Biosecurity Guidelines for Post-harvest Greenhouse Tomatoes: Prevention of Post-harvest and Storage Rot

Submitted to

B.C. Ministry of Agriculture
&
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by

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Disclaimer

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1. Introduction:

Fungal storage rots are an important cause of post-harvest loss in conventional and organic greenhouse tomatoes. Prevention is the most useful and powerful strategy against tomato storage rots and other greenhouse tomato diseases.

Preventing storage rots in greenhouse tomatoes requires a comprehensive and coordinated approach throughout crop growing and handling including all steps in the pre- and post-harvest production pathways (PHPP). The PHPP used by producers include the process from the time of crop picking until the time that the product arrives at the customer. Two PHPP flow charts were developed from a grower survey conducted during April-June, 2011 (Figures 1 & 2). The exact pathway that harvested tomatoes follow will depend on the grower, client, type of tomato and availability of equipment. Growers are advised to use these Biosecurity Guidelines as they apply to their own greenhouse operation.

The overall objective of the project was to develop Best Management Practices (BMPs), i.e. Biosecurity Guidelines, for growers and packers that can be used to reduce storage rots in greenhouse tomato crops with the emphasis on greenhouse sanitation, climate control, disease and pest management, proper crop handling and shorter packing and storage time. The principle of preventing post-harvest fruit rot is to avoid the 3 D's:
1) damaging the fruit; 2) disease build up and 3) dampness on the fruit surface.

2. Correctly identify storage rot fungi

Post-harvest storage rots often begin at stem scars and wounds on the tomato surface which become infected by pathogenic and saprophytic fungi and bacteria. These fungi overwinter in crop debris and organic matter in and outside the greenhouse then spread into the greenhouse and storage areas by windborne spores, greenhouse staff and visitors, and pests such as fruit flies and fungus gnats. Soilborne fungal spores could be a source of storage rot fungi in organic greenhouses where seedlings are grown directly in the soil where soil surface is exposed. Late blight is not listed below and is not found in commercial greenhouses where climate is controlled and plant foliage remains dry. Fungal spores causing storage rots can build up in a greenhouse, especially in an organic greenhouse when leaf debris remains moist and becomes colonized by fungi (Punja, 2012).

Results from BC greenhouse surveys of diseased tomato fruit in 2011 found three *Penicillium* species, *P. olsonii, P. polonicum, P. solitum*, were most common during May to June. They were also the most common fungi isolated from fruit surfaces and calyx tissue. The next most common fungi isolated from disease tomato fruit were *Alternaria alternata,* then *Botrytis cinerea, Rhizopus stolonifer* and *Geotrichum candidum* respectively. *Rhizopus stolonifer* was the most common fungus isolated from diseased tomato fruit collected in summer (July- August). Lab experiments at 90- 92% RH found that wounding the fruit had the most effect on increasing the lesion size of *Botrytis, Rhizopus* and *Geotrichum. Rhizopus* had the largest lesion size at 18˚ C and 10˚ C. Storage of harvest tomato fruit at 10˚ C slowed storage rot development (Punja, 2012).

2.1 Correct disease identification

Correctly identify the crop disease by sending a sample to the B.C. Ministry of Agricultures' Plant Diagnostic Laboratory, 1767 Angus Campbell Road, B.C. V3G 2M3, Tel: 604-556-3127 OR any other reputed Diagnostic Laboratory.

2.2 Common greenhouse tomato storage rots:

1. Black mold rot (*Alternaria alternata*),
2. Blue mold rot (*Penicillium* spp.)
3. Gray mold rot (*Botrytis cinerea*)
4. Leaf mold rot (*Cladosporium fulvum*)
5. Hairy rot (*Rhizopus stolonifer*)

2.2.1 Black mold (*Alternaria alternata*)

Tiny lesions form on the shoulders or near the stem scar of the fruit where there is cuticle cracking or damaged surfaces. Green fruit are quite resistant. Lesions start as water-soaked then become brown to black with tiny, pimple-like fruiting structures (perithecia) and may have a covering of dark gray to olive green spores. Lesions can form even at low temperatures but grow rapidly at 24-28°C. Spread can occur by fruit-to-fruit contact in storage. Decaying plant debris is a major source of the fungus; the fungus is ubiquitous and spreads by windborne spores.

2.2.2 Blue mold (*Penicillium olsonii, P. polonicum, P. solitum*)

Disease symptoms are like grey mold rot except the fungal growth on fruit is a distinct bluish-grey colour. Disease tomato fruit with spotting, necrotic lesions and fruit softening were sampled from B.C. greenhouses from May- August, 2011. The most common tomato fruit rot and the most common fungi from tomato fruit and calyx were three *Penicillium* species: *P. olsonii, P. polonicum and P. solitum* (Punja, 2011).

2.2.3 Grey mold (*Botrytis cinerea*)

Grey mold rot is an important pre- and post-harvest disease and often penetrates the pericarp directly if growing from a food base such as plant debris or decaying petals. Lesions are gray-brown slightly water-soaked near the stem end of the tomato. A grayish mold forms at breaks in the skin surface or sparsely over the fruit surface. The disease grows rapidly at 18- 24 °C and continues even at 0 °C. Tomatoes held in cold storage for a prolonged period are at risk to grey mold rot, especially damaged fruit.

2.2.4 Leaf mold rot (*Cladosporium fulvum*)

Green and mature fruit can form a black, leathery rot similar to black rot at the stem end. The lesions do not have definite margins and can cover large portions of the fruit.

2.2.5 Hairy rot (*Rhizopus stolonifer*)

Hairy rot is often thought of as a disease of ripe fruit but it can affect all fruit ripeness stages. Lesions rapidly enlarge as water-soaked spots at wounds, stem scars or open stylar pores but they are not normally discoloured. Infected area is soft and watery and covered with coarse, grayish-white strands of the fungus. Nests of rotted fruit will form in packages with an odor like eggs not the sour odor of bacterial soft rot.

3. Pre-harvest sanitation (Woodske and Sabaratnam, 2008, Ferguson, 2006)

Following strict sanitation is critical in preventing storage rots and other greenhouse diseases.

- Start with a thorough year-end cleaning and disinfecting of all empty, greenhouse facilities so that diseases do not carry over to the new crop.
- Maintain a high level of sanitation throughout crop growing, harvesting and transporting phases.
- Adopt standard operating procedures (SOP) ensuring that sanitation programs are in place, maintained and recorded.
- Keep logs with records documenting all sanitation procedures and maintenance.
- Follow the U.S. Food and Drug Administration guide for preventing food safety hazards of tomatoes. Guidelines will also prevent greenhouse storage rots (U.S. FDA, 2009).

3.1 Year-end greenhouse sanitation

- When the crop is finished, remove all plants and crop debris from inside the greenhouse and dispose off-site in a landfill or composting site or bury and cover.
- Before washing out the greenhouse remove all equipment.
- Thoroughly clean the empty greenhouse structure, including all surfaces, heating pipes, walkways, and benches.
- Clean and sanitize all equipment, trays, carts and trolleys.
- Apply a high pressure wash with detergent to remove any soil residue, dust and debris, salt and organic matter build up from all interior surfaces.
- Before sanitizing with a disinfectant spray be sure to rinse off any soap residue as soap can inactivate disinfectants.
- Allow time for washed surfaces to dry.
- Disinfectants can be inactivated by organic matter, improper pH and sunlight. Carefully read and follow the label directions, rates and worker safety precautions (protective wear). In organic production, first determine which disinfectants are accepted.
- Treat dry surfaces with disinfectant not wet surfaces which will dilute the disinfectant.
- Never mix bleach and ammonia as toxic chlorine gas will result.
- Use one of the disinfectants listed below to sanitize all interior surfaces taking care to reach all horizontal surfaces (rafters, ledges, piping) and other hard to reach spots.
- Ideally mix disinfectants in warm water (approximately 20 °C) and apply the solution to dry surfaces during the evening in a warm greenhouse. The disinfectant works more effectively at higher temperature and requires less contact time than at lower temperatures. Use the same disinfectant if repeating the application.
- To prevent the build up of pests and diseases leading to storage rots, also refer to B.C. Ministry of Agriculture factsheets: "On-Farm and Greenhouse Sanitation and Disinfection Practices" http://www.agf.gov.bc.ca/cropprot/cleanup.htm

- In organic production, pasteurize soil using steam to heat the soil to 60˚C for 30 minutes to eliminate pests and diseases. www.ontario.ca/crops

3.2 Pre-harvest sanitation

3.2.1 Visitors and workers

Visitors and workers use foot baths (for a minimum of 30 seconds) and hand wash stations at all entrances and exits to the greenhouse. Use footbaths between greenhouse compartments. Visitors wear sanitation suit or lab coat and plastic booties.

- Workers use gloves that can be regularly dipped in a sanitizing agent. Or thoroughly and frequently wash hands with soap and water and dry with a single use towel for all phases of production when crop is handled.
- Disposable gloves are more effective than hand washing or the use of hand sanitizers.
- Wash hands thoroughly or change gloves when handling materials other than tomatoes or if gloves become damaged or contaminated.
- All staff wears clean clothing daily, or uniforms and separate foot wear for greenhouse use.
- When harvesting, workers wear gloves or thoroughly and frequently wash hands and have short fingernails and no jewelry.

3.2.2 Greenhouse

- As frequently as possible, sanitize all pruning tools, knives and picking containers. Dip cutting knives in disinfectant for minimum of 2 minutes between plants. Use two knives and alternate by leaving one in the disinfectant.
- Keep all walkways and surfaces as clean as possible.
- Promptly remove prunings and crop debris from the greenhouse and dispose off-site in a landfill or composting site or bury and cover.
- Remove entire dead or dying plants by securing them in bags when removing them from the growing row. Use a separate wheelbarrow or cart to remove bagged plants from the greenhouse. Dispose off-site. Disinfect the wheelbarrow or cart regularly.
- Eliminate puddles and wet surfaces by maintaining good drainage. This will reduce breeding sites for insects such as fungus gnats and shoreflies which could spread diseases.
- Keep at least a 10-metre strip of mowed lawn around the greenhouse and keep weeds around the greenhouse controlled as these may harbor pest and diseases.
- Control other pests including rodents which can spread disease and injure fruit.
- Clean and sanitize picking containers (totes, bins etc.) regularly by pressure washing and treating with KleenGrow® (formerly Chemprocide®). Ensure that they remain clean and dry before reuse. Picking containers are contaminated by airborne fungal spores.

4. Post-harvest sanitation

4.1 Packinghouse

- Clean and sanitize empty packinghouse facilities as outlined for year-end greenhouse sanitation.
- Clean and sanitize all interior surfaces including walls, floors, equipment, containers, totes, bins and pallets to ensure that harvested tomatoes do not become contaminated.
- Maintain strict sanitation in all holding areas, packinghouse, storage and distribution facilities.
- Regularly sanitize and maintain all harvesting, sorting and packing equipment according to manufacturer directions and the disinfectant label.
- Packinghouse machinery and sorting, weighing and packing lines from the Dutch manufacturer Aweta BV are customized to grower specifications. Clean and sanitize equipment according to manufacturer's guidelines and the U.S. FDA guidelines to minimize microbial food safety hazards and storage rots.
- Inspect and ensure packaging is clean and uncontaminated by storing in a sanitary manner off the floor.
- Empty cull bins, debris and waste at least daily and dispose off-site or bury. If composting, be sure to entirely cover the cull pile with soil or a tarp.
- Clean and sanitize reusable plastic containers (RPC) according to SOP before reuse and include accurate label information regarding reuse.
- Ensure that insect and rodent pests are controlled.
- Visually inspect the trucks before loading. Clean and then sanitize by spraying KleenGrow® if the truck is unclean. (see 5.5)

4.2 Distribution center

- Distribution centers used by large producers require the same high sanitation standards as other greenhouse facilities described above.

5. Greenhouse disinfectants

5.1 Disinfectants, based on chemical type, for year-end greenhouse sanitation

Alcohols	Halogens	Peroxides	Quaternary ammonia
Ethanol	bleach	Hyperox®	KleenGrow®
Isopropanol		Virkon®	Virocid®

5.2 Disinfectants for pruning tools and knives

Dip tools frequently for 2 minutes using one of the disinfectants**:**

- 70% alcohol
- 10% bleach (corrosive to metals)
- 0.1- 0.2% KleenGrow® (periodically rinse afterwards)
- 5% Virkon®

5.3 Disinfectants for greenhouse production areas, facilities and equipment

- KleenGrow® is the only registered disinfectant for use in greenhouse production areas. Follow label directions and thoroughly wet surfaces with KleenGrow® but do not rinse off. Use 8ml/L product for surfaces and equipment. Use 30ml/L of product for wood, painted and concrete surfaces.

5.4 Disinfectants for picking totes and cull bins

- First remove debris by pressure rinsing,
- Secondly, thoroughly wash totes with detergent and then rinse.
- Sanitize as the final step by using KleenGrow® or a bleach dip and rinse all fruit contact surfaces.

5.5 Disinfecting vehicles

- Steam clean in a commercial facility before loading.
- KleenGrow® : use 4ml/L; or Virkon®: use 1 ml/L; follow label directions

6.0 Avoid fruit damage

- Avoiding tomato fruit damage is an absolute requirement to reduce storage rots. Avoid any fruit damage during production, harvesting and handling. Do not bruise or puncture fruit when harvesting and handling.
- Avoiding fruit damage also applies to insect pests that may feed and damage the fruit surface. Follow integrated pest management practices to control insect pests.
- Harvest and handle with care so that fruit is not damaged and TOV’s are not punctured by the truss or stems.
- Reduce the amount of handling of fruit.
- Picking and packing directly into shipping container is an example of reduced fruit handling. Carefully handle and pack fruit to ensure there is no disease, wounded or damaged fruit.
- No debris should fall into containers.
- Do not over-pack harvest and market containers (clamshells).
- Slight injury such as fine cracking, light bruising and wounding provide openings for fungal infection and storage rots.
- Use sharp tools and avoid puncturing, nicking or pocking fruit when pruning or picking.
- Ensure that fruit doesn’t rub against clamps used to attach plants to the support twine.
- Some packaging, such as clamshells, appears to have higher incidence of post-harvest rot.
- Carefully tip totes onto packinghouse conveyor belts so that fruit is not damaged.
- Meticulously inspect and sort out damaged fruit as culls.
- Be sure to cover cull bins and empty them frequently off site.
- Carefully load pallets onto trucks to ensure stability and avoid any physical damage to the fruit during transport.
- Trucks with air ride suspension may help reduce fruit damage by reducing truck vibration during transport.

7.0 Tomato varieties

Check with seed company specialists for varieties with improved physiological characteristics including: shelf life, resistance to storage rots, high yield, free from cracking, disease resistance and quality.

- Storage rots were reported on all seven types of tomatoes grown in B.C., including beefsteak, TOV's, cherry, cocktail, grape, Roma and tomato berries.
- TOV and beefsteak varieties were reported to have more problems with storage rot. TOV's are very susceptible to gray mold, especially when film-wrapped in a tray.
- Quality issues with large fruited tomatoes could lead to storage rot.
- Ensure the truss of TOV varieties is healthy and without wounds or signs of disease when harvested. The truss and calyx can be a major source of diseases.

8.0 Cuticle cracking

Concentric cuticle cracking has been reported on all types of tomato varieties grown and is due to a complex interaction of genetic, climatic and cultural factors. Research is needed to determine the effects of relative humidity, temperature, mineral nutrient requirement and irrigation practices on fruit water status in relation to cuticle cracking. (Dorais et al, 2004). Cuticle cracking in tomato was studied in relation to fruit development and a model was developed based on environmental and cultural conditions (Ehret, 2008).

8.1 Cuticle cracking symptoms

- Cuticle cracking is also known as russeting, hair cracking, swell cracking, shrink cracking, micro-cracking, rain check, crazing and cuticle blotch.
- A network of very fine hair-like cracks (0.1- 2.0 mm in length) occurs in the cuticle and the first layers of epidermal cells.
- Cuticle cracking is located around the stem scar or multidirectional on the sides and bottom of fruit.
- Tomato fruit quality is reduced by cuticle cracking which creates wounds where storage rots enter and develop.

8.2 Minimize cuticle cracking

- Ensure high fruit load is balanced with adequate plant vigour.
- Avoid high temperatures, large day/night differentials and high relative humidity.
- Avoid direct exposure of fruit to solar radiation and large fluctuations in fruit water status.
- Keep the greenhouse as cool as possible when the outdoor weather is hot.
- Reduce water flow at the end of hot days.
- Balance fertilizer concentrations.

9.0 Climate control

9.1 Greenhouse

Temperature and relative humidity are essential in reducing greenhouse diseases.

- Keep an optimal vapour pressure deficit between 0.5- 0.8 kPa

- Follow recommended tomato temperature schemes (19°C optimum 24-hour temperature) and relative humidity (ideally below 85%)
- Keep plant foliage dry. Do not allow condensation (free water) to form on plant surfaces as this encourages disease development.
- Avoid condensation on plants by increasing air temperature before sunrise relative to outdoor temperature, followed by limited opening of vents.

9.2 Packinghouse

- Ensure that there is good lighting where tomatoes are sorted so that small defects and fruit blemishes can be seen and fruit culled.
- Passive air movement systems are used for cooling
- Locate packinghouse close to greenhouse.
- Deliver product to the packinghouse as soon as possible.
- Cooler temperature and faster turn around times are best.
- Growers report packinghouse temperatures of 15, 20 and 24 °C with turn around times from product arrival to shipping of 2 days, 8 hours and up to16 hours respectively, depending on the facility and time of year.
- Reduce turn around times in the packinghouse as temperatures increase.
- Product may need to be placed in a cooler when temperatures are higher but there is a risk of condensation and storage rots due to fruit cooling and warming.
- When the temperature is cooler (15 °C) product may be held up to two days in the packinghouse.
- Stack pallets of packed tomatoes to ensure there is good air circulation and product temperature before and during shipping and in distribution centers.

9.3 Distribution center

- Large producers with distribution centers hold their product at controlled temperatures of 11°C, 12°C or 15.5°C depending on maturity stage and ripening time.
- Reduce the length of time from harvest to consumption.
- Ship tomatoes as quickly as possible.
- Hold tomatoes no longer than 10 days to two weeks under ideal storage and cooling temperatures.

9.4 Cooling for storage

- Pre-cooling is required only when fruit temperature is >26-27 °C and ripening is to be delayed.
- Rapid cooling soon after harvest is essential for keeping quality.
- 12.5°C (55°F) is the precooling endpoint. Room cooling is more common but forced-air cooling is the most effective practice.
- Good air circulation is needed for effective heat removal over time in storage.
- No lower than 10°C (50°F).
- 2-3 weeks storage
- Optimum relative humidity is 90-95% to prevent water loss (desiccation) but this favors stem scar and surface molds; use ~85%.

9.5 Climate control in storage (Suslow, 1996)

- Cooling is the most effective way of extending tomato shelf life.
- Promptly cool fruit after picking to approximately 20 °C. Do not leave harvested fruit for long periods of time in a hot greenhouse.
- Rapid cooling after tomatoes are harvested is essential for optimal post-harvest keeping quality. Temperature regulation once the product is cooled is essential to maintaining quality and shelf life.

9.6 Maintaining the 'cold chain'

The most important step in safeguarding shelf life and reducing storage rots is to maintain the cold chain (temperature and humidity management) during the post-harvest production pathway (PHPP). Ideally, ensure tomatoes are cooled shortly after picking and then maintain that temperature until they reach the customer.

- Maintain the cold chain during cooling, storage and transport so that fruit temperature is kept cool throughout the PHPP (Figure 1, 2).
- Start in the packinghouse by immediately sorting fruit at room temperature. Do not keep produce in a cool area or cooler in the packinghouse before sorting and packing
- Once the fruit is sorted and packed it can be quickly moved into storage areas that are cooled and regulated or loaded immediately onto a precooled truck ensuring that there is room between the pallets for proper circulation of refrigerated air.
- Load the pallets of tomatoes towards the truck center and place insulating plastic strips inside the door of the truck
- Monitor and maintain the temperature inside the truck during transport.
- A refrigerated loading and unloading area would be useful during hot conditions but likely cost prohibitive.
- After the fruit has been cooled the temperature should not be increased until it arrives at the customer.

9.7 Ripening temperatures

- 18- 21 °C (65- 70 °F) and 90- 95% RH for standard ripening
- 14- 16 °C (57- 61 °F) for slow ripening (i.e. in transit)

9.8 Control moisture condensation

- Allow air circulation and ventilation of the produce during storage
- Maintain refrigeration coils within 1°C of the air temperature and maintaining the cold chain reduces the risk of condensation.
- Prevent condensation caused by warmer, moist air striking cold produce.
- Avoid moving product from cool to warmer storage as condensation on the fruit surface will form.
- The surface of dry fruit is protected by a layer of air and/or natural wax that can impede infectious microorganisms such as storage rot fungi. Fungi require a film of water or wounded plant surfaces to infect the fruit.
- Do not reduce relative humidity below 85% to try to control condensation as this will lead to excessive water loss (desiccation) of fruit.

10. Chilling injury

- Once the tomato has been cooled in storage it shrinks slightly in size. If product is warmed up again it can swell slightly and the tomato skin can crack similar to the greenhouse where osmotic pressure in the fruit changes and the skin cracks. Shrink cracks are wounds that allow entry of storage rots.
- Tomatoes are sensitive to chilling injury if exposed to temperatures from -1 to 12.5 °C depending on the length of time and the stage of fruit maturity.
- Green tomatoes are more susceptible to chilling injury than ripe tomatoes.
- Tomato fruit with chilling injury won't ripen properly or form full color and flavor. The fruit surface damaged by cold temperature will become blotchy, soft, pitted and the seeds will turn brown.

10.1 'Mature green' and 'color breaking' stage

- 'Mature green' or fruit harvested at 'color breaking' stage should not be held lower than 12 °C (55 °F) as chilling injury will adversely affect quality and ripening.
- Store mature green fruit at 13-15 °C for one to two weeks
- Short term storage or transit temperatures below the range of 10-12.5 °C are sometimes used but will cause chilling injury in several days.
- Turning or color breaking tomatoes can be stored at 10-13 °C for one to two weeks

10.2 Ripe tomatoes

- Fully ripe tomatoes may be held at 2-5 °C for a few days prior to consumption but not longer as color loss and softening occur.

11. Optimum storage temperatures

11.1 Mature green: 12.5-15°C (55-60°F)

- Mature-green tomatoes can be stored up to 14 days prior to ripening at 12.5°C (55°F) without significant reduction of sensory quality and color development.
- Decay is likely to increase following storage beyond two weeks at this temperature.

11.2 Light red (USDA color stage 5): 10-12.5 °C (50- 55 °F)

11.3 Firm red (USDA color stage 6): 7- 10°C (44- 50°F) for 3-5 days.

- Typically 8-10 days of shelf life are attainable within the optimum temperature range after reaching the Firm-ripe stage.
- Short-term storage or transit temperatures below this range are used by some but will result in chilling injury after several days.

12. Controlled atmosphere (CA) technology

- Extended storage with controlled atmosphere (CA) has been demonstrated (Jan, 2003).
- Low O_2 (3-5%) levels delay fruit ripening and mold incidence on fruit surface and near the stem scar; fruit quality is acceptable.
- 3% O_2 and 0-3% CO_2 is typically used for up to 6 week's storage prior to ripening.
- Elevated CO_2 above 3-5% is not tolerated by most cultivars and will cause injury.
- Low O_2 (1%) will cause off-flavors, odors and other condition defects, such as internal browning.

13. Responses to ethylene

- Tomatoes are sensitive to external ethylene and exposure of mature-green fruit initiates ripening and shortens the storage time.
- Tomatoes release ethylene at a moderate rate while ripening.
- Avoid co-storage and shipping with lettuce and cucumbers and other sensitive produce.

14. Shipping

- Industry stores and ship tomatoes at 11-15.5 °C depending on ripening stage and expected length of time in storage.
- Use sanitized, temperature regulated trucks with incorporated temperature sensor to move pallets of tomatoes from the greenhouse to an off-site packinghouse and from the packinghouse to distribution centers or directly to market.
- Place a separate temperature recording device in the truck to monitor temperatures in transit.
- The buyer may decide where to place the temperature recorder or place it mid-way in the truck or next to the door.
- Storage temperatures may be lowered to 10°C if tomatoes are shipped with other crops such as bell peppers or cucumbers but could cause chilling injury.

15. Summary

20 Steps to Prevent Greenhouse Tomato Storage Rots

1. **Avoid the three D's: 1) damaging the fruit; 2) disease buildup and 3) dampness on the fruit surface**.
2. Prevention is the most useful and powerful strategy against tomato storage rots and other greenhouse tomato diseases.
3. Correctly identify the disease causing storage rot.
4. Prevent storage rot fungi during crop growing and handling including all steps in the pre- and post-harvest production pathways (Figure 1, 2) by following best management practices: sanitation, climate control, disease and pest management, proper crop handling and short packing and shipping time to customer. Ensure that stem and calyx tissues are healthy. Rhapsody (*Bacillus subtilis* QST 713) applications during production, particularly prior to harvest, helped prevented spread of these storage rot fungi onto the fruit. (Chatterton, 2012; Punja, 2013) Storage rot was reduce to 0 – 5% when Rhapsody applications were made every 3 weeks and fruit stored at 13º C for no more than 12 days (Punja, 2013).
5. Follow strict year-end greenhouse sanitation and continue throughout the pre-harvest and post-harvest production pathways.
6. Workers and visitors keep strict sanitation protocols: foot baths (minimum time of 30 seconds), clean clothing or uniforms, dedicated footwear and hand sanitizing or gloves.
7. Grow tomato varieties that have less storage rot problems.
8. Promptly remove crop debris and prunings from the greenhouse.
9. Ensure that the truss of TOV's is healthy and without wounds or disease when harvested. The truss and calyx can carry the major microbial load.
10. Optimize growing conditions and production practices to minimize cuticle cracking and any wounds, bruises or damage to fruit surfaces.
11. Control condensation. Do not allow free water to form on plant or fruit surfaces.
12. Reduce turn around times in the packinghouse, distribution center and shipping.
13. Shorten the time from harvest to customer.
14. Ensure there is good lighting in the packinghouse for sorting and culling fruit.
15. Immediately sort fruit in the packinghouse and do not keep in cooler before sorting and packing. Cooling and warming fruit increase the risk of condensation on the fruit surface and can create ideal conditions for storage rot.
16. Avoid chilling injury by keeping fruit at the optimal storage temperature (see section 11.). Do not store below the pre-cooling endpoint of 12.5 °C.
17. Maintain the cold chain once tomatoes and packed and cooled and shipped to the customer. Ensure that tomatoes are cooled shortly after picking and then maintain that temperature until they reach the customer.
18. Controlled atmosphere (CA) storage: 3% O_2 and 0-3% CO_2 is typically used for up to 6 weeks storage prior to ripening tomatoes.
19. Hold tomatoes no longer than 10 days to two weeks under recommended storage and cooling temperature.
20. Ship using sanitized, temperature regulated (12.5 °C) trucks with air ride suspension ensuring that load does not shift during transport.

16. New technologies

16.1 Pre-harvest treatments

Follow accepted integrated pest management and best management practices throughout the growing season and PHPP to reduce fruit wounds, damage and storage rots.

Biocontrol:

Rhapsody ASO (*Bacillus subtilis*)

Laboratory and greenhouse tests by Dr. Zamir Punja in 2011 and 2012 showed that tomato storage rot fungi were significantly reduced when *Bacillus subtilis* (Rhapsody ASO) was applied to either pre-harvest or post-harvest tomatoes. The ability of biocontrol bacteria or fungi in reducing fruit colonization as well as post-harvest decay is worthy of further study (Punja, 2012).

Greenhouse Testing of Rhapsody (*Bacillus subtilis)* for Tomato Fruit Rot Control
Dr. Z.K. Punja, Simon Fraser University, November 30, 2012

Rhapsody ASO registered in Canada on greenhouse tomatoes:

Rhapsody ASO (*Bacillus subtilis)*, PCP 28627, is an aqueous suspension biofungicide used as a broad spectrum, preventative treatment to suppress ornamental, crop, turf and lawn diseases. Registered use in Canada includes crop group 8, greenhouse tomatoes, for the suppression of grey mould (*Botrytis cincerea*). Use Rhapsody ASO as a foliar spray in a rotational program with other registered crop protection products. Apply Rhapsody prior to or at the onset of disease development for maximum product effectiveness. Use maximum label rates and shorter spray intervals (7- 10 days) when disease pressure is increasing. Thorough coverage of plant parts is needed for disease protection. Rhapsody ASO can be applied up to and including the day of harvest. Spray solution must be agitated during mixing and application to maintain uniform product suspension. Do not allow spray mixture to settle overnight or for prolonged periods.

Dr. Punja tested the biofungicide Rhapsody (*Bacillus subtilis*) for control of tomato fruit rot in two commercial greenhouses in B.C. from July-October, 2012.

Research summary:

• Rhapsody (*Bacillus subtilis*) was applied once a month over a 4-month period (July-October, 2012) in two commercial greenhouses in B.C. Fruit samples were collected weekly and data collected on fungal populations on fruit, *Bacillus* populations on fruit, and development of post-harvest fruit decay on tomatoes after storage for 12 or 16days at 13º C and 21º C.

• Following each Rhapsody application, populations of bacteria reached 8-10 ($x10^5$) colony-forming-units (CFU)/cm^2 of fruit surface and declined over the ensuing 4 weeks to 2-4 ($x10^5$) CFU/ cm^2. **Fungal populations were reduced to undetectable levels.**

• **Disease incidence and severity were both significantly reduced in treated fruit in**

each week of the study. Variation in the level of disease was seen over time, perhaps due to variation in fungal populations as well as unidentified cultural and environmental factors.

• Both temperature and incubation time had a significant effect on disease. Rhapsody-treated fruit incubated at **13º C had 0-5% fruit infection** compared to **0-18% at 21º C** after 16 days. By comparison, untreated control fruit had 10-50% fruit infection. Disease levels (incidence and severity) were 30-50% lower after 12 days compared to 16 days of incubation.

• Increasing the frequency of **Rhapsody** application to once every 2-3 weeks compared to once month **reduced disease incidence** further by 20-30 % in only 8 out of the 18 weeks of the study. *Bacillus* populations were maintained at a higher level (above 60 $CFU/cm^2 x10^4$) in-between the application dates as compared to a once-a month schedule.

• Disease levels differed between the two greenhouses. In addition to cultivar differences, **the greenhouse with the lower disease had leaf litter removed every 7-10 days and applications of Rhapsody were made every 3 weeks to the rest of the greenhouse. Total fungal populations were also lower in this greenhouse**. Disease levels were not well correlated with maximum weekly temperatures.

• The fungi most frequently encountered as causing fruit decay were ***Penicillium, Botrytis, Rhizopus*** **and** ***Alternaria*****. A major source of inoculum of these fungi in harvested fruit was the stem and calyx tissues, leading to infection through the stem end. Rhapsody applications, perhaps, prevented spread of these fungi onto the fruit.**

•**Rhapsody applications made every 3 weeks are sufficient to maintain high populations of *Bacillus* on the fruit surface. When combined with a storage temperature of 13º C for no more than 12 days, disease can be reduced to 0-5 % compared to 50% in untreated fruits. This effect was observed in both greenhouses.**

16.2 Post-harvest treatments

16.2.1 Physical

Temperature and humidity management

- Cold chain (temperature and humidity) management-uses optimal temperature and relative humidity (RH) throughout the post-harvest production pathway (PHPP). Forced-air cooling is good for small operations for pre-cooling and is the most effective way to extend shelf life in horticultural crops (Kader, 2003). One B.C. greenhouse is evaluating an active, forced air cooling system.

Intermittent warming

- Mature-green and early breaker tomato fruit treated to intermittent warming for one day per week at 20˚ C and storage at 6, 9 and 12˚ C for 28 days reduced chilling injury and decay of tomato fruit (Artes, 1994).

Modified/Controlled atmosphere
- Storage rot prevention with modified atmosphere (MA) technology uses films and coatings to alter the air composition around the produce and/or to reduce water loss (Srinivasa, 2006).
- Controlled atmosphere (CA) technology is commercially used in refrigerated marine containers for apples and pear storage in longer term transportation (Li, 2003)

UV sterilizing systems
- UV irradiation system for tomato packinghouse to induce resistance rather than decontaminate fruit surfaces may be a better strategy to reduce storage rot. Fruit quality was affected by treatments (UV steribeam). www.steribeam.com

16.2.2 Chemical

Paper wraps
- Impregnated paper wraps with oil, copper or ethoxyquin is used in the U.S. against tree fruit storage rots. Keyes Packaging Group, Wenatchee, WA manufactures these products. These have not been tested against tomato storage rots.(Keyes. 2011)

Smart Fog and Ultrapure systems
- Smart Fog and Ultrapure systems are dry fogging systems used to introduce antimicrobial sprays onto fruit surface but there is a problem as the bottom of the fruit surfaces (contacting the container) are often unexposed and remain unsterilized (Ultrapure, 2011).
- Smart Fog Conveyor System® (Nevada, USA) is built over the existing conveyer system and applies a disinfectant fog to product to eliminate viruses, bacteria, molds and other organisms without wetting the fruit surface (SmartFog, 2011). The disinfectant is Biosafe's StorOx™, 27% hydrogen peroxide (Health Canada. 2011).
- Ultrapure Technology® produces a dry fogging chamber but does not use an organic disinfectant (Ultrapure, 2011).

AutoJet Modular Spray System: model 1550, Spray Systems Co., Phase 1
- The AutoJet Modular Spray System is a self-contained unit that provides liquid delivery and spray nozzle control for non-flammable, non-volatile liquids for use in a fog tunnel. The system has electric valves, manual pressure regulators and gauges to control pneumatic nozzle actuation and liquid spray pressure. The PulsaJet electric air atomizing spray nozzle type is a flat spray (SUJF1) consisting of a fluid cap (J2850) and an air cap (J73420). This nozzle is designed to spray as little as 0.25 gph and as much as 3.36 gph (Miller, 2012). The screening tests used a flat 'fluid nozzle' prototype system with 15 PSI liquid pressure/28 PSI air pressure and liquid spray rate of 113 ml/min for maximum surface spray coverage. (Miller, 2012)

Products evaluated using the Autojet Modular Spray System included the sterilant StorOx 1:50, 1:100 (27% hydrogen peroxide), food preservative Natamycin, six biocontrol products Rhapsody ASO (*Bacillus subtilis* QST 713), Subtilex (*Bacillus subtilis* MBI600), Mycostop (*Streptomyces griseoviridis* K61), Mycostop (*Streptomyces griseoviridis* K61) and Actinovate (*Streptomyces lydicus* WYEC 108) and PreStop (*Gliocladium catenulatum*), the fungicide Scholar (fludioxanil), plant-based Regalia (aqueous extract of giant knotweed), Influence LC (garlic powder), Cyclone (citric and lactic acid as bacterial fermentation products) and CO_2. The Biocontrol product Shemer (*Metschnikowia fructicola*) is still on request from Koppert Biological systems Inc., Netherlands. (Miller, 2012)

Post-harvest Tomato Fruit Treatment: Phase 2
AutoJet Modular Spray System: model 1550, Spray Systems Co.
Dr. Sally Miller et. al., Ohio State University (January, 2013)

In phase 2 laboratory tests, Dr. Sally Miller evaluated the relatively inexpensive, prototype spray tunnel system describes above to apply test products for tomato fruit rot control.

Tomatoes were first inoculated with one of the fruit rot fungi, *Botrytis cinerea*, *P. olsonii*, *P. polonicum* and *P. soliticum*, *Alternaria* spp. and *Rhizopus* spp. Inoculated tomatoes were air dried for 3 hours at room temperature. The tomatoes were then sprayed with a test product using the AutoJet Modular Spray System, a ‘twin fluid nozzle’ prototype spray tunnel system. After treatment the tomatoes were stored for 13, 18 and 21 days at 13ºC/55ºF with ~ 70% RH. No phytotoxicity was observed on non-inoculated or treated tomatoes.

Research summary:

A. *Botrytis* fruit rot of TOV tomato test results

• The six most effective products against tomato fruit rot due to *Botrytis cinerea* included four biocontrol products**,** Actinovate (*Streptomyces lydicus* WYEC 108), Mycostop (*Streptomyces griseoviridis* K61), Rhapsody ASO (*Bacillus subtilis* QST 713) and Subtilex (*Bacillus subtilis* MBI600), the fungicide Scholar (fludioxanil) and the plant-based product Influence LC (garlic) (Table 1.)

• Four other products which were moderately effective against tomato fruit rot due to *Botrytis cinerea* were the sterilant StorOx 1:50 and 1:100 (hydrogen peroxide), the food preservative Natamycin, Cyclone (citric and lactic acid) and CO_2. The biocontrol product PreStop (*Gliocladium catenulatum*) and plant extract Regalia (giant knotweed) were slightly effective.

Table 1. Effective treatments in reducing tomato fruit rot severity caused by *Botrytis cinerea* using a prototype spray tunnel system (at 15 PSI liquid pressure/28 PSI air pressure) to treat inoculated tomato fruit.

Biocontrol products:

Actinovate (*Streptomyces lydicus* WYEC 108)
Mycostop (*Streptomyces griseoviridis* K61)
Rhapsody ASO (*Bacillus subtilis* QST 713)
Subtilex (*Bacillus subtilis* MBI 600)

Fungicide:

Scholar (fludioxanil)

Plant-based product:

Influence EC (1.8, 3.6%) (garlic extract)

B. ***Penicillium* fruit rot of TOV tomato test results**

• Cyclone and both rates of Influence LC 1.8% and 3.6% (garlic) applied using the spray tunnel system did not reduce tomato fruit rot caused by the three *Penicillium* species (*P. olsonii*, *P. polonicum* and *P. soliticum*). However, Cyclone and both rates of Influence, 15 days after application of the product, did reduce the number of coalescing lesions on the fruit caused by the three *Penicillium* species. None of the three treatments (Cyclone and both concentrations of Influence LC) significantly reduced *Penicillium* disease severity compared to the inoculated, non-treated control. *P. polonicum* 01 and *P. solitum* 510 were more pathogenic and caused more coalescing lesions and fruit rot than *P. olsonii*.

In 2012, 10 test products for *Penicillium* fruit rot control were evaluated using the same fogging system as described previously.

• The 9 effective products in reducing *Penicillium* fruit rot included the four biocontrol products Mycostop (*Streptomyces griseoviridis* K61), PreStop (*Gliocladium catenulatum*), Rhapsody ASO (*Bacillus subtilis* QST 713), Subtilex (*Bacillus subtilis* MBI600), $CO_{2,}$ food preservative Natamycin, the fungicide Scholar (fludioxanil), plant extract Regalia, (giant knotweed), sterilant StorOx 1:50 and 1:100 (hydrogen peroxide) (Table 2.)

• Natamycin, Rhapsody and Scholar were the three most effective products in reducing *Penicillium* rot on the basis of per cent disease.

• Actinovate (*Streptomyces lydicus* WYEC 108) was the only ineffective treatment against *Penicillium* fruit rot of tomato.

• None of the ten treatments significantly reduced the number of coalescing lesions on tomato fruit compared to the non-treated control.

Table 2. Nine effective treatments in reducing tomato fruit rot severity caused by *Penicillium spp.* using a prototype spray tunnel system (at 15 PSI liquid pressure/28 PSI air pressure) to treat inoculated tomato fruit.

Sterilant

StorOx (hydrogen peroxide)

Food preservative:

Natamycin

Biocontrol products:

Mycostop (*Streptomyces griseoviridis* K61)
PreStop (*Gliocladium catenulatum*)
Rhapsody ASO (*Bacillus subtilis* QST 713)
Subtilex (*Bacillus subtilis* MBI600)

Fungicide:
Scholar (fludioxanil)

Plant extract:
Regalia (giant knotweed: *Reynoutria sachalinensis*)

CO_2

C. *Alternaria* fruit rot of TOV tomato test results

Twelve test products for *Alternaria* fruit rot control were evaluated using the same fogging system as described previously. The high rate of StorOx 1:100 (hydrogen peroxide), Mycostop *Streptomyces griseoviridis* K61), Scholar (fludioxanil) and Prestop (*Gliocladium catenulatum)*, low rate of Influence 1.8% (garlic) and Regalia (giant knotweed) were very effective in significantly reducing fruit rot severity and the number of disease lesions. The low rate of Influence 1.8 % was also effective. Actinovate (*Streptomyces lydicus* WYEC 108), CO_2, Natamycin, Rhapsody (*Bacillus subtilis* QST 713), Cyclone (citric and lactic acid) and Subtilex (*Bacillus subtilis* MBI600) were ineffective. However, the results were inconclusive due to very low disease pressure observed on the inoculated, untreated control fruits.

Table 3. Effective treatments in reducing tomato fruit rot severity caused by *Alternaria alternata* using a prototype spray tunnel system (at 15 PSI liquid pressure/28 PSI air pressure) to treat inoculated tomato fruit.

Sterilant:
StorOx 1:50 (hydrogen peroxide)

Biocontrol products:
Mycostop (*Streptomyces griseoviridis* K61)
Prestop (*Gliocladium catenulatum*)

Fungicide:
Scholar (fludioxanil)

Plant-based product:
Influence LC (garlic)
Regalia (giant knotweed)

D. *Rhizopus* fruit rot of TOV tomato test results

Fourteen treatments were evaluated for efficacy against Rhizopus fruit rot of tomato with the same fogging system as previously described. Only two products, the biocontrol Rhapsody (*Bacillus subtilis* QST 713) and fungicide Scholar (fludioxanil) were effective in reducing disease severity caused by *Rhizopus*. *Rhizopus* disease severity at the end of the experiment and storage long disease progress were significantly lower in tomatoes treated with StorOx 1:50 (hydrogen peroxide), Natamycin, Subtilex (*Bacillus subtilis* MBI600), Mycostop (*Streptomyces griseoviridis* K61) and Regalia (giant knotweed) than in non-treated control tomatoes. StorOx 1:100, Actinovate (*Streptomyces lydicus* WYEC 108), Prestop (*Gliocladium catenulatum*), CO_2 and Influence LC (garlic) were ineffective.

Table 4. Effective treatments in reducing tomato fruit rot severity caused by *Rhizopus* using a prototype spray tunnel (at 15 PSI liquid pressure/28 PSI air pressure) to treat inoculated tomato fruit.

Biocontrol products:
Rhapsody ASO (*Bacillus subtilis* QST 713)
Fungicide:
Scholar (fludioxanil)

Summary

The fungicide Scholar was effective against all of the tomato storage rot pathogens. (Table 5.) Rhapsody (*Bacillus subtilis* QST 713) was highly effective against all of the pathogens except *Alternaria*. Four products, Subtilex (*Bacillus subtilis* MBI600), Mycostop (*Streptomyces griseoviridis* K61), Actinovate (*Streptomyces lydicus* WYEC 108), Natamycin and CO_2, were highly to moderately effective against *Botrytis, Penicillium*, and *Alternaria* on tomato fruit. A combination of these products may provide tomato fruit rot control.

Table 5. Summary of activity of tested products against post-harvest pathogens of greenhouse tomatoes, measured by disease severity at the end of the storage period (+++ = highly effective; ++ = moderately effective; + = slightly effective; - = ineffective (not significantly different from non-treated control)) (Miller, 2013)

	Pathogen			
Treatment	***Botrytis***	***Penicillium****	***Alternaria***	***Rhizopus***
Storox 1:50	++	+	+++	-
Storox 1:100	++	+	-	-
Natamycin	++	+++	-	-
Subtilex	+++	++	+++	-
Rhapsody	+++	+++	-	+++
Mycostop	+++	++	+++	-
Actinovate	+++	++	+++	-
Scholar	+++	+++	+++	+++
Pre-Stop	+	++	+++	-
Regalia	+	+	+++	-
CO_2	++	+++	+++	-
Cyclone	++	-	-	-
Influence 1.8%	+++	-	+++	-
Influence 3.6%	+++	-	-	-

*All data in this column except for Cyclone and Influence were generated in Phase I.

Organic treatments: Plant essential oils

- Vapours of essential oils, thyme, oregano and lemongrass (carvacrol, thymol, citral and trans-2-decenal respectively) showed complete growth inhibition *in vitro* of *Botrytis cinerea* and *Alternaria arborescens.* Some oil vapors appeared to induce phytotoxicity on treated fruit under long periods of exposures. Emulsions of oils of thyme and oregano as dip treatments reduced disease development on tomatoes inoculated with these two fungi (Plotto, 2010).

Inorganic treatments: Hydrogen peroxide

- StorOx (27% hydrogen peroxide) has been tested and found effective against certain tomato storage rots (Miller, 2013).

16.2.3 Biological

Biocontrol products registered in Canada

- *Bacillus subtilus* QST 713 and Rhapsody® (PCP # 28627, Health Canada. 2011) is registered in Canada as a spray for greenhouse tomato bacterial blight (*Pseudomonas syringae*) at 1-2 L/100L. Subtilex *B. subtilis* MBI600, (PCP # 28705-28708, Health Canada. 2011) is registered in Canada as Promix HP for Pythium damping off and root rot for use in vegetable transplant growing mixes. Serenade (*Bacillus subtilis* QST 713) is registered in the UK for postharvest use on potato prior to storage. These products work against *Alternaria* but do not specify effectiveness against tomato storage rots. (US EPA. 2011).

- PreStop, *Gliocladium catenulatum* Stain J1446, (PCP # 28820, Health Canada. 2008) is registered in Canada as a preventative foliar, soil and growing media treatment for flowering bedding plants, vegetables and herbs. PreStop suppresses *Pythium* and *Rhizoctonia* damping off and crown and root rot due to Pyth*ium* sp. on vegetables, including tomato.

- Actinovate, *Streptomyces lydicus* WYEC 108, (PCP # 28673, Health Canada. 2007) is registered in Canada as a foliar spray for the suppression of a number of field and greenhouse crop diseases including powdery mildew on field and greenhouse tomato and cucurbit vegetables and Botrytis fruit rot and powdery mildew on strawberry.

- Mycostop, *Streptomyces griseoviridis* K61, (PCP# 26265, Health Canada.) is registered in Canada as a growing media treatment (sprayed, drenched or by drip irrigation) for the suppression of damping-off, root and crown rot and wilt caused by *Fusarium* on greenhouse ornamentals and vegetables including tomato. It also suppresses root and stem rot and wilt caused by *Phytophthora* on greenhouse ornamentals, tomato and pepper.

Biocontrol product not registered in Canada

- *Metschnikowia fructicola*, Shemer WDG, is a yeast used in Israel for *Botrytis* (grey mold), *Rhizopus*, and *Penicillium*. AgroGreen (Israel) manufactures Shemer and is now owned by Koppert Biological Systems.

Bioengineering

- Genetically engineered varieties with increased shelf life resulting from the gene-spliced 'Flavr Savr' resulted in delayed ripening tomatoes approved by the USDA but failed commercially as a GM food. (http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/favr_savtm_tomato-tomate_flavr_savrmd-eng.php)

- 'Daniela' is a cultivar grown in Spain, Morocco and Israel for export markets due to its' greater post-harvest longevity (DJ Cantliffe, 2009).

- ESL, extended shelf-life trait is partly due to either the presence of the *rin* or *nor* gene (Yang, 2012)

Figure 1. Production pathway of greenhouse grown tomatoes from picking to packaging.

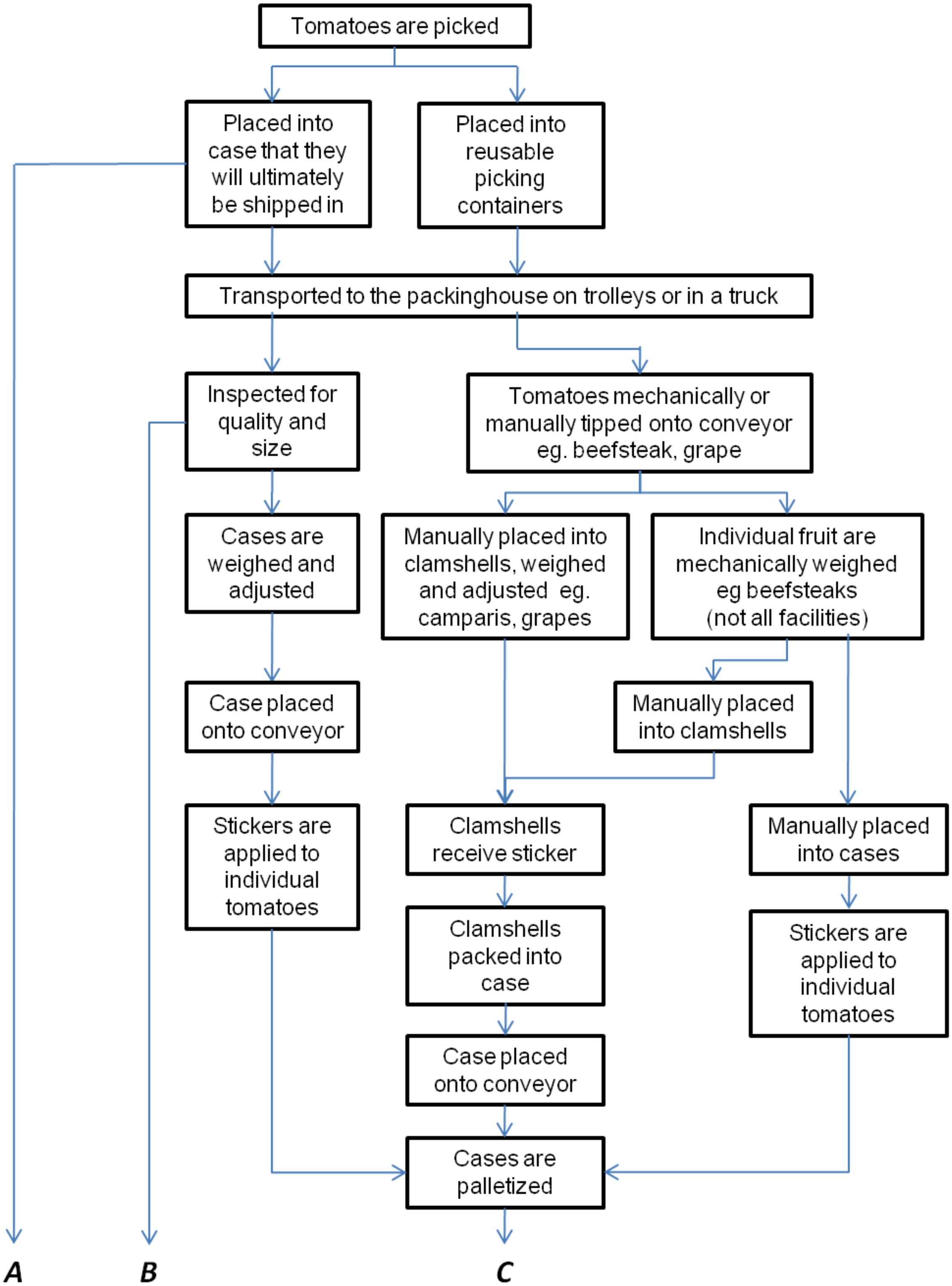
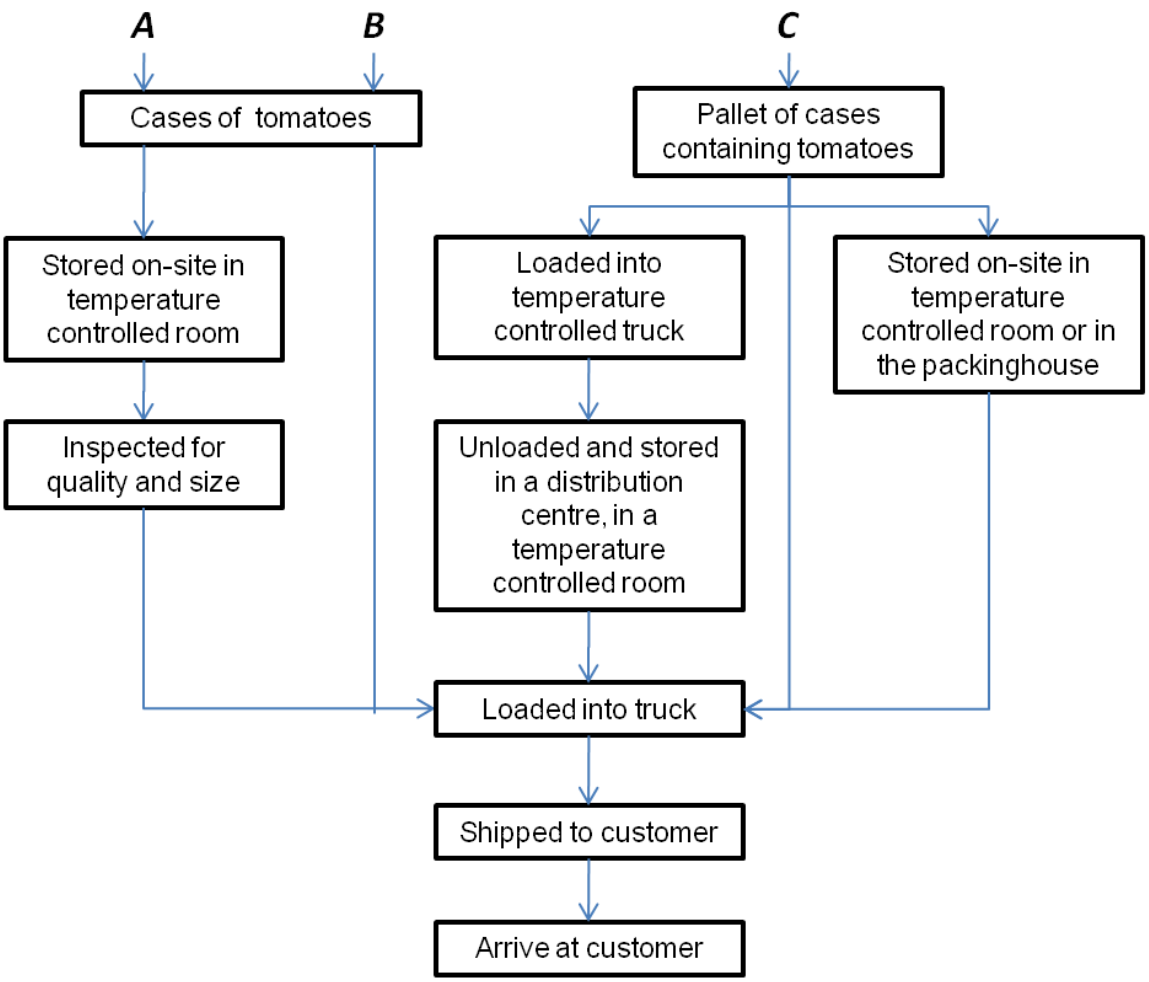


Figure 2. Post-harvest production pathway of greenhouse tomato from packing to shipping.



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