Managing the Microflora of Harvested Fruits and Vegetables to Enhance Resistance

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Accepted for publication 15 March 1989.

We are accustomed to thinking of the microbial deterioration of postharvest commodities, but not of microbial preservation. Are certain microbes associated with harvested fruits and vegetables capable of enhancing resistance to postharvest pathogens? Can we manipulate the microflora on the surfaces of harvested commodities to enhance their resistance? Although these questions have to be left primarily to speculation, some intriguing avenues are open for investigation. Campbell et al (6) mention several instances where epiphytic microorganisms and ectomycorrhizae function as part of the plant's defense. Naturally occurring microflora on plant surfaces can impart resistance to pathogen infection (5,26). Also, antagonists can be artificially introduced onto plant surfaces to impart resistance against pathogens (35). This paper considers various ways that epiphytic microorganisms might be associated with resistance in fruits and vegetables to postharvest pathogens and how we might manipulate such resistance.

HOW EPIPHYTIC MICROORGANISMS RELATE TO PLANT RESISTANCE

To effectively manipulate resistance imparted by epiphytic microorganisms we need to understand the nature of such resistance.

Plant-mediated or antagonist-mediated resistance? Is the resistance that is expressed primarily under the genetic control of the antagonist or the host plant? Resistance to postharvest pathogens can be expressed directly by artificially introduced epiphytic antagonists without apparent host mediation. This appears to be the case with *Bacillus subtilis*, which controls the brown rot pathogen, *Monilinia fructicola* (G. Wint) Honey through the production of an antibiotic (21). Other epiphytic antagonists may be under the genetic control of the host plant.

What is the evidence that resistance imparted by epiphytic microorganisms can be under the genetic control of the host? It has been suggested that exudates from plant surfaces, both above and below ground, might selectively promote a specific microbial population on and near plant surfaces and that such exudates could be under the genetic control of the host plant (3). Atkinson et al (3) and Neal et al (19) have examined the genetic control of the rhizosphere microflora of wheat as it relates to antagonists. They found that the genotype of the host governs the magnitude and composition of bacterial populations in the rhizosphere "with surprising specificity."

Bird et al (4) have developed cotton breeding lines at Texas A&M University that are resistant to a variety of pathogens and insects as well as adverse environmental conditions. This resistance has been termed Multi-Adversity-Resistance (MAR). Bird et al also have evidence that microorganisms in and on tissues (both below and above ground) play a role in MAR resistance in cotton. The microbial populations isolated from the surfaces of MAR resistant cotton varieties contained more antagonists than those from susceptible varieties (Bird, *personal communication*). Gough (12) found that winter wheat leaves sprayed with streptomycin become more susceptible to *Septoria tritici* Roberge in Desmaz. He speculates that bacterial antagonists under the genetic control of the host may be responsible for resistance to leaf spot of wheat.

If certain antagonistic microorganisms on plant surfaces are under the genetic control of the host, then the resistance expressed could be considered part of the plant's resistance. Such plant-mediated resistance would be subject to management through gene manipulation of the host. It is conceivable that you could breed and select fruits and vegetables with an epiphytic population that would impart resistance.

Microorganisms as inducers of host resistance. Induced resistance is being recognized as an important and manipulable form of resistance in vegetative plant tissues (17). Similar mechanism of resistance are probably operable in harvested commodities, yet they have received little attention. Induced resistance has been suggested as playing a role in the protection imparted through

UV irradiation (27) and heat treatments (24; and Couey, *personal communication*). If induced resistance is part of a fruit or vegetable's response to irradiation, effective prophylactic treatments may be possible with lower and consequently safer levels of irradiation.

Part of the mode of action of epiphytic antagonists to plant pathogens may be through the induction of resistance responses in the host plant. Swinburne (28,29) found that unripe Bramley Seedling apples produced benzoic acid in response to invasion by *Nectria galligenia*. He determined that proteases from the pathogen would elicit this response. Evidence has been found that part of the mode of action of antagonists against certain citrus fruit rotters may be through induction of resistance in the fruit (Wilson and Chalutz, *unpublished data*). The use of microorganisms to induce resistance in harvested commodities warrants exploration. Induction may be possible through use of nonbiological elicitors or from elicitors derived from pathogens or antagonists (15,23).

In considering microbially induced resistance in postharvest commodities, we are presented with some interesting questions. For instance, do preharvest infections by microorganisms induce resistance responses in fruits and vegetables to postharvest infections? Induced resistance in vegetative cucumber and watermelon plants has been transmitted to the fruit (7; and Kuć, *personal communication*). In tobacco plants, induced resistance to tobacco mosaic virus has been transmitted through the seed (22). Transmission of resistance from grafted root stock to scions has been demonstrated (17). A major barrier to considering this approach to disease control may be that, as Kuć says, "People have difficulty in accepting the reality that plants can be systemically immunized against disease (17)."

EFFECT OF PREHARVEST AND POSTHARVEST MANAGEMENT PRACTICES ON EPIPHYTIC MICROBIAL POPULATIONS

If we can better understand the impact of various preharvest and postharvest management practices on epiphytic microbial populations of harvested fruits and vegetables, we can perhaps modify these practices to promote a disease-suppressing population of antagonists.

Preharvest management practices. Microflora on the surfaces of fruits and vegetables after harvest will be affected undoubtedly by management practices before harvest. Pesticide use and fertilization may affect microbial antagonists on fruit and vegetable surfaces (1,32). Also, the nutritional makeup of fruits and vegetable surfaces going into storage may selectively promote certain microorganisms (18).

Andrews (1) found that the natural epiphyllous microflora of apple was altered quantitatively and qualitatively when standard pesticide applications were made. Fluorescent pseudomonads and lactic acid-type bacteria were among those microbial populations that were depressed by pesticides. Andrews and Kenerley (2) suggest that we may be suppressing a natural antagonistic population through pesticide applications.

Pesticide applications that have been shown to affect preharvest epiphytic antagonist populations should also affect postharvest populations and, presumably, rot development in storage. Little is known about what affect soil fertilization has on the phyllosphere population of the plant. In one instance, however, Turner et al (32) found that N and P fertilization enhanced the ratio of fungi to bacteria in the rhizosphere of rye grass.

Nutrients applied onto plant surfaces, as well as the soil, may affect epiphytic microbial populations. Morris and Rouse (18) found that the application of simple organic compounds to bean leaf surfaces such as glutamine and alanine can alter the epiphytic bacterial populations. They were able to alter the population size of fluorescent pseudomonads and reduce disease severity caused by *Pseudomonas syringae*. Fokkema et al (11) in contrast, found that spraying nutrients on rye leaf surfaces had little effect on the epiphytic population unless there was a simultaneous addition of microorganisms.

Postharvest management practices. The treatment that fruits and vegetables receive after harvest affects profoundly their epiphytic microbial population. We have some knowledge of these treatments on pathogen populations, but know little about what happens to other accompanying microorganisms. The dumping of commodities into biocidal solutions and spraying them with fungicides, waxes, and antioxidants should eliminate a number of microorganisms from fruit and vegetable surfaces as well as introduce new ones. Spotts et al (25) found that dump tanks are an important source of pathogens for the inoculation of fruit. It would also seem that the dump tank solution could be manipulated to facilitate inoculation of commodities with beneficial microorganisms. Antagonists could possibly be used in dump tanks to reduce pathogen inoculum in the tank.

Storage conditions affect the development of postharvest pathogens and presumably antagonists and other microorganisms on plant surfaces. Tronsmo and Hoftun (31) found that the way carrots were stored affected the type of disease that developed. In an ice bank cooler Mycocentrospora and Rhizoctonia rots were markedly reduced as compared with cold room storage. However, *Botrytis cinerea* caused severe damage in the former type of storage facility and was less of a problem in cold room storage. Tronsmo and Dennis (30) were able to control Botrytis rot of strawberries in storage by treatment with an antagonistic *Trichoderma* species. Control was enhanced by the selection of an isolate of *Trichoderma* that was better adapted to cold temperatures.

MANAGEMENT STRATEGIES FOR ENHANCING RESISTANCE TO POSTHARVEST DISEASES WITH MICROORGANISMS

It is clear that preharvest and postharvest management practices have a significant impact on the epiphytic microbial population of harvested fruits and vegetables. If natural microbial populations exist that can reduce disease development, as the evidence indicates, we should be able to manage them to our benefit through cultural manipulations. We also have the capability of artificially introducing antagonists and managing them to suppress postharvest diseases.

Managing naturally occurring microbes as plant "bodyguards." Do naturally occurring microbial populations on the surfaces of fruits and vegetables act to suppress disease development? It has been commonly observed that washed commodities develop more rot than unwashed commodities (9,10). When we wash fruits and vegetables could we be removing a microbial population that imparts resistance? Washing probably affects rot resistance in a number of other ways such as removing protective waxes and causing wounds. We shouldn't disregard the possibility that we may be removing protective populations of microorganisms.

Chalutz and Wilson (9) found that citrus fruit that were washed, dried, and stored rotted much more rapidly than unwashed fruit. They also found that bacteria and yeasts predominated when undiluted washings from citrus fruit were plated out. It was only after the washings were diluted that fungal rot pathogens appeared. This suggested that there may be an epiphytic microbial population on citrus fruit that is naturally suppressive to rot pathogens.

Managing introduced antagonists to suppress postharvest diseases. A number of postharvest diseases of peaches (20,21), cherries (33), apples (14–16,36), pears (16), citrus (10), and strawberries (30) have been controlled by introduced antagonists. Biological control with antagonists in the postharvest environment has advantages over biological control in the field. Greater control of the environment under postharvest conditions appears to be the major advantage. Also, antagonists can be more easily targeted to where they are needed when they are applied to harvested commodities, compared with field or soil applications (35).

Most antagonists against postharvest pathogens have been tested against a limited number of pathogens on a single fruit or vegetable species. It would be advantageous to find and develop antagonists with a broad spectrum of activity against a large number of pathogens or a wide variety of commodities. The osmophilic yeast, *Debaryomyces hansenii* has been found to be effective against the major rots of citrus (10), apple (36), grapes, and tomato (Chalutz and Wilson, *unpublished data*). It is also a normal inhabitant of fruit surfaces and foods such as milk and cheese.

Antagonism by microorganisms against pathogens is expressed in a variety of ways (8). Tronsmo and Dennis (30) found that *Trichoderma* controls postharvest Botrytis rot of strawberry, presumably by direct parasitism and antibiotic production. Pusey and Wilson (21) found that *Bacillus subtilis* expressed its antagonism against *Monilinia fructicola* by the production of an iturin antibiotic (13). Janisiewicz and Roitman (16) found that an isolate of *Pseudomonas cepacia* was effective against Botrytis, Penicillium, and Mucor rots of apples and pears. This isolate produces a powerful antibiotic, pyrrolnitrin, that can control these pathogens in the absence of the antagonists.

Antagonists of postharvest diseases have also been found that do not show antibiotic activity in vitro against the pathogens that they control. Examples are *Debaryomyces hansenii* (36) and *Enterobacter cloacae* (34), which controls Rhizopus rot of peaches. Antagonism expressed by *D. hansenii* against rot pathogens of citrus and tomato can be overcome in proportion to the amount of nutrients added to the wound site where infection occurs (Chalutz, *personal communication*).

CONCLUSION

Epiphytic microorganisms on the surfaces of fruits and vegetables could be managed to enhance resistance to postharvest diseases. Fruits and vegetables have natural epiphytic microbial populations that may be under genetic control of the host. If such populations suppress disease development, they could be managed through plant breeding and selection.

Preharvest and postharvest management practices affect the epiphytic microbial populations of fruits and vegetables. It may be possible to manage a disease-suppressing microbial population on the surfaces of fruits and vegetables through cultural manipulations.

We have only a rudimentary understanding of induced resistance in stored commodities. Induced resistance may be part of the mode of action of hot water treatments and irradiation. Elicitors produced by pathogens and antagonists may be used to enhance resistance of harvested commodities through induced resistance.

Postharvest rot diseases of fruits and vegetables have been effectively controlled by artificially applying antagonists. This procedure promises to be an effective way to control postharvest diseases since in the postharvest environment both the antagonist and environment can be managed for enhanced resistance to the pathogen.

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