6 mm and the second, one week later. . The grapes from the two vineyards were stored at zero °C for 5 weeks after which they were evaluated for decay. The results from both vineyards demonstrate significant reduction in decay (Fig. 2B), confirming that reduction in cracking also improves the natural resistance of the berries to pathogens. It should be stated however that treatment with GA should not be considered as sufficient for commercial postharvest treatment against decay, as tolerance to decay in commercial practice is well below 1% and the treatment may only improve the efficacy of additional postharvest treatments.

Disinfection after harvest

If opportunistic pathogens encounter a natural opening such as a fresh crack, they will initiate filamentous growth into the berry. The commercial practice is to store the grapes with SO_2 paper sheets which protect against decay-development during storage. However, there is a window of time, from harvest until the initiation of SO_2 activity when the berries are less protected during which the disinfection technologies may play a significant role.

Clusters of 'Scarlotta' grapes were dipped in ethanol solution after harvest or stored in the presence of SO_2 sheets. This ethanol dipping treatment has a double action as it cleans the clusters and disinfects them. After 6 weeks of cold storage and 3 days at 20°C the clusters of the control treatment suffered high levels of decay compared to the treated clusters (Fig. 3). The number of decayed berries in the control was 60 which approximated 60% decay by weight. The SO_2 treatment, which reached a level of 6 to 8 ppm during storage, did not supply sufficient protection against decay with 10% of the berries suffering decay (severity was however much lower than the control). It should be stated that in commercial practice, the SO_2 treatment is considered very effective, with less than 1% decay after storage[2]. The ethanol dipping treatment gave in this case 3% decay with much cleaner appearance of the clusters. It should be emphasized that if *Botrytis* spores inoculated the flowers, no external disinfection treatment after harvest can prevent fruit decay or limit the spread of the disease during storage.

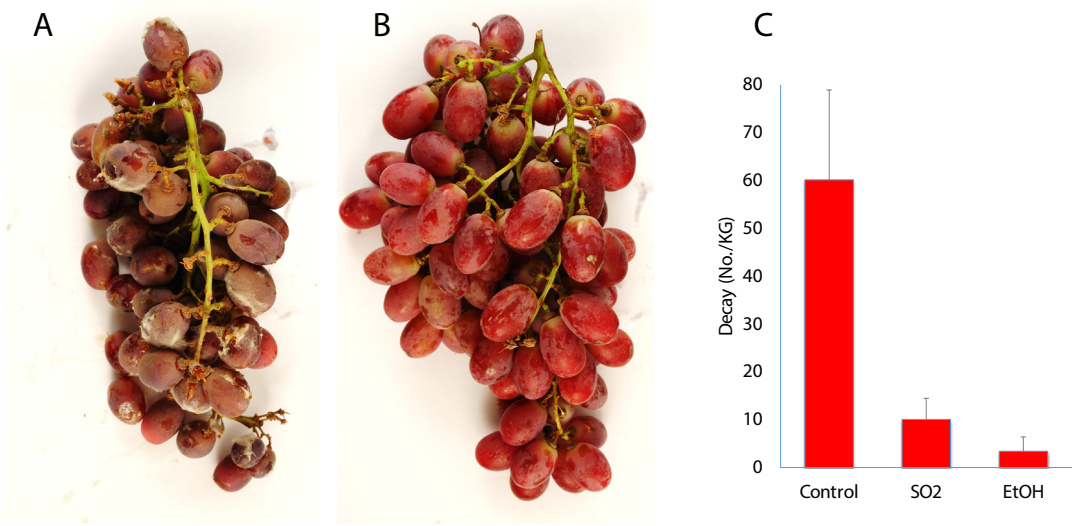


Figure 3: *The appearance of clusters of 'Scarlotta' grapes and the level of decay in the treatments after cold storage. A. Control untreated cluster. B. Ethanol (33%) treated cluster. C. The level of decay after 6 weeks of cold storage at 0°C and 3 days at 20°C. The grapes were stored in 9 kg boxes with SO_2 sheets above them. Ethanol was applied by dipping in a solution of 33% and drying for one hour. The boxes were wrapped with low density polyethylene during storage.*

.....reduction in the level of cracking by application of gibberellic acid during late fruit set can affect the level of postharvest decay

Conclusions

The results demonstrate that reduction in the level of cracking by application of gibberellic acid during late fruit set can affect the level of postharvest decay. This reduction in cracking is not sufficient to prevent postharvest decay by itself but when combined with other approaches it can allow the use of milder postharvest treatments. Additionally, disinfection of table grapes after harvest can have a dramatic effect on prevention of decay in grapes. This approach can be implemented in several ways but it also has an advantage by removing dirt that accumulates on the berries throughout the season. These two approaches are part of a 'multiple hurdle approach' which takes into account that there is no single step or protocol that can allow full protection against postharvest losses and that protection against postharvest losses is possible during all stages of fruit development.

References

[1] Lavee, S. (1960) Effect of gibberellic acid on seeded grapes. Nature 185, 395.

[2] Lichter, A., F.M. Gabler, and J.L. Smilanick (2006) Control of spoilage in table grapes. Stewart Postharvest Review 2, 1-10.

[3] Opera, L.U., C.I. Studman, and N. Banks (1996) Fruit skin splitting and cracking. Hort. Rev 19, 217-Z262.

[4] Reynolds, A., D. Wardle, C. Zurowski, and N. Looney (1992) Phenylureas CPPU and thidiazuron affect yield components, fruit composition, and storage potential of four seedless grape selections. Journal of the American Society for Horticultural Science 117, 85-89.

About the main Author

Dr Amnon Lichter is scientist in the Department of Postharvest Sciences since 1998 and served as the chair of the department from 2010 to 2013. Table grapes are the major focus in the current research program of Dr Lichter and it includes a wide range of activities: understanding the effects of pre-harvest developmental and horticultural factors on the postharvest quality; search for new varieties with improved postharvest properties; prevention of decay by *Botrytis cinerea* during storage; stem browning during storage; adoption of new technologies for phenotyping postharvest traits of grapes including image analysis and auto-fluorescence. A major part of the current program is better understanding of flavor in grapes from the perspective of tannins, volatile compounds and how we can maintain and improve unique flavor components. Past research also included abscission in bunch tomatoes, postharvest of litchi fruit, sanitation technologies and involvement in diverse postharvest research programs.

.....when combined with other approaches it can allow the use of milder postharvest treatments.

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Non-chemical approaches for postharvest quality management of root, tuber and bulb vegetables

Dani Eshel
Department of Postharvest Science of Fresh Produce,
Email: dani@agri.gov.il

We focus on environmentally friendly approaches for postharvest storage of root, tuber and bulb vegetables. A fast curing method, using heat and high humidity, was developed to improve skin stability in onions. Partially effective doses of nonchemical methods for disease management can be combined to obtain synergistic and effective control of postharvest diseases in carrots. The use of essential oils for sprouting control is effective and necessary in an integrated method to preserve potato quality in storage. Effort should be made to replace chemical treatments used today in root, tuber and bulb vegetables with integrated environmentally friendly methods.

Underground vegetables (UVEG), including roots, tubers and bulbs, are considered commodities, produced on a large scale and consumed year round. Among these, onions (*Allium cepa* L.), carrot (*Daucus carrota* L.) and potato (*Solanum tuberosum* L.) are some of the world's largest food crops in terms of fresh produce. In developing countries, many farmers are highly dependent on UVEG crops as sources of food, nutrition and income. In developed countries UVEG are harvested on a massive scale using mechanical machines, leading to vegetable bruising and skin injury, which is hidden by the residual soil. Some of the UVEG are generally not washed before storage, and thus the soil, contaminated with microorganisms, can alter vegetable quality.

UVEG function as vegetative reproductive organs which, immediately after detaching from the mother plant, tend to sprout and produce roots. In some cases, such as potato tubers, sprouting occurs after a dormancy period. The duration of the endodormancy period is primarily dependent on the genotype, but other factors, such as the growth conditions of the crop and storage conditions after tuber harvest, are also important. Due to increased concern for consumer health and safety, there is considerable interest in finding effective sprout controllers that are safe for humans and have a negligible environmental impact.

The aim of our research is to suggest an environmentally friendly approach, which will synergistically improve postharvest quality of UVEG. The three selected vegetables (onions, carrots and potatoes) represent different approaches to postharvest curing, disease and sprouting control, as dictated by their physiological requirements.

…….observation showed that fast curing keeps the onion tunic and inner fleshy scales intact and ensures postharvest quality

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Onion Curing to improve skin stability

Most of the onions (*Allium cepa* L.) grown in southern Israel are treated with maleic hydrazide before storage, and are cold-stored for up to 8 months with minimal losses to rot or sprouting. Nevertheless, in most cases the complete dry outer skin (tunic) cracks and loosens, and tends to fall off during storage. To improve onion postharvest quality, we developed a harvest and curing protocol[2]. Bulbs are harvested at 80 to 100% green leaf drop (top-down), leaving about 10 cm of neck above the bulb. The early harvest reduced skin cracks in 93% of the bulbs, resulting in a sturdy tunic beneath the muddy outer skin. We apply fast curing (FC) at 30 °C and 98% RH for up to 9 days postharvest: the onion neck became narrower after 6 days, similar to the effect of 5 months of cold storage (Figure 1). FC also changes the color of the treated onion bulbs' outer skin to a darker reddish brown. FC of onions harvested with long neck and stored for 290 days reduced weight loss and rot by 80% compared to non-FC onions. The better onion quality induced by FC was accompanied by an increased number of onion skin layers (from an average of 1.8 to 4) and a higher force needed to tear the tunic. FC compressed the effects of 5 months of cold storage into a few days, since most of the bulb neck and tunic changes measured during FC occurred only after long cold storage without FC (Figure 1). Histological observation showed that FC keeps the onion tunic and inner fleshy scales intact and ensures postharvest quality, even after 8 months of cold storage. The high temperature used for FC can be reached in hot-climate storage areas with minimal energy investment.

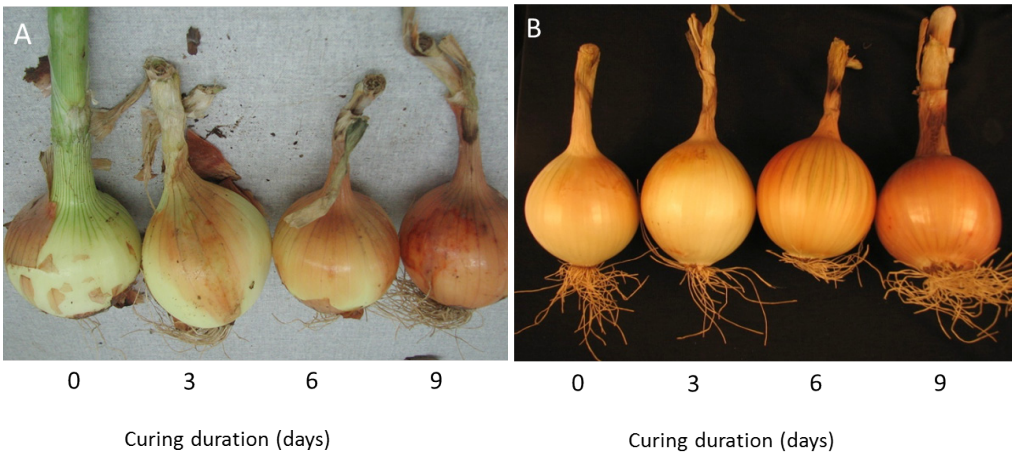


Figure 1 *Effect of duration of postharvest onion bulb curing on bulb neck diameter (A) and bulb tunic color (B). Onions are presented after 0, 3, 6 and 9 curing days and after an additional 5 months of cold storage (A and B, respectively).*

……sublethal dosage of steam or hydrogen peroxide followed by the yeast commercial product ShemerTM, before storage, improved efficacy of disease control.

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Synergistic control of carrot disease

In the last few years, most carrot growers resort to brush carrots to remove the peel epidermis before storage in order to improve the product's appeal: this practice increases the frequency of some postharvest diseases and increases tissue susceptibility to chemical and physical damage. In the case of carrots, there is no point for curing, since this will cause unmarketable carrot skin colors. The tissue wounds caused by postharvest brushing of carrots increase the incidence of black root rot disease, a postharvest disease caused by the fungus *Thielaviopsis basicola*[1].

The use of heat treatments has been found effective at controlling postharvest disease, but can damage the treated plant tissue if not applied carefully. We developed a real-time thermal imaging inside a treatment chamber to monitor temperatures on carrot surfaces, proven to be an efficient tool for optimizing heat level and uniformity over the entire surface area of the commodity[1].

Hydro-cooling carrots before storage, a practice that is aimed at reducing root respiration, can be used to protect tissue against damage by heat treatments. Steam treatment was shown to cause less damage to the carrot tissue when applied immediately after hydro-cooling. The use of this precision steam-application prototype for research and practical applications can be expanded to additional products, with the aim of reducing the use of postharvest chemical treatments.

When two control measures are applied together, the resultant effect on the pathogen can be antagonistic, additive, or synergistic. Antagonistic effects result in the efficacy of the integrated measures being lower than the sum of their efficacies as individual components, while additive and synergistic effects are equal to or larger than, the sum of the components' separate effects respectively. Application of a sublethal dosage of steam or hydrogen peroxide followed by the yeast commercial product Shemer™, before storage, improved efficacy of disease control compared to each of the treatments alone[1] (Figure 2).

Figure 2. *Synergistic control of black root rot disease, a postharvest disease caused by the fungus Thielaviopsis basicola, by combining control methods. 'Dordogne' carrots were exposed to the following treatments: A. water spray, B. yeast commercial product (ShemerTM), C. 3 s of steam and D. steam followed by Shemer, before storage in commercial polyethylene bags for 30 days at 0.5°C plus 8 days under shelf conditions (20°C).*

Potato sprouting control

Undesirable sprouting during storage is a serious problem for the UVEG fresh market, prior to industrial processing, and in the storage of potato seed-tubers. Cold-temperature storage (2-4°C), delays sprout development in potato, but does not delay unacceptable tissue sweetening.

Chlorpropham (isopropyl N-[3-chlorophenyl] carbamate; CIPC) is the most effective postharvest sprout inhibitor registered for use in potato storage and has been used successfully as a sprout inhibitor for more than 50 years. However, there have been reports of residual levels of CIPC in processed potato products. Random sampling has shown the potential to exceed the maximum residue limit, even when applications were performed according to best practices (http://www.pro-potato.com). Alternatives to CIPC are also needed for both the organic and non-organic markets, where CIPC is either not permitted or its residue level is limited, respectively.

1,4-Dimethylnaphthalene (DMN), a natural product found in potato tissues, inhibits sprouting and recently, there has been renewed interest in its application. To date, only one monoterpene, carvone, a chemical produced from caraway (*Carum carvi*) seeds, or mint (*Mentha spicata*) leaves and identified as a volatile sprout suppressant has been developed for commercial use in Israel and worldwide (Figure 3). We found that *R*-carvone application causes damage to the meristem membrane, leading to local necrosis of the bud apical meristem and a few weeks later, axillary bud growth is induced in the same sprouting eye. Surprisingly, application of a very low dose of *R*-carvone catalyzed axillary bud sprouting in the tuber[3]. The application of essential oils to control sprouting might have the additional benefit of suppressing postharvest diseases.

Figure 3. *The effect of mint essential oil on potato sprouting: Untreated (A) and treated (B) tubers after 6 months of cold storage at 8°C.*

Conclusions and recommendations for future experimental work

Combining environmentally friendly approaches for sprouting and disease control with proper curing of harvest wounds may enable the production of UVEG with no application of synthetic chemicals postharvest. The dose and sequence of application should be optimized for each type of vegetable individually.

…….carvone, a chemical produced from caraway (Carum carvi) seeds, or mint (Mentha spicata) leaves is identified as a volatile sprout suppressant.

References

[1] Eshel D., Regev R., Orenstein J., Droby S., Gan-Mor S. (2009). Combining physical, chemical and biological methods for synergistic control of postharvest diseases: a case study of Black Root Rot of carrot. *Postharvest Biology and Technology* 54:48-52.

[2] Eshel D., Teper-Bamnolker P. , Vinokur Y., Saad I. , Zutahy Y., Rodov V. (2014). Fast curing: a method to improve postharvest quality of onions in hot climate harvest. *Postharvest Biology and Technology* 88:34-39.

[3] Teper Bamnolker P., Dudai N., Fischer R., Belausov E., Zemach H., Shoseyov O. and Eshel D. (2010). Mint essential oil can induce or inhibit potato sprouting by differential alteration of apical meristem. *Planta* 232:179-186.

About the main Author

Dr. Dani Eshel is a Research Scientist at the Department of Postharvest Science of fresh produce of the Postharvest and Food Sciences Institute. He is involved in *in-depth* research of roots, tubers and bulbs storage and their progressing physiological age. His recent breakthrough studies are in the field of connecting programmed cell death of meristem cells to apical dominance and stem shape of stored propagation organs.

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

"Rock-Ad"—an improved and healthier rucola

Dalia Maurer, Daniel Chalupowicz, Alona Ovadia, Shahar-Ivanov Yelena and David Kenigsbuch*.
Department of Postharvest Science of Fresh Produce,
**E-mail: davke@volcani.agri.gov.il*

There is an ongoing need to extend postharvest storability of field crops intended for export. This challenge is particularly significant in leafy vegetables and herbs. The attractive form and distinctive flavor of rucola leaves have made this crop plant a popular choice, both for salads and as a garnish; however its production and marketing have been limited by early flowering and poor storability. The newly developed rucola cultivar, named 'Rock-Ad', was developed by our group through advanced breeding techniques from wild rocket seeds, involving screening of progenies for desired characters which have a linked regulation. 'Rock-Ad' has the potential to be the cultivar of choice for growers and customers due to its enhanced characteristics including late flowering, which enables several harvests per season. It is also suitable for sea shipment due to improved storability.

Rucola or wild rocket is native to the Mediterranean region and is cultivated as a crop plant in Israel. The rucola leaves are used in salads or in cooked dishes such as pasta or pizza. It adds a spicy pungent flavor to the popular packages of mixed "baby leaves" intended for fresh salads. The rucola leaves are high in nutrients containing vitamin C, iron, fibers, flavonoids and the cancer preventing agents - glucosinolates. Rucola is also considered to be a depurative medicinal herb having diuretic and laxative properties, having also been described since Roman times as being an aphrodisiac[1].

Rucola is a fast growing, cool-season crop which flowers under long days and high temperatures[2]. The main obstacles to commercial production of this crop are early flowering during spring and autumn and limited storability. Flower stems are not desirable at harvest time, because they continue to grow during storage and reduce the quality of adjacent leaves. Limited storability prevents the economically preferable sea shipment. The minimum requirement for sea shipment is 14 days in storage and shelf life with the commercial cultivar of rucola turning yellow and decaying within this time.

We have developed a new rucola cultivar from the commercial cultivar, by employing a speeded up version of a natural evolutionary process called mutagenesis. Previous studies with Arabidopsis plants showed that the control of vegetative growth and postharvest traits is linked to the regulation of early germination traits[2]. This enabled us to select mutant lines carrying the desired traits at the germination stage. After several generations of screening the mutants, we selected a new, improved line that was named 'Rock-Ad'. 'Rock-Ad' has a two week delay in flowering as compared to the commercial cultivar plants.

This delay allows for rucola plants with a higher number of leaves in the rosette to be harvested, with more harvests being completed before flower buds appear.

***Figure 1**: The common commercial cultivar of rucola (left bed) and 'Rock-Ad' the newly developed rucola cultivar (right bed). Both cultivars were sowed and planted at the same time in a net-house. The commercial cultivar presents earlier flowering then 'Rock Ad'.*

The 'Rock-Ad' leaves are dark green, rough and lobe - shaped as required by the consumers with only a few un-lobed leaves comparable to other Eruca varieties. Under postharvest conditions the 'Rock-Ad' leaves remained green in contrast to the yellowing leaves of the commercial cultivar. This difference was seen even in hard storage conditions (ventilated atmospheres). This means the 'Rock-Ad' cultivar can be exported by sea shipment.

'Rock-Ad' also showed an advantage in preserving important natural health metabolites. The cultivated rucola plants contain respectable levels of antioxidants and glucosinolates, but these levels decrease significantly after storage. However in the 'Rock-Ad' cultivar after two weeks of storage and shelf life the antioxidants and the glucosinolates quantities were not significantly lower than their initial levels at the time of harvest.

***Figure 2**: Bunches of the commercial cultivar and the newly developed cultivar of rucola after storage: the commercial rucola (left) is much yellower than the 'Rock-Ad' (right).*

In summary, we developed a new rucola cultivar, named 'Rock-Ad' which has the potential to be the cultivar of choice for growers and customers due to late flowering, and a growth pattern allowing several harvests per season. It is also suitable for sea shipment and has better qualities after storage than the commercial cultivar.

Rock-Ad' cultivar can be exported by sea shipment.

References

[1] Bianco VV, Boari F (1997) Up-to-date developments on wild rocket cultivation. In S Padulosi, D Pignone, eds, "Rocket: a Mediterranean crop for the world" 1996 Legnano (Padova), pp 41-49.

[2] Kenigsbuch D., Ovadia A., Shahar-Ivanova, Y., Chalupowicz, D. and Maurer, D. (2014) "Rock- Ad", a new wild rocket (*Diplotaxis tenuifolia*) mutant with late flowering and delayed postharvest senescence. Scientia Horticulturae, 174:17-23.

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Control of Alternaria black spot symptoms in persimmon fruit by growth regulators

Dov Prusky*, Esti Biton, Oleg Feygenberg, Haya Friedman
Department of Postharvest Science of Fresh Produce
**Email: dovprusk@volcani.agri.gov.il*

In Israel, black spot, caused by *Alternaria alternata*, is the main postharvest factor that reduces quality and impairs storability of persimmon fruits *Diospyros kaki* cv. Triumph. The fungus infects the fruit in the orchard and remains quiescent until harvest or renews its development just before harvest, following rain or high humidity, and then preferentially colonizes the stem side of the fruit. Recent findings suggest the importance of ethylene and respiration, early during fruit growth, as factors influencing maturity, crack development, and susceptibility to Alternaria black spot (ABS) colonization in the stem end side of the fruit. We tested the effects of the growth regulator Superlon – a mixture of gibberellin (GA_{4+7}) and benzyl adenine (BA) – on fruit physiological responses during growth on ABS incidence. Superlon treatments during early stages of fruit growth, i.e. starting 40 days after fruit set (dafs), inhibited ethylene and CO_2 evolution of the stemd end side. Application of the treatment at later state of fruit development, i.e. 100 dafs, enhanced cell proliferation of the external layers of the exocarp under the fruit cuticle. In both cases, Superlon delayed chlorophyll degradation, and reduced fruit cuticle cracks and ABS susceptibility during late stages of fruit growth and during storage. These results suggest that the phytohormone, acting as a modulator of host physiological responses that resulted in delayed fruit maturation, is a main factor in enhanced resistance to ABS at harvest and during storage.

Black spot, caused by *Alternaria alternata* has been described as the most economically devastating postharvest disease of persimmon fruits *Diospyros kaki* cv. Triumph, in all growing regions of Israel [3,4]. The primary mode of infection of persimmon fruits by *A. alternata* is either through small wounds under the sepals attached to the fruit stem and/or directly into the fruit cuticle. We demonstrated that Alternaria black spot (ABS) incidence was enhanced by the presence of cracks on the stem end side of the fruits, in contrast to non-ABS symptoms on the bottom end side, where cracks hardly occur. Moreover, our study indicated that sensitivity of the stem end to Alternaria is also determined by higher maturity of this end in comparison to the bottom end. Analysis of the various factors that affect this differential fruit response revealed 400 and 100% greater ethylene and CO_2 evolution respectively in the stem end of the fruit than in the bottom end at early fruit development. The resulting enhanced ethylene content is modulated by transcriptional activation of *ACC* oxydase, which begins in the calyx lobes, diffuses to other fruit tissues, where it stimulates autocatalytic ethylene biosynthesis. This transcriptional activation of *ACO2* and increased ethylene evolution in the stem end of the fruit contribute to early maturation and enhanced susceptibility to ABS at harvest and during storage.

ABS symptom development may be affected by: growth regulators, which reduce host susceptibility to fungal attack; pre- and postharvest fungicide treatments

that protect against and/or eradicate fungal infections; or a combination of approaches during high incidence of infection. Harvesting of persimmon fruits is selective and is done according to their external color. At a late stage of maturation, i.e., about 2 weeks prior to harvest, the fruit color shifts from green to slightly orange, and at this time the fruit are treated with gibberellin (GA_3) at 50 µg L^{-1}, to prevent softening and to reduce ABS incidence during storage. The GA_3 delayed maturity of the fruit, postponed the green-to-orange color change, enhanced erection of the sepal lobes and thereby reduced local humidity and black spot incidence, and also affected the fruit cell-wall composition. The cell walls of GA_3-treated fruits inhibited the capability of *A. alternata* endoglucanase (EG) to digest those cell walls, resulting in the prevention of fungal colonization.

Postharvest dip treatment is the main means of disease control during storage and shipment. After harvest, prior to storage at 0˚C fruit are subjected to a dip treatment with a chlorine compound that is released from sodium troclosene tablets. Although the chlorine treatment is effective for control of black spot in fruits stored at 0°C for up to 2 months, decay incidence increases significantly as the storage duration is extended beyond 2.5 months.

Our objective in the present study was to develop approaches to reduce ABS colonization, based on the use of the growth regulator Superlon. This study shows that treatment during fruit development with this phytohormone reduced ABS development at harvest and during storage; it inhibited ethylene and CO_2 evolution during early stages of fruit growth, increased cell proliferation, reduced fruit cracks and delayed chlorophyll degradation. The results highlight the importance of modulation of fruit physiological changes that promote slow maturation on the upper side of the fruit, as a factor for modulating crack development and reducing susceptibility to Alternaria black spot.

Susceptibility of persimmon fruits to ABS has been shown to be associated with physiological factors in the tissue that led to cracks through which the pathogen might penetrate and initiate colonization[1]. Differentially increased maturity in the stem end side of the fruit, compared with the bottom end side that occurred as a result of ethylene evolution and respiration, led to increased cracked area and the consequent ABS development. Furthermore, the stem end displayed higher sensitivity to ABS colonization, irrespective of the cracks.

The present study showed that treatments with the growth regulator Superlon significantly altered several of the host responses that affect ABS development. These alterations included: (i) 85 and 75% inhibition of ethylene and CO_2 evolution, respectively, at an early stage of development; (ii) 40–60% inhibition of *ACO2* expression throughout the period of fruit growth in both the upper and lower ends of the fruits; (iii) enhanced cell numbers by 17 and 10.2% in the upper and lower tissues, respectively, compared with those in untreated fruits; (iv) delayed fruit maturation, as indicated by a 30% delayed decrease in chlorophyll content; (v) reduction by 45% in the cracked area of the fruit; and (vi) reduction by 40–50% in the incidence of naturally occurring ABS after 3 months of storage.

However, inhibition of ethylene evolution and increased cell density are the most critical factors in modulation of fruit response and ABS development induced by Superlon observed during the late stages of growth[2]. In Superlon-treated fruit, ethylene and CO_2 evolution were inhibited by 86 and 63%, respectively, and the *ACO2* relative expression was inhibited by factors of 5 and 2.3 in the upper and lower ends, respectively, which suggests that the phytohormone treatments had a significant effect on ethylene biosynthesis during fruit growth. It is not clear which of the two phytohormones – BA or GA_{4+7} – contributed more to this ethylene inhibition. Benzyl adenine treatment of *Arabidopsis* inhibited morphogenetic changes in the hypocotyl – changes that are characteristic ethylene responses – not by affecting sensitivity of the tissue, but by affecting production of ethylene. In the present study similar results were observed in Superlon-treated fruit. Inhibition of ethylene by Superlon, during early stages of fruit development was accompanied by significant inhibition of *ACO2* relative expression during most of the fruit growth period until a month before harvest. In light of the present finding that at harvest the stem end of untreated persimmon fruits showed a 32-times greater increase in *ACO2* relative expression than the bottom end, we suggest that Superlon might be involved in the inhibition of the signal release from the fruit lobes, and thereby in prevention of the stem end maturation and of development of cracking and ABS.

However, this effect of Superlon on ethylene production could not be observed when the treatments started 100 dafs – when ethylene and CO_2 evolution had declined – but the treatment still affected the cracked area and ABS development. Since persimmon fruits are characteristically low producers of ethylene although they are, nevertheless, ethylene sensitive, there might be differential ethylene sensitivity in the upper and lower ends of the fruit that is also modulated by Superlon when applied late during fruit growth.

However, the effects of early and late Superlon treatments (40 and 100 dafs) on the delay of chlorophyll decrease indicate that Superlon action might also have significant effects on fruit maturation by other mechanisms. In persimmon fruits Superlon treatment delayed chlorophyll metabolism during the last month of fungal growth, i.e., from 115 through 190 dafs. It is interesting that, although in the present study no detectable ethylene evolution was measured in the last month before harvest, the relative expression of *ACO2* in the upper and lower ends of the fruits were, respectively, 164 and 31 times higher than the lowest expression of *ACO2* found in the lower end of the fruits at harvest. It is also interesting that Superlon treatment reduced *ACO2* relative expression by factors of 1.5 to 2 in all the tissues. This may indicate that the presence of ethylene produced during the initial months of fruit growth, enhanced tissue crack development and ABS susceptibility in untreated fruits.

One host factor that clearly modulated ABS was the resistance of the bottom end of the fruit. Although a clear positive correlation was observed between the cracked area and ABS susceptibility in the upper (stem) end of the fruits, the lower end was fully resistant to ABS, irrespective of Superlon treatment or the cracked area, and no differences in

cuticle thickness between the two ends of the fruit were observed that might account for this differential susceptibility.

Analysis of the incidence of ABS development following 3-month storage of Superlon-treated fruits showed 50% inhibition compared with untreated control fruits (Table 1 and Fig.1), which suggests that growth regulators are efficient physiological modulators of fruit susceptibility. Pre-harvest application of a plant-growth regulator such as Superlon could serve as a simple and practical way for growers to effectively delay incidence of ABS, similarly to the practice used in other crops.

	Alternaria colonization (Surface %)		Firmness (Index 1-10)	
Treatment	**Out of storage**	**Shelf Life**	**Out of storage**	**Shelf Life**
Control	**4.21a**	**8.5 a**	**6.36a**	**3.60b**
40 dafs	**2.49b**	**4.2c**	**5.92a**	**3.8ba**
70 dafs	**3.58b**	**5.8b**	**6.52a**	**4.60a**
100 dafs	**2.37b**	**3.8d**	**5.65a**	**3.70b**

Table 1: *Effect of pre-harvest Superlon treatment on the incidence of black spot caused by Alternaria alternata after 3 months of storage at 0°C and after 4 days of shelf life at 20°C. Superlon sprays at 40 µg mL-1 were initiated at 3 different stages after fruit set (dafs) and were applied once a month during 3 consecutive months. The commercial GA3 treatment was applied commercially to all the treatments in the experiments 10 days before harvest. Values followed by different letters in each column are significantly different at $P \leq 0.05$ according to the Tukey-Kramer Multiple Comparison Test.*

References

[1]. Biton, E., Kobiler, I., Feygenberg,O., Yari,M., Kaplunov,T., Ackerman,M., Friedman, H., Prusky, D. 2014. The mechanism of differential susceptibility to alternaria black spot, caused by *Alternaria alternata*, of stem- and bottom-end tissues of persimmon fruit. Postharvest Biology Technology: 94:74-81.

[2]. Biton, E., Kobiler, I., Feygenberg,O., Yari, M., Friedman, H., Prusky, D. 2013. Control of alternaria black spot in persimmon fruit by a mixture of gibberellin and benzyl adenine, and its mode of action. Postharvest Biology Technology. 94:82-88.

[3]. Prusky, D., Ben-Arie, R., Guelfat-Reich, S., 1981a. Etiology and histology of Alternaria rot of persimmon fruits. Phytopathology 71, 1124–1128.

[4]. Prusky, D., Eshel, D., Kobiler, I., Yakoby, N., Beno-Moualem, D., Ackerman, M., Zuthji, Y., Ben Arie, R., 2001. Postharvest chlorine treatments for the control of the persimmon black spot disease caused by *Alternaria alternata*. Postharv. Biol. Technol. 22, 271–277.

Superlon could serve as a simple and practical way for growers to effectively delay incidence of Alternaria black spot

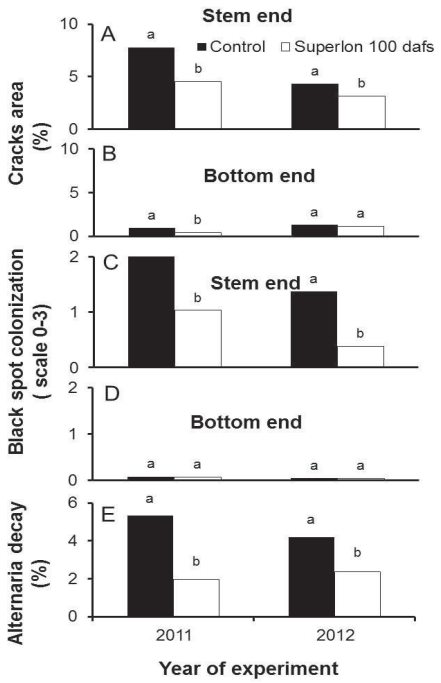


Figure 1: *Effect of Superlon treatment during two consecutive years on: the area of crack incidence (A, B); susceptibility to ABS (C, D); and natural incidence of Alternaria black spot colonization during storage (E). Superlon was applied starting 100 days after fruit set (dafs) and inoculation with 1 µL of a suspension containing 106 spores/mL at 24 spots per fruit was applied at harvest. The inoculated fruit were incubated at high humidity at room temperature and were evaluated 5 days after inoculation, according to the percentage of surface area covered with spots.*

About the Main Author

My research focuses on the basis of resistance and susceptibility of fruits and vegetables to postharvest disease as a universal model system for understanding the mechanism of pathogen attack and host spoilage of fruit and vegetables. We study the mechanism of host/pathogen regulation of the activation of initial fungal infections that cause spoilage in fruits and vegetables all over the world. We pioneered the discovery of the mechanism of pathogen quiescence in resistant fruits to postharvest pathogens and identified the chemical basis for fruit resistance of unripe fruits compared to ripe susceptible fruits. At the same time our lab developed a new pioneering understanding of the mechanistic process leading to the activation of quiescent infection for full active attack. Our studies on the mechanism of host pH environment regulation, lead to the discovery that pathogens activate metabolic pathways involved in the secretion of small effecting molecules that modulate the host pH environment and became key signals for activation of pathogenicity factors. The secreted metabolite either ammonia or organic acids, were found to i. modulate the host pH around the infection point, ii. to activate the transcript activation of pathogenicity factors and to iii. induce host cell H_2O_2 production and cell death resulting in the enhanced active infection in ripening fruits. The understanding of these mechanisms has resulted in the development of new approaches for the amelioration of post-harvest disease that are applied today in the field.

Alternaria black spot incidence was enhanced by the presence of cracks on the stem end side of the fruits

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Elucidating the ripening control mechanism in fruits for the development of molecular tools to improve fruit quality and security

Friedman Haya
Department of Postharvest Science of Fresh Produce,
Email: hayafr@volcani.agri.gov.il

Fruit ripening is a coordinated process which is executed by ethylene in climacteric fruit, leading to: fruit softening, sugar accumulation, aroma development, chlorophyll reduction and increased sensitivity to pathogen. Based on knowledge from the model plant tomato, it is clear that ripening is controlled by multiple transcription factors acting upstream of ethylene production. In banana we discovered a transcription factor which controls ripening, in its absence ripening is delayed. Currently we are examining the involvement of homologous transcription factors in apple and peach softening. In addition, we are studying ethylene and other hormones' involvement in fruit ripening. Several genes encoding enzymes in the ethylene biosynthesis pathway have been extracted from the Apple genome sequence and their role in fruit storage capacity was examined. We determined that only one gene, encoding 1-aminocyclopropanecarboxylic acid ACC synthase, is correlated with high ethylene production in an apple cultivar with low storage capacity. Identifying components within the ripening control pathway(s) will be useful in the future to engineer or breed cultivars with delayed ripening and hence reduced fruit deterioration.

Fruits are an essential component of a balanced diet and their consumption is necessary for human's wellbeing. Accordingly, they supply the body demand for vitamins, minerals, fibers, and health-promoting substances. Fruits are usually consumed after they reach maturity and after harvest they continue the ripening program and the metabolic processes which accompany this process eventually lead to fruit deterioration. To avoid food loss many farmers harvest the fruit too early, but this practice may act as a boomerang, since at low maturity, fruit fail to develop a good taste and deter consumers from recurrent purchases.

The ripening program, which is characterized by fruit softening, sugar accumulation, aroma development, chlorophyll reduction, color development, but also an increase in sensitivity to pathogen, has been elucidated by using the tomato as a model for fleshy fruits[1]. The tomato is a climacteric fruit, characterized by an abrupt increase in respiration and ethylene production. Several components which regulate ethylene production have been identified[1]. However, it is still not clear if all fruits use similar components to execute ripening.

Our mission is to extend the shelf life of fruit, while maintaining their quality. Towards this end we study the mechanism of fruit ripening in several fruit and search to identify components which control ripening. This will enable, in the future to select for markers which can be used in breeding programs, and it will enable to develop knowledge-based novel treatments to extend fruit postharvest

shelflife and hence promote food security. Our studies are conducted on apricot, apple, banana, cherry, persimmon and peaches and strides were made to understand the ripening pathway. This report highlights examples from apple and banana.

Ethylene biosynthesis in apple

Different varieties of apples differ in their storage capacity. The apples of the variety 'Ana' 'Golden Delicious (GD)' and 'Galaxy' develop on June, August and September, respectively. At harvest and after storage 'Ana' develops more ethylene than the other two cultivars (Fig. 1). 'Ana' has inferior fruit qualities to the other cultivars and almost no storage capacity, probably due to the high ethylene production.

Ethylene is produced from methionine by two major enzymes ACC synthase (ACS) and ACC oxidase (ACO). In apple we found 11 ACC synthase and 7 ACO oxidase genes encoding these enzymes which are expressed in fruit. We found that only the expression pattern of one of the *ACC synthases* is correlated with higher ethylene production by 'Ana'. This information can be used in molecular engineering or breeding for apples with better storage capacity.

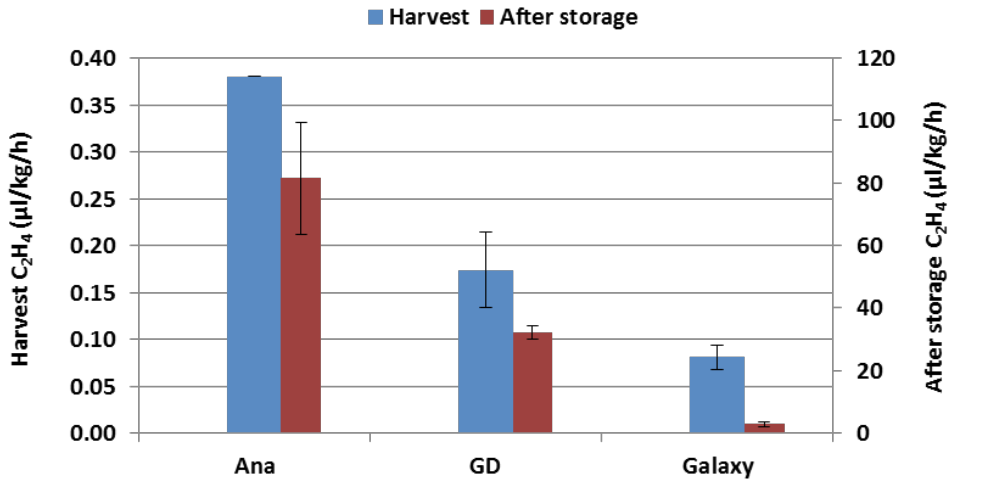


***Figure 1**: Ethylene production at harvest (axis on the left hand side) and after storage (axis on the right hand side) of three different cultivars. GD- Golden Delicious.*

Control of ethylene production in banana

Banana is a staple fruit in many developing countries and hence any developments to extend the shelf life will improve food security in those communities in which their diet is dependent on banana. Banana fruit is harvested at three quarters of its final size, when the fruit is still green and it can ripen at postharvest due to internal ethylene production.

Based on studies in tomato, it was discovered that the genes of the ethylene biosynthesis pathway are controlled by transcription factors responsible for

the induction of ethylene biosynthesis genes. Few of these transcription factors belong to a family of MADS-box genes, and in tomato, a mutation in RIN-MADS delayed fruit ripening. As a matter of fact, this mutation exists in most of the modern tomato varieties used today in commerce. We identified 6 banana *MaMADS* genes, which are differentially expressed in peel and pulp[2]. We also studied their interactions with ethylene. It is clear that *MaMADS1* is not induced by ethylene in the pulp, but it does in the peel. On the other hand, *MaMADS2* expression is independent of ethylene in both peel and pulp (Fig. 2). This suggested that *MaMADS2* acts upstream of ethylene biosynthesis and hence represents a major

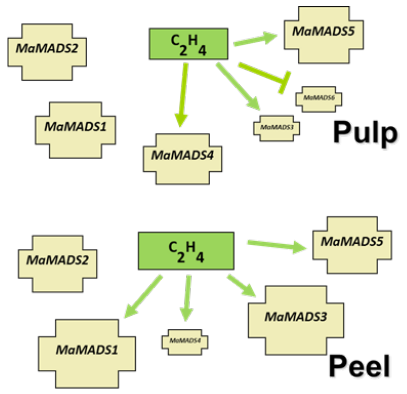


***Figure 2**: Schematic description of the interactions between ethylene and MaMADS genes in pulp and peel. The size of the shape indicates the levels of expression. Arrows shows induction and perpendicular lines indicate inhibition.*

candidate for the control of ripening which might function similarly to RIN-MADS in tomato.

Suppressing the expression of *MaMADS2* transcription factor delayed ripening (Fig. 3). The fruit obtained from the suppressed lines did not differ in sugar content from those of the control at the yellow stage. Moreover, fruit of the suppressed lines were firmer than those of the control. Hence, fruit of the suppressed line have equal/better characteristics than control fruit. In this study we established a proof of concept for silencing of MADS genes as a technology to extend fruit shelf life.

***Figure 3**: Banana of control and of MaMADS2 suppressed lines 20 and 26 days after harvest.*

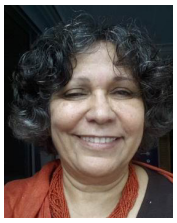
In summary, by exposing the molecular mechanisms of ripening in various fruit we expect to provide tools for obtaining fruit with better shelf life.

References

1. **Giovannoni JJ** (2001) Molecular biology of fruit maturation and ripening. Ann Rev Plant Physiol Plant Mol Biol 52: 725-749

2. **Elitzur T, Vrebalov J, Giovannoni JJ, Goldschmidt EE, Friedman H** (2010) The regulation of MADS-box genes expression during ripening of banana and their regulatory interaction with ethylene. J Exp Bot 61: 1523-1535

About the first Author



Haya Friedman, Ph.D. Dr. Friedman is a Researcher in the Department of Posthavest Science of Fresh Produce, Institute of Postharvest and Food Science, Agriculture Research Organization (ARO)-Volcani Center.

Dr. Haya Friedman gained her Ph.D. from the Hebrew University of Jerusalem at the Faculty of Agriculture, Israel and trained as Post-Doctoral fellow at Yale University, USA. Since 1995 she is a research scientist in the field of Postharvest Science, using molecular tools to understand ripening and senescence processes under stress. She is working on banana, apples, cherry, pear, peaches, apricot, plum and persimmon and developes protocols for handling of these fruits to improve fruit quality. Her developments are based on molecular understanding of processes related to ripening and to stress encountered during storage.

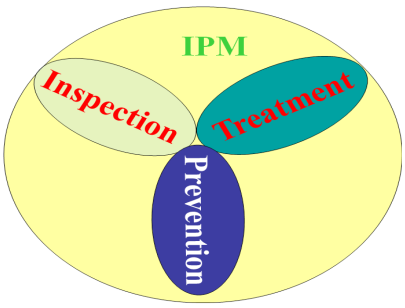
.....only the expression pattern of one of the ACC synthases is correlated with higher ethylene production by 'Ana'. This information can be used in molecular engineering or breeding for apples with better storage capacity

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Integrated Protection as Alternative to the Traditional Methods of Grain Pest Control

Moshe Kostyukovsky,
Department of Food Quality and Safety,
**Email: inspect@volcani.agri.gov.il*

One of the most significant factors for the global food crisis is grain losses during storage caused by pest insects, which may reach 30-40%. Today, there are two main chemical methods for stored product insect pest control: fumigation and grain protection by contact insecticides. For both these methods phyto-sanitarian, healthy and ecological disadvantages are well documented. The global tendency is to prevent/decrease the wide use of common chemical insecticides with high toxicity to humans, especially for control of pests in food. Therefore, there is an urgent need for developing an alternative eco-friendly approach for stored insect pest control, aimed to prevent grain quantity losses and quality degradation of stored food and feed grain and dry food products caused by stored product insect pests. Based on results from our lab and other labs worldwide, we suggest an integrated protection approach as an alternative to the traditional methods of grain pest control. This approach includes prevention measures, systematic inspection and monitoring for grain storage risk factors; the use of eco-friendly volatiles and protectants; and improved technologies for currently used fumigants. The implementation of this approach in Israel resulted in a dramatic reduction in stored grain losses to < 0.5%.



Insect damage in stored grain and other durable commodities may account to 5-10% in developed countries and 30-40% in developing countries, where modern storage technologies have not been introduced. High levels of grain losses during storage aggravate the food security situation and in turn augment the global food crisis. Currently, food industries rely mainly on the usage of fumigants and contact synthetic insecticides as effective measures for insect pest control in grain and other dry food commodities. In spite of high efficacy, relatively low cost and developed technologies for their use, for both of these methods the phyto-sanitarian, health and ecological disadvantages are well known [4, 7, 8]. Among fumigants, methyl bromide is mostly phased out in developed

countries due to its ozone depletion effects. Phosphine, which is widely used today, has many disadvantages: It is a very toxic gas; phosphine fumigation has to be maintained for 7-10 days; treatment is ineffective at low temperatures; and some insects have developed resistance to phosphine. A high toxicity to mammals, pesticides residues in the food and insect resistance are the most negative sides of the currently used contact insecticides (protectants). Today, the global. Today, the global tendency is to prevent/decrease the wide use of common chemical insecticides with high toxicity to humans and the environment, especially for pest control in food. Therefore, there is an urgent need for developing an alternative, safe to humans, eco-friendly approach for stored insect pest control aimed to prevent grain quantity losses and quality degradation of stored food and feed grain as well as dry food products caused by stored product insect pests. Based on the results of our and worldwide research, we suggest an integrated protection (integrated pest management – IPM) as alternative to the traditional methods of grain pest control. This approach should include prevention measures, systematic inspection and monitoring for grain storage risk factors, the use of eco-friendly volatiles and protectants and improved technologies for currently used fumigants.

The approach is based on the understanding of the ecosystem of the stored grain or any other dry food product. The outside factors, such as the region and location of the stored site, and the inside factors, both abiotic (temperatures of the commodity and air, moisture content and air relative humidity) and biotic (mainly insects and microflora) will determine the environmental pressure on the stored grain and the insect infestation.

Preventative measures

Today we have a wide range of prophylactic measures which prevent stored product insect pest-outbreaks or significantly reduce their populations in the stored grain/dry food. Among these measures are included: the structure construction and maintenance; sanitation; aeration; cooling; heating; and drying. All these measures may affect abiotic and biotic factors creating the proper conditions for stored grain/food and unfavorable conditions for insects. Aeration and cooling will reduce commodity temperature and moisture content, resulting in the prevention or reduction of the insect population. The same results may be reached by drying or heating of the stored commodity. The proper structure and regular cleaning of the machinery and equipment, inside and surrounding territory, before, during and after storage, will prevent penetration of insects into the stored grain.

Inspection and monitoring

Inspection and monitoring is an inseparable part of the IPM strategy. Systematic inspection of grain storage sites, detection and monitoring of the risk factors, allow for the management of an insect infestation and will prevent/reduce the quantity and quality stored grain loss. The volume of grain consumption in Israel is estimated at 4.5 million ton

with a financial turnover of $1.5 billion. The local and imported grains serve partly as an emergency stock in Israel. The inspection includes three main steps: pre-storage, grain receiving and the grain storage period. At the first step the grain storage structures have to be checked and permitted for emergency stock storage. Attention is mainly given to the cleanness from food residues and insects, prevention of water penetration, the possibility to aerate, fumigate and to provide monitoring for risk factors in the grain bulk. At the next step – the filling of the storage facilities, strict control is given to the grain quality, especially the grain moisture content. Under the climatic conditions in Israel, the moisture content of grain for emergency stock storage has to be lower than 12.5%. The most important step of the inspection is the monitoring of risk factors during the storage period. According to the understanding of the grain bulk ecosystem, there is a need to monitor the main abiotic factors, such as temperature and moisture content of the stored grain, as well as ambient temperature and relative humidity of the air. The main biotic factors for monitoring are the pest insects and the moulds. On site, except the visual evaluation of the general situation and the sanitation level, we provide measurements of grain and air temperature, relative humidity of air and water penetration. For evaluating insect infestations we utilize pheromone traps, pit-fall traps and grain sieving. The samples of the grain are checked in the laboratory for moisture content, insect infestation (species, numbers, location), mould development. Based on received information, we may manage the grain storage. Some treatments, such as aeration, fumigation, transfer or evacuation, may be conducted, if the need arises. The strict systematic monitoring of the risk factors allows us to prevent the self-heating of the grain, cracking, insect and mould damage and therefore reduce losses to very low level of 0.1-0.5% without grain quality degradation.

Eco-friendly volatiles as alternatives to currently used danger fumigants.

Essential oils

Our research group lead by Prof. Eli Shaya in cooperation with Prof. Uzi Ravid - has focused in recent years on the use of plant oils for the protection of grain and other agricultural products, due to their low mammalian toxicity and low persistence in the environment. A large number of essential oils (EOs) and their constituents were screened against stored products insects. Two EOs obtained from Labiatae plants, were found as highly potent fumigants. The main component of the oils was Pulegone and the other was identified and titled SEM-76. The first has some toxicity to mammals; the second is generally recognized as safe (GRAS). Space fumigation tests of these volatiles caused total adult mortality of insect pests at a very low concentration of 0.5 g/m^3 air, at exposure time of 24 hours. Somewhat higher concentration was needed to kill the larvae of the insects tested. Pilot experiments in 250-kg bins filled with 70% wheat using SEM-76 at 100 g/m^3 supplemented with CO_2, and exposure time of 7 days caused 100% mortality of all tested insects. (Addition of CO_2 is essential also in conventional chemical fumigation to enable gas penetration) [4, 8].

The findings suggest that certain plant essential oils and their active constituents, mainly terpenoids, possess high bioactivity against a range of storage insect pests. They are specifically selective to insects, since they target insects' octopaminergic receptor, a non-mammalian target [1].The plant EOs and their terpenoids are worldwide-available. Their common use as flavoring agents in foods and beverages is a good indication of their safety. There is a good basis to believe they can be introduced as complementary, or alternative methods to currently used toxic fumigants for the preservation of grains and dry foods, for crop protection and for integrated pest management.

Mating disruption by using sex pheromone

Indian meal moth (IMM) *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) is a common and harmful insect pest in stored grain and dry food in Israel. Usually, chemical treatment, such as fogging, residual insecticides or fumigation is used for IMM control. Despite the high efficacy of these traditional measures, their negative impact on the environment and public health have led to intensive research focused on safe and eco-friendly alternatives. In recent years, the use of sex pheromone for mating disruption (MD) of IMM was suggested [5].

We conducted a study in small-scale (15 m^3 filled with 3 ton of wheat grain) and two commercial-scale warehouses (each one of 3000 m^3 filled with 2000 ton of wheat grain) in the central part of Israel. The Prescription Treatment® brand ALLURE® MD (Whitmire Micro-Gen Research Laboratories, Inc., MO, USA) with 93% of active ingredient (Z-9, E-12-Tetradecadien-1-yl acetate) was used for mating disruption. Evaluation of IMM populations was performed using two methods: the first, based on pheromone traps containing pheromone dispensers SP LOCATOR (AgriSense BCS Ltd, UK) and the second, based on food traps containing culture media used for IMM rearing. The small-scale warehouse was initially tested as a control (without mating disruption) and then as a treatment (1 dispenser per 15 m^3). Two commercial warehouses were used for control and treatment (1 dispenser per 30 m^2) in parallel. It was found that mating disruption resulted in significant suppression of IMM in the first generation and even higher in the F_2 generation (Table 1).

Treatment	No of adults/trap	No of F_1 adults in traps	F_1 population decrease % of control	No of F_2 adults in traps	F_2 population decrease % of control
MD	23 ± 6	231	70	682	85.2
Control	144 ± 38	716	-	4595	-

***Table 1**: Efficacy of MD at low initial IMM population (40 introduced pupae) in small-scale warehouse (3 ton wheat grain in 15 m^3)*

Aeration and cooling will reduce commodity temperature and moisture content, resulting in the prevention or reduction of the insect population

However, when high initial populations were tested (300 introduced pupae), the treatment was ineffective.

Field trials were conducted in commercial warehouses. Initial IMM population levels were low (one male per pheromone trap). After the first month of MD treatment, the number of males caught by pheromone traps in the warehouse was 1.5 ± 0.3 in the treated warehouse versus 7.0 ± 2.7 in the control. However, at the end of the second month the number of males caught in the traps in the treated and untreated warehouses was not significantly different: 19.3 ± 5.9 and 20.5 ± 6.3 respectively.

We can conclude that mating disruption can indeed suppress populations of IMM in warehouses, however when populations reach high levels chance encounters prevail and thus mating disruption should be implemented within an integrated pest management program thereby maintaining pest populations at low levels.

Eco-friendly protectants as alternatives to currently used danger contact insecticides

Today, the wide use of protectants for grain protection against stored product insects based on the contact insecticides belonging to the phosphororganics and pyrethroids. High toxicity to human, residues in the food, development of insect resistance and others negative consequences of their use have led to the search for eco-friendly alternatives, such as insect grows regulators (IGRs), inert dusts and others.

A promising IGR is Novaluron, a novel chitin synthesis inhibitor belonging to the benzoyl phenyl ureas group of IGRs with a broad-spectrum activity on various insects, but with very low toxicity to mammals (LD50 > 5000 mg/kg). The compound has a specific mode of action, affecting only chitin-contenting organisms[2]. In the laboratory, pilot and field experiments, a high efficacy of Novaluron at very low concentrations of 1-2 ppm was found against the major stored product insects, including the internal feeders: *Sitophilus oryzae* and *Rhyzopertha dominica* (Fig. 1). Novaluron prevented progeny emergence of internal feeders by influencing egg hatching. Few survived F_1 insects completely died after 3 – 4 months. The damage caused by the pests after 190-days storage was insignificant. Duration of Novaluron activity was at least 1 year.

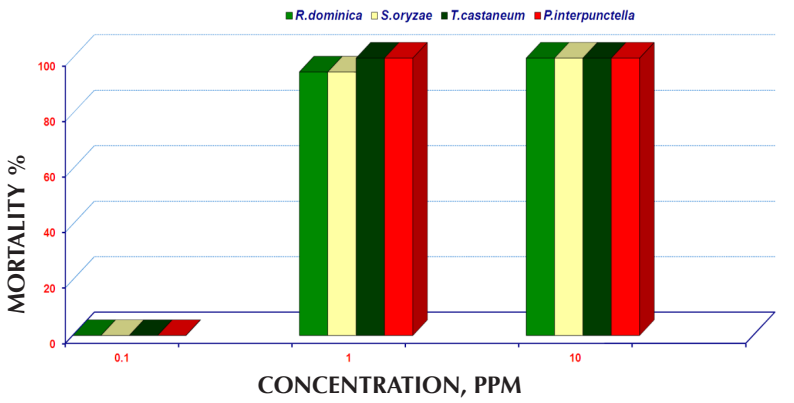


Figure 1 *Effect of IGR Novaluron against stored product insect pests (adult stage).*

Diatomaceous earth (DE) is known as one of the most promising alternatives to traditional residual insecticides. DE is a non-toxic, safe, natural origin material with a unique, non-chemical mode of action against insects which die through desiccation. Today, DE is in wide use for various products and processes, from toothpaste to cigars, plastics to paprika, filter media in swimming pools to home fish tanks, as well as insect and parasite control in animals and grain.

In the current study (Fig. 2), the laboratory and field evaluation of the sensitivity of the main external and internal stored product insect pests to commercial formulation of DE Detia Degesch Diatomaceous Earth – DDDE- Inerto was conducted. Among adults, *S. oryzae* and *O. surinamensis* were found to be the most susceptible to DE, regardless of the dose rate. After two and three weeks of exposure to DE, even at the lowest concentration of 0.5 g/kg, mortality of *S. oryzae* was 82 and 92%, respectively. Larvae of *T. castaneum,* were very susceptible to DE. Even if larvae survived and reached the adult stage, no progeny was produced. Nine weeks after treatment, the F_1 was 100% suppressed. The field trials proved that DE is effective against stored-grain pests, at the dose rate of 1-2 kg/t. A longer exposure may alleviate the need for increased doses in order to control species that are less susceptible to DEs. DE does not react much with the environment, which makes DEs ideal candidates for long-term grain protection [3].

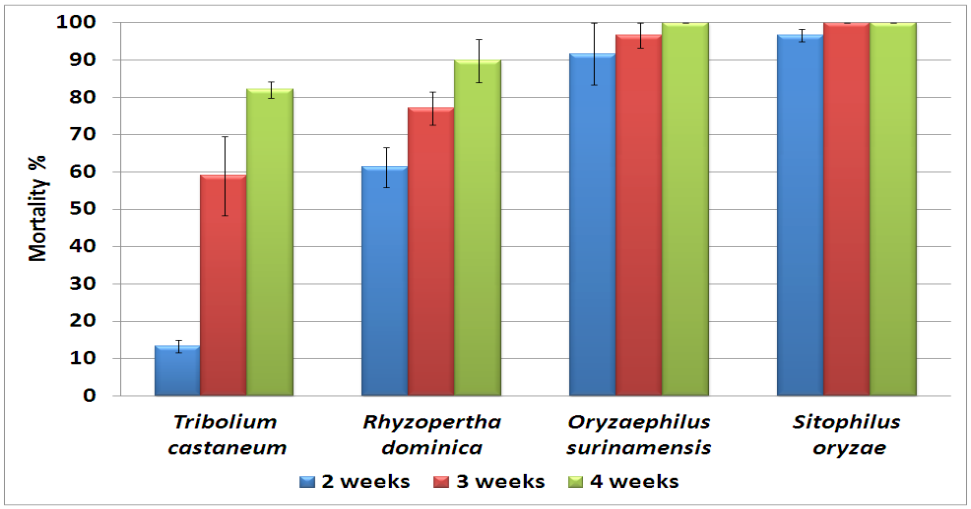


Figure 2: *Effect of DE on the tested insects, adults, 1 g/kg*

Inspection and monitoring is an inseparable part of the IPM strategy

Improvement of Phosphine fumigation by using speedbox

Fumigant Phosphine is mainly in use today for stored product insect pest control. However, some limitations, such as low temperatures and relatively long exposure time, limit its use. In order to overcome these difficulties, a special devise, called "Speedbox" has been developed by Detia Degesch Germany and can be used with Phosphine Degesch Plates®. The Speedbox is a small waterproof aluminium box containing a heater and a ventilator for injection and recirculation of the phosphine gas into the treated space. Our studies showed a high efficacy and advantage of the Phosphine fumigation by speedbox against stored product insects, compared with the common Phostoxin tablets and the others phosphine formulations [6]. The dynamics of Phosphine concentrations following fumigations using Speedbox and compared with different types of Phosphine formulations in use at the rate of 4 g/m^3 is shown in Table 2a. Using the Speedbox, Phosphine concentrations of 200 ppm and 830 ppm were reached 2 h and 8 h respectively following the fumigation compared with 35-102 ppm and 200-520 ppm with the other formulations.

Treatment	Phosphine concentration, ppm hrs						
	1	2	4	8	24	48	72
Al tablets	20	35	90	200	610	960	910
Mg tablets	30	102	185	300	590	730	650
Mg plates	40	85	210	520	1150	920	700
Mg plates by speedbox	80	200	470	830	980	830	630

Table 2a: *Phosphine concentrations following 72 hours fumigation using different types of formulations, 4 g/m^3 in the 15 m^3 fumigation room.*

Al: Phostoxin, Mg: Magtoxin

The accumulative Phosphine concentrations by using Speedbox were much higher during 72 h of the fumigation compared with the non Speedbox technologies (Table 2b).

Treatment	Phosphine concentration, ppm hrs						
	1	2	4	8	24	48	72
Al tablets	20	55	205	865	3185	12345	23775
Mg tablets	30	132	467	1522	3692	13127	19167
Mg plates	40	125	475	2155	6545	18665	28325
Mg plates by speedbox	80	280	1070	3840	7600	19070	28620

Table 2b: Accumulative concentration of Phosphine following 72 hours fumigation using different types of formulations, 4 g/m3 in the 15 m3 fumigation room.

Al: Phostoxin, Mg: Magtoxin

As a result of using Speedbox a high Phosphine concentration was reached practically at the beginning of fumigation, 100% mortality of all tested insects at all developmental stages were recorded after only 48 h treatment at the rate of 6 g/m^3 or 72 h at 4 g/m^3.

By using Speedbox, it was possible to reduce the time of Phosphine treatment from 7-10 days to 2-3 days to control all developmental stages of the major stored product insects [6].

To conclude, the implementation of an IPM approach which includes preventative measures, systematic inspection and monitoring for grain storage risk factors, the use of eco-friendly volatiles and protectants and improved technologies for currently used fumigants, decreases stored grain losses to very low level of less than 0.5%.

References

1. Kostyukovsky M, Rafaeli A, Gileadi C, Demchenko N, Shaaya E (2002) Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of activity against insect pests. *Pest Manag Sci* 58:1–6

2. Kostyukovsky, M. and Trostanetsky, A. (2006). The effect of a new chitin synthesis inhibitor, Novaluron, on various developmental stages of *Tribolium castaneum* (Herbst). *J. Stored Prod. Res*. 42: 136–148.

3. Kostyukovsky M, Trostanetsky A, Menasherov M, Yasinov G, Hazan T (2010) Laboratory evaluation of diatomaceous earth against main stored product insects. *Proceedings of the 10th International Working Conference on Stored Product Protection* 22-26 July 2010 Estoril, Portugal.

4. Kostyukovsky M and Shaaya E (2012) Advanced Methods for Controlling Insect Pests in Dry Food. In: I. Ishaaya et al. (eds.), *Advanced Technologies for Managing Insect Pests*, Springer, Dordrecht

5. Kostyukovsky, M., Trostanetsky, A., Quinn, E., Bernstein, S. and Rafaeli, A. (2014). Suppression of *Plodia interpunctella* moth populations infesting wheat warehouses in Israel using mating disruption Proceedings of the 9th Conference on Integrated protection of Stored Products. 01 – 04 July, 2013, Bordeaux, France. *IOBC-WPRS Bulletin*, 98: 99-104

6. Kostyukovsky, M, Trostanetsky, A, Quinn, E, Bernstein, S and Hazan, T (2014) Improved speedbox as an effective instrument for phosphine fumigation. Proceedings of the 9th Conference on Integrated Protection of Stored Products. 1–4 July, 2013, Bordeaux, France. *IOBC-WPRS Bulletin*, 98: 315-320

7. Raja N, Albert S, Ignacimuthu S, Dorn S (2001) Effect of plant volatile oils in protecting stored cowpea *Vigna unguiculata* (L.) Walpers against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infestation. *J Stored Prod Res* 37:127–132

8. Shaaya E, Kostyukovsky M (2006) Essential oils: potency against stored product insects and mode of action. *Stewart Postharvest Rev* 2(4):5:1–6

Acknowledgements

I am very thankful to the inspection unit team:

Dr. Anatoly Trostanetsky, Eng. Yigal Carmi, Mr. Yoram Gollani, Mr. Elazar Quinn, Ms. Solange Berenstein, Mrs. Chen Sela, Mr. Amir Dekel; to scientific collaborators: Prof. Eli Shaaya, Prof Ada Rafaeli, Prof. Nahman Paster, Prof. Uzi Ravid, Dr. Talat Hazan. The researches were founded by the Chief Scientist of the Israeli Ministry of Agriculture and Rural Development and Makhtishim-Agan Ltd.

About the author:

Dr. Moshe Kostyukovsky received his PhD degree in the Plant Protection Institute, Kiev, Ukraine. After repatriation to Israel, he joined in 1992 the ARO, Volcani Center. He is a leading researcher in the field of grain storage, stored product insects management, quarantine treatments, grain quality and sunn pest *Eurygaster integriceps*. He also serves as a Head of the Inspection Unit of the grain food and feed emergency stock in Israel.

Novaluron, a novel chitin synthesis inhibitor…… prevented progeny emergence

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Novel approaches to improve microbial quality and safety of dairy products

Moshe Shemesh
Department of Food Quality and Safety,
moshesh@volcani.agri.gov.il

Microbial damages caused by bacteria in the dairy industry are a fundamental threat to the safety and quality of milk products. Many bacteria in industrial settings tend to form multicellular communities known as biofilms. Individual cells in the biofilms are deeply embedded and protected by a self-produced matrix that consists mainly of sugars and proteins, which form a physical barrier. Biofilms represent one of the most successful strategies for bacteria to survive unfavorable environmental conditions, for instance in the food industry. In order to ensure the safety and quality of dairy food, there is a fundamental requirement of effective cleaning and sanitizing procedures. Otherwise, residual spores and bacteria on inadequately cleaned surfaces can quickly form multicellular biofilms that are extremely difficult to remove. Biofilms are not only a potential source of contamination, but can also increase corrosion rate, reduce heat transfer and increase fluid frictional resistance. Therefore, mitigation of biofilm forming species will enable the development of novel means and technologies for preventing biofilm formation and subsequent contamination of dairy products. We are currently developing three different approaches to control biofilm formation: (i) a model system to evaluate the cleaning and sanitizing effectiveness of milking equipments on dairy farms; (ii) a novel super-hydrophobic surfaces which minimizes bacterial adhesion and subsequent biofilm formation; (iii) searching for natural molecules capable of inhibiting the signal transduction pathway responsible for biofilm formation.

In spite of advances in food preservation techniques, bacterial spoilage remains a leading cause of global food loss. Nearly one-third of all food produced worldwide is estimated to be lost postharvest, and much of this loss can be attributed to microbial spoilage. Dairy products constitute one of the leading sectors impacted by food losses. The microbial quality of raw milk is crucial for the production of qualitative dairy products. Bacterial contamination can adversely affect the quality, functionality and safety of milk and its derivatives. It appears that the major source of the contamination of dairy products is often associated with biofilms on the surfaces of milk processing equipment. Biofilms are highly structured multicellular communities, which allow bacteria to survive in hostile environments. Dairy biofilms regularly contain significant milk residues, particularly protein and minerals such as calcium phosphate. Biofilms are not only a potential source of contamination, but can also reduce heat transfer and increase corrosion rate of metal pipes and equipment used in the milk industry. Thus, contamination of dairy products due to the presence of

bacterial biofilms is a major concern to modern dairy manufacturers, especially with current trends of longer production runs, the use of complex equipment, automation of plants and increasingly stringent microbiological requirements.

Bovine milk is highly nutritious and this makes it an ideal medium for the growth of microorganisms. It contains abundant water and nutrients (such as lactose, proteins and lipids) and has a nearly neutral pH. Since microorganisms in milk may hold spoilage and health risks, milk manufacturing is subject to extremely stringent regulations. These regulations include: (a) pasteurization at high temperatures, which kills most bacteria; (b) milk storage at low temperatures, which limits the growth of many bacteria; (c) dairy farm pipelines are regularly cleaned with alkaline and acidic liquids at high temperatures in a cleaning-in-place (CIP) procedure. Despite these stringent conditions, some bacteria are able to overcome these obstacles. For instance, thermophilic and spore-forming bacteria are able to survive pasteurization procedures, and psychrotropic bacteria thrive at the low temperatures in which milk is stored. Moreover, bacterial spores can survive treatment with reagents commonly used in CIP procedures. Some of these bacteria produce enzymes (proteases and lipases), resulting in off-flavors and curdling in the final product. Members of the *Bacillus* genus, which can undergo sophisticated differentiation pathways to generate different types of cells (Fig. 1), are of the most common bacteria found in dairy farms and processing plants. Spores produced by *Bacillus* species are highly resistant to a variety of stresses as well as hydrophobic, which allows them to adhere easily to food processing equipment. *Pseudomonas* species are considered as another important group of problematic bacteria in clinical as well as in industrial settings. Biofilms of *Bacillus* and *Pseudomonas* species are thus jointly regarded as the most significant microbiological problem in the food industry, because the damage they impose on the quality and safety of food products may impact public health as well as the economy. Their ubiquitous nature, combined with their ability to grow even at refrigerator temperatures, make them extremely difficult to control.

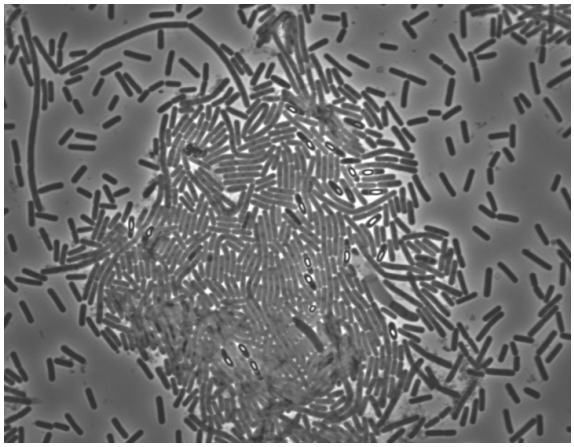


***Figure 1**: Phase contrast image of a Bacillus subtilis population which contains several different types of bacterial cells: motile single cells, chaining and bundling cells and sporulating cells. Magnification: approximately x1000.*

Preventing biofilm formation would be a much more desirable option than affecting it in the maturation stage, therefore a range of antimicrobial strategies have been proposed to control biofilms. However, conventional cleaning and disinfection regimens or present antimicrobial strategies may contribute to inefficient biofilm control and to the dissemination of resistance. Hence, presently there is no known technique that is able to successfully prevent or control the formation of unwanted biofilms without causing adverse side effects. In our lab, we are currently developing in parallel three different approaches to control biofilm formation associated to the food industry: (i) a model system to evaluate the cleaning and sanitizing effectiveness of milking equipments on dairy farms; (ii) the wax-based super-hydrophobic surfaces which minimizes bacterial adhesion and subsequent biofilm formation; (iii) identification of natural molecules inhibiting the signal transduction pathway responsible for biofilm formation.

Developing a model system to evaluate the cleaning effectiveness of milking equipments on dairy farms

There is need to establish a model system to evaluate the effectiveness of cleaning detergents in removal of biofilms from the surfaces of stainless steel, which is a predominant substrate in the food industry. Thus, we have developed the model system to evaluate the cleaning outcome based on *Bacillus* spores (Fig.2), which are surrounded by exopolymeric substances produced by bacteria during biofilm formation in special media[3]. This project is performed in collaboration with Avraham Harel from the Israel Dairy Board. The spores applied on sampling plates are mounted on T-junctions protruding either 1.5 or 3-times the milk pipe diameter from the main loop to resemble different levels of cleaning difficulty. The cleaning tests are conducted using commercial alkaline cleaning solutions at conditions which are relevant to actual farm environment. The cleaning effect is evaluated by comparing the numbers of spores (attached to sampling plates) before and after cleaning. The ability of the developed system to evaluate properly the cleaning efficiency of the tested solutions has been further validated using simplified laboratory scale flow system. Thus, our results indicate that the developed model system can simulate actual farm conditions for proper evaluation of the effectiveness of cleaning and disinfection solutions.

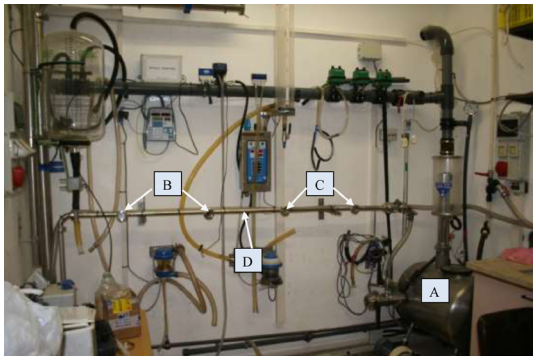


Figure 2: *The model system developed for evaluation of the effectiveness of cleaning solutions. The system was designed and developed in collaboration with Avraham Harel from the Israel Dairy Board. In the picture can be seen part of the system: releaser, A; two 75mm T junctions, B; two 35 mm T junctions, C; and the main pipeline (Ø25mm), D.*

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Developing novel surfaces which minimize bacterial adhesion and subsequent biofilm formation

Since adhesion is the initial and most important stage for successful colonization of bacteria, we aim to devise a technique that could be applied on surfaces used in food and other industries. Thus, we have developed novel surfaces that would prevent the adhesion of bacteria and consequently reduce biofilm formation. This project is carried out in collaboration with Prof. Boaz Pokroy from the Technion, Israel. Using bio-inspired smart technology, we successfully identified several wax-based super-hydrophobic surfaces, which minimize bacterial adhesion[2]. To test the efficacy of the generated surfaces, we examined the interactions of bacteria with wax-coated stainless steel, glass, and polystyrene substrates. It was demonstrated that our modified surfaces passively (with no toxic effect) and almost completely prevent the formation of biofilms by two different pathogenic bacteria, *B. cereus* (Gram positive) and *P. aeruginosa* (Gram negative) (Fig. 3), both considered to be extremely problematic bacteria in clinical as well as in industrial settings. Moreover, we further found that such wax surfaces can be formed on a great variety of materials and intricately shaped surfaces, making the technology potentially feasible for various medical and industrial applications.

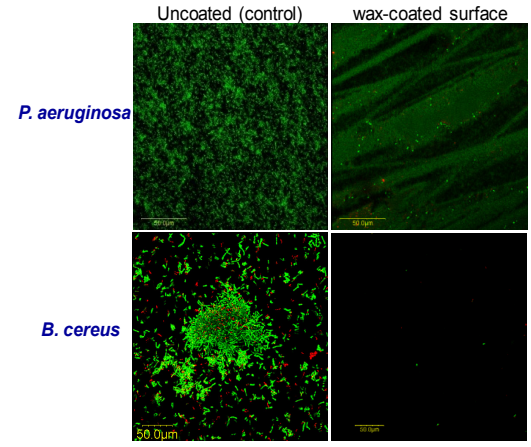


***Figure 3**: The inhibitory effect of developed surfaces on biofilm formation by Gram positive Bacillus cereus and Gram negative Pseudomonas aeruginosa. The samples were stained using a FilmTracerTM LIVE/DEAD Biofilm Viability Kit (Molecular Probes, OR) and visualized under an Olympus IX81 confocal laser scanning microscope (CLSM, Japan). Live cells were stained green and dead cells were stained red.*

Identifying natural molecules capable of blocking the signal transduction pathway responsible for biofilm formation

Biofilm bacteria established in milking installations are a continuous source of dairy products contamination. Biofilm formation depends on the synthesis of an extracellular matrix that holds the constituent cells together. In *Bacillus subtilis*, the model organism within the Gram positive bacteria, the matrix has two main components, an exopolysaccharide (EPS) synthesized by the products of the *epsA-O* operon, and amyloid fibers encoded by *tasA* located in the *tapA-sipW-tasA* operon. Both operons are under indirect control of the master transcriptional regulator, Spo0A, the activity of which depends

on its phosphorylation state. Spo0A phosphorylation is controlled by members of the Kin family, which include five histidine kinases. These kinases respond to different environmental and physiological cues, but the nature of these cues and how the kinases respond to them are not known in most cases. We have recently identified some of the signaling molecules that trigger biofilm formation, which enabled understanding the molecular mechanism for biofilm formation by the members of *Bacillus* genus[1]. Understanding the signaling mechanism for biofilm formation allows us to develop innovative approaches to prevent or delay the adherence and establishment of bacteria to surfaces used in the food industry. We are now identifying different natural molecules capable of inhibiting the signal transduction responsible for biofilm formation (Fig. 4), which will enable the development of approaches to prevent or inhibit the adherence of bacteria to the surfaces used in the food industry. For instance, our results indicate that some natural flavonoides may notably inhibit biofilm formation by *Bacillus* species (Fig. 5).

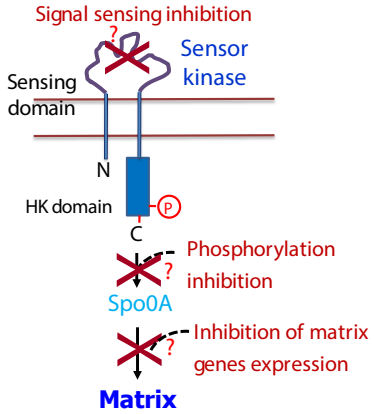


Figure 4: *Schematic representation of possible blocking mechanisms in the signal transduction pathway, which leads to matrix production and biofilm formation.*

Untreated *B. cereus* **Treated with flavonoide phloretin**

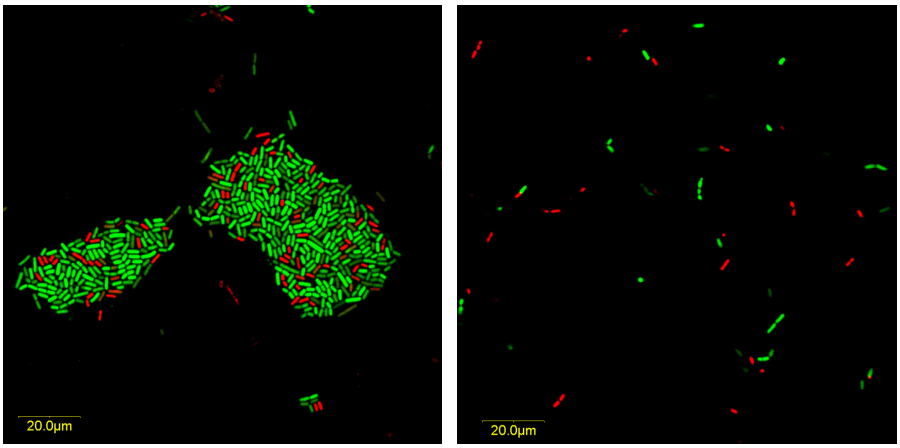


Figure 5: *Confocal laser scanning microscopy analysis of Bacillus cereus biofilms in response to flavonoide phloretin. The samples were stained using a FilmTracerTM LIVE/DEAD Biofilm Viability Kit (Molecular Probes, OR) and visualized under an Olympus IX81 CLSM (Japan). Live cells are stained green and dead cells are stained red.*

In conclusion, since biofilm bacteria represent a considerable threat to dairy food safety and quality, it is expected that development of novel means and technologies for the inhibition of biofilm formation will open opportunities for important innovations leading to the reduction of dairy food losses.

References

[1] Pasvolsky, R., Zakin, V., Ostrova, I. and Shemesh, M. (2014) Butyric acid released during milk lipolysis triggers biofilm formation of *Bacillus* species. *International journal of food microbiology* **181**, 19-27.

[2] Pechook, S., Sudakov, K., Polishchuk, I., Ostrov, I., Zakin, V., Pokroy, B. and Shemesh, M. (2015, in press) Bioinspired passive anti-biofouling surfaces preventing biofilm formation. *Journal of Materials Chemistry B.*

[3] Shemesh, M. and Chai, Y. (2013) A combination of glycerol and manganese promotes biofilm formation in *Bacillus subtilis* via histidine kinase KinD signaling. *Journal of bacteriology* **195**, 2747-2754.

Acknowledgments

I am grateful to my past and present group members, especially Solang Bernstein, Dr. Ronit Pasvolsky, Varda Zakin, Ievgeniia Ostrov, Danielle Asaf, Hilla Oknin and Dana Inbar. I am very grateful to my collaborators: Avraham Harel from the Israel Dairy Board and Prof. Boaz Pokroy and his team from Technion. I would like to thank Prof. Shlomo Sela and Dr. Uzi Merin (from ARO), Dr. Gabriel Leitner (from Kimron Veterinary Institute) as well as Doron Steinberg and Eran Lavi (from The Hebrew University of Jerusalem) for helpful discussions. I am very grateful to Drs. Shmuel Fridman, Moor Freed and Adin Shwimmer (from Israel Dairy Board) for their supportive suggestions and discussions. I would like also to thank Dr. Y. Chai from the Northeastern University for some *Bacillus* strains and helpful discussions. I am also thankful to Eduard Belausov for excellent technical assistance with confocal microscopy. Part of the presented work was supported by Grant 421-0251-13 from the Chief Scientist of the Ministry of Agriculture (Israel). The work was also partially supported by the Israel Dairy Board grants 421-0254-13 and 421-0270-14.

About the first Author

Moshe Shemesh received his Ph.D. from The Hebrew University of Jerusalem. His postdoctoral studies he pursued at Harvard University to understand mechanisms that governing the formation of complex communities of bacterial cells. Starting from 2011 he is a research scientist in the Department of Food Quality and Safety at ARO. His research interests are focused on investigating adaptation and survival mechanisms of spore forming bacteria in dairy food. His research is also dedicated to develop novel means to improve microbial quality and safety of dairy products.

VolcaniVoice
Agricultural Research Organization - Volcani Center Annual E-Newsletter

Minimizing Losses in Preserved Forage Crops

Zvi G. Weinberg* and Yaira Chen, Forage Preservation and By-Products Research Unit,
Department of Food Quality and Safety,
**Email: zgw@volcani.agri.gov.il*

Forage crops are used for feeding ruminant animals such as cows, beef, sheep and goats. The digestive system of ruminants includes the rumen, a large compartment in which billions of microorganisms help the animals digest the fibrous feed (roughage) which comprises whole crop forages. The main forage crops worldwide include maize, alfalfa, various grasses, and in Israel also wheat[13]. In areas where pasture is abundant all year round, animals get their roughage through grazing. However, where pasture or forage crops are not available throughout the year due to weather limitations such as frozen winters or dry seasons, forage crops are harvested in seasons when yields and quality are optimal and the large quantities must be preserved to ensure continuous supply throughout the year[13]. Methods to preserve forages should be efficient and economical. These include hay making and ensiling. The following paragraphs describe the pros and cons of each method, point out new developments and summarize recent studies of our Unit.

Hay making

Hay making is based on field drying of the harvested crops. Sunny and breezy conditions are paramount for the success of this process. The crops are harvested with a mower which sometimes conditions the plants, i.e., the crops are crushed and scratched between rollers in the mower in order to hasten the drying process. The harvested crops are spread over the field with occasional turning and tedding in order to allow drying of the lower layers (**Figure 1**); then they are windrowed and picked up for baling. Bales are usually stored under roof to avoid rain damage [13].

Figure 1 *Hay tedding*

Drying to below a critical moisture content (<15%) should be quick in order to minimize losses by the continued respiration of the plant material and to avoid molding. Not all plants are suitable for drying and those with wide stems are difficult to dry (corn plants for example). Weather conditions during harvesting and drying are not always favorable and rain might interfere with drying, damaging the crops and enhancing mold development. In some tropical areas the rainy season is not over when the plants are at the best quality for feeding; if one waits for the

rain to be over the crops might be too mature (lignified, woody) at harvest and an alternative preservation method should be applied. An additional problem during hay making relates to legume hay: the thin leaves in which most of the protein is found, dry fast, become brittle and drop before the stems have a chance to dry, thus contributing to field losses. Good quality hay has a green color, slightly pleasant smell with no signs of molds. Spoiled hay heats up, the color darkens to brown and molds are apparent. In rare events fires may break out in hay bales with moist spots due to intensive heating[13].

Ensiling

Ensiling is a preservation method of moist crops. It is based on anaerobic lactic acid fermentation whereby lactic acid bacteria (LAB) convert soluble sugars into organic acids, mainly lactic acid; as a result the pH decreases (the biomass becomes acidic) and the crops are preserved as long as no air penetrates. It is crucial that the LAB become the dominant microbial population in the ensiled biomass, and this is achieved by adequate moisture content of the harvested plants (usually 60-70%), adequate concentration of sugars for the fermentation (at least 3-5% in dry matter) and anaerobic conditions[6].

Ensiling is less weather dependent than hay making. However, it requires more investment in infrastructure (silos) and machinery. The steps of the ensiling process include harvesting the forage crop, chopping it, application of silage additives (organic acids or selected cultures of LAB [inoculants]), shipping to a silo (horizontal bunker silo, **Figure 2**, or upright towers), compaction to expel air and sealing with polyethylene sheeting. In a tower silo the biomass consolidates by its own weight and there is no place for sealing. In horizontal silos compaction is achieved with heavy tractors which move back and forth over the layers of chopped crop that are brought in. In recent years silages are prepared also in small bales and bags (sleeves) which enable more flexible use (**Figures 3 and 4**)[7].

Figure 2: *Silage in a bunker silo*

Figure 3: *Bagged silage*

Figure 4: *Baled silage (curtsey Racheli Gavrieli)*

Air is detrimental to silage because it enables aerobic microorganisms to spoil it, especially yeasts and molds[11]. The latter might also produce mycotoxins which involve potential negative health implications to animals and humans. In a bunker silo, the areas adjacent to the walls, the top and the corners between the walls and the top are the most susceptible for air penetration and thus spoil readily, if procedures are not followed precisely. Our studies[7] - revealed that air penetrates 1-2 m from the silage face into the silage. A rapid consumption of the silage helps to avoid face spoilage and losses. Top dry matter losses might reach over 75% of the top layers as compared with only 12% in the center which is the furthest from the walls and top[2] -. Recently an oxygen barrier (OB) plastic sheeting has been developed in Europe and experimental results indicate that it is effective in protecting the top layers of the silage.

In cool temperate climates the crops are very moist at harvest (75-80% moisture); the resulting silage suffers from high effluent losses and from inadequate fermentation which is referred to as secondary fermentation with high levels of butyric acid and unpleasant odors. The effluent (seepage) represents an environmental nuisance with potential pollution to fresh water sources.

Good quality silage has a typical color, smell and flowing texture, does not heat and has no signs of molds; moldy parts of silage are dark, they stick together and their smell is obnoxious.

Studies in our Unit

Silages are exposed to air during storage and more so when the silage is unloaded for feeding. In warm and dry climate such as in Israel, aerobic yeasts and molds are the causes of major silage preservation problems. Therefore, studies in our Unit focus on measuring aerobic spoilage and finding solutions to this problem. We developed a sustainable unit comprised of recycled soft-drink bottles in which we measure production of carbon dioxide, changes in pH and numbers of yeasts and molds as spoilage indicators during aerobic exposure[1]. Increase in temperature above that of the ambient temperature is also an aerobic spoilage indicator. It is possible to slow down aerobic spoilage with suitable additives which inhibit yeasts and molds (mainly volatile fatty acids which include acetic, propionic and butyric acids).

We discovered that application of LAB inoculants, which produce only lactic acid in the silage enhance aerobic spoilage[8]. That is because aerobic yeasts can utilize this acid. However, aerobic yeasts and molds are inhibited by volatile fatty acids such as acetic and propionic acids. Following this finding another bacteria, *Lactobacillus buchneri*, which converts lactic to acetic acid was introduced as silage additive[5].

Studies in our laboratory tested the effect of various parameters on aerobic stability: the effect of the stage of maturity of wheat at harvest[3], interaction with additives[10] and recently the effect of silage age and re-location of silage on aerobic stability[4, 12] . Our findings indicate that the longer the storage period of the silage, the better is its aerobic stability, because acetic acid is produced by *L. buchneri* only after 3 months of storage. Surprisingly, relocation of silage does not cause substantial damage or losses if the moved silage is of good quality. Another study indicated that baled silages of total mixed rations for lactating cows preserved well and their aerobic stability improved during the warm summer months in Israel[11]

Summary

The methods of forage crop preservation include hay making and ensiling. The preservation method should be chosen according to crop structure and composition and according to climate conditions. Both methods require strict adhesion to Good Managing Practices in order to avoid spoilage and losses.

Cited Literature

[1] Ashbell, G., Weinberg, Z.G., Azrieli, A., Hen, Y. and Horev, B. (1991). A simple system to determine the aerobic deterioration of silages. *Can. Agric. Engn.* **33**, 391-394.

[2] Ashbell, G. and Weinberg, Z.G. (1992). Top silage losses in horizontal silos. *Can. Agric. Engn.* **34**, 171-175.

[3] Chen, Y. and Weinberg, Z.G. (2009). Changes during aerobic exposure of wheat silages. *Anim. Feed Sci. Technol.* **154**, 76-82.

[4] Chen, Y. and Weinberg, Z.G. (2013). The effect of re-location of whole-crop wheat and corn silages on their quality. *Journal of J. Dairy Sci.* **96**, 406-410.

[5] Driehuis, F., Oude-Elferink, S.J.W.H. and Spoelstra, S.F. (1999). Anaerobic lactate degradation in maize silage inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. J. Appl. Microbiol. **87**, 583-594.

[6] Muck, R.E., Moser, L.E. and Pitt, R.E. (2003). Postharvest factors affecting ensiling. Chapter 6 in: Buxton, D.R., Muck, R.E. and Harrison, J.H. (eds.), Silage Science and Technology, Agronomy monograph 42, American Society of Agronomy, Madison, WI, USA. Pp. 251-304.

[7] Savoie, P. and Jofriet, J.C. (2003). Silage Storage. Chapter 9 in: Buxton, D.R., Muck, R.E. and Harrison, J.H. (eds.), Silage Science and Technology, Agronomy monograph 42, American Society of Agronomy, Madison, WI, USA. Pp. 405-468.

[8] Weinberg, Z.G., Ashbell, G., Hen, Y. and Azrieli, A. (1993). The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. *J. Appl. Bacteriol.* **75**, 512-518.

[9] Weinberg, Z.G. and Ashbell, G. (1994). Changes in gas composition in corn silages in bunker silos during storage and feedout. *Can. Agric. Engn.* **36**, 155-158.

[10] Weinberg, Z.G., Khanal, P., Yildiz, C., Chen, Y. and Arieli, A. (2010). Effects of stage of maturity at harvest, wilting and LAB inoculants on the aerobic stability of wheat silages. *Anim. Feed Sci. and Technol.* **158**, 29-35.

[11] Weinberg, Z.G., Chen, Y., Miron, D., Raviv, Y., Nahim, E., Bloch, A., Yosef, E., Nikbakhat, M. and Miron, J. (2011). Short communication: preservation of total mixed rations for dairy cows in bales wrapped with polyethylene stretch film – a commercial scale experiment. *Anim. Feed Sci. and Technol.* **164**, 125-129.

[12] Weinberg, Z.G. and Chen, Y. (2013). Effects of storage period on the composition of whole crop wheat and corn silages. *Anim. Feed Sci. and Technolog.* **185**, 196-200.

[13] Wilkinson, J.M., Bolsen, K.K. and Lin, C.J. (2003). History of Silage. Chapter 1 in: Buxton, D.R., Muck, R.E. and Harrison, J.H. (eds.), Silage Science and Technology, Agronomy monograph 42, American Society of Agronomy, Madison, WI, USA. Pp. 1-30.

[14] Woolford, M.K. (1990). The detrimental effects of air on silages. *J. App. Bacteriol.* **68**, 101-116.

About the first Author

Dr Zwi Weinberg did his Ph.D. degree at Cornell University in Food Science. His research pertains mainly to applied aspects of forage preservation and utilization of by-products for ruminant feeding. In recent years he was involved in studying the effect of cultivation of cattle manure on the survival of *E. coli* in the manure, the extent of antibiotic resistance of silage bacteria and the effect of lactic acid bacteria which are used as silage inoculants on the survival of *E. coli* in rumen fluid, as part of their propbiotic effects on ruminants.

ARO
Volcani Center
Bet Dagan
50250, Israel

Vision:

"Excellence in research and development
for the promotion of agriculture and sustainability
of the environment"

Editors:
Prof. Ada Rafaeli & Dr. Nirit Bernstein

We're on the Web!
Visit us at:
www.agri.gov.il