Europ.J.Hort.Sci., **73** (2). S. 73–75, 2008, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Cantaloupe Cultivar Differences as Opportunistic Hosts for Human Pathogenic *Escherichia coli* O157:H7 and *Salmonella*

B. Duffy[1)], S. Ravva[2)] and L. Stanker[2)]
([1)]Agroscope Changins-Wädenswil (ACW), Plant Protection Division, Wädenswil, Switzerland, and [2)]U.S. Department of Agriculture, Agricultural Research Service, Foodborne Contaminants Research Unit, Albany, USA)

Summary

A significant influence of plant genotype at the cultivar level was observed for survival and opportunistic root colonisation by *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Thompson on canteloupe. Our results demonstrate the potential for cultivar selection as a novel approach to reduce preharvest contamination of canteloupe plant surfaces by human pathogenic bacteria.

Key words. foodborne pathogens – food safety – *Salmonella enterica* serovar Thompson

Introduction

Fresh produce has increasingly been linked to outbreaks of foodborne infections worldwide caused by human pathogenic fecal coliforms (Beuchat 2002). Salmonellae and *Escherichia coli* O157:H7 are among the most common and problematic culprits. Although exact determination of infectious dosages is difficult (Latimer et al. 2001), it is commonly agreed that in vulnerable groups relatively few bacterial cells are required to initiate acute infections, often with debilitating disease sequellae. Over the past decade, a number of disease outbreaks have been traced to contaminated cantaloupe (Stone et al. 1993; Mohle-Boetani et al. 1999). Epidemiology has focused on improper post-harvest handling processes that favor rapid pathogen regrowth. Bacteria that land on the fruit rind before or at harvest are transferred during cutting to the edible melon (Ukuku et al. 2001b). How the bacteria get to the rind in the first place is uncertain. Likely sources are contaminated irrigation water and land application of untreated municipal or animal waste (Pell 1997). Once the pathogen enters a production field, survival even at low numbers on growing crops could provide an inoculum reservoir for continued dispersal during subsequent irrigation and transfer to developing fruits that are in contact with soil. Recent studies have reported plant colonisation by human pathogens on foliage (Brandl and Mandrell 2002; Wachtel et al. 2002), fruits and flowers (Guo et al. 2001). Ours is the first study of crop cultivar effects on pathogen colonisation and one of the few to examine root colonisation.

We investigated the ability of two humans pathogens associated with produce-related outbreaks to colonise cantaloupe plant surfaces. Both *E. coli* O157:H7 Odwalla strain (Anonymous 1996) and *Salmonella enterica* serovar Thompson (Campbell et al. 2001) have been linked to recent human disease outbreaks from unpastuerised apple juice and cilantro, respectively. To enable monitoring, strains were marked with spontaneous resistance to rifampicin (*E. coli* O157:H7 Odwalla, 100 mg L^{-1}) and nalidixic acid (*Salmonella* Thompson RM1987, 75 mg L^{-1}). In addition, both strains were marked with stable plasmids carrying genes encoding a green fluorescent protein (GFP) conferring kanamycin resistance (Brandl and Mandrell 2002).

Material and Methods

The inoculum was prepared by scraping 1 day old bacterial cultures, grown at 37 °C on LB agar (Difco, Detroit, MI) with 25 mg L^{-1} kanamycin, into sterile phosphate buffer (PBS, pH 7.1), centrifuging, and then re-suspending the cells in PBS to give approximately 1 x 10^5 colony forming units (CFU) ml^{-1}.

Cantaloupe cultivars 'Burpee's Ambrosia', 'Hale's Best', 'Hearts of Gold', 'Israel old original', and 'Sweet 'n Early' were used. Seeds were surface disinfested in 3 % sodium hypochlorite (vol/vol) for 10 min, and pre-germinated on 0.85 % water agar in darkness for 2 d at 20 °C. Plants were grown in microcosms constructed by filling polystyrene plastic tubes (20.5 cm length x 4 cm diameter; Cone-tainers™, Stuewe and Sons, Corvallis, OR) with approximately 115 g natural silt loam soil. Two seeds were placed on the surface and covered with an additional 10 g soil. Inoculum was introduced by watering over the seeds with 15 ml of bacterial suspension to give a final concentration of 1 x 10^4 CFU g^{-1} soil. Soil was kept moist and plants were grown in a growth chamber equipped with a HEPA filtration system to minimize pathogen exposure. Plants were maintained at 22 °C night: 24 °C daytime temperature with 12 h light period, and 50 % relative humidity.

Pathogen colonisation was determined 18 d after planting. While still in the polystyrene tubes, shoots were

removed, and the two oldest true-leaves were placed in 25 ml of PBS. Entire root systems were then removed from the tubes, shaken vigorously to discard all but the most firmly adhering soil, and placed in 25 ml PBS. Samples were sonicated for 60 s to dislodge bacteria. Dilutions were plated onto antibiotic amended LB agar, 100 mg L^{-1} rifampicin or 75 mg L^{-1} nalidixic acid for *E. coli* O157:H7 and *Salmonella* Thompson, respectively. Each treatment consisted of four replicates with eight plants and the experiment was repeated once. Data were subjected to ANOVA.

Results and Discussion

Cantaloupe cultivars differed significantly in their susceptibility to root colonisation by *Salmonella* ($P<0.004$) and *E. coli* O157:H7 ($P<0.001$). Cultivars 'Burpee's Ambrosia' and 'Hale's Best' had the highest and 'Israel old original' had the lowest populations for both bacteria (Fig. 1). Colonisation of cultivars 'Hearts of Gold' and 'Sweet 'n Early' was similar to 'Israel old original' (Fig. 1). *Salmonella* populations were substantially greater than *E. coli* populations on all cultivars (by 3 to 8 fold), suggesting that this pathogen is more rhizosphere competent. Similar results have been reported for other pathogen strains in a recent study with alfalfa sprouts showing that *Salmonella* strains were generally more aggressive colonizers (Barak et al. 2002). Very few bacteria were found to colonise the seedling aerial surfaces, and no differences were observed between cultivars. Bacteria were detected in only five of twenty leaf samples of 'Burpee's Ambrosia', 'Hale's Best', and 'Israel old original' (data not shown). In these samples, bacterial counts ranged from 1 to 12 CFU and 1 to 5 CFU $plant^{-1}$ for *Salmonella* and *E. coli* O157:H7, respectively. The overall absence of pathogens on leaves, together with the fact that the cultivars with the highest and lowest root populations had sporadic foliar colonisation suggests that transfer from contaminated soil to aerial plant parts during emergence is an unlikely risk factor. The potential role of root colonisation as a reservoir for pathogens (Berg et al. 2005) to contaminate soil and thus potential be distributed to fruit by vectors such as nematodes (Caldwell et al. 2003) or other means remains to be investigated.

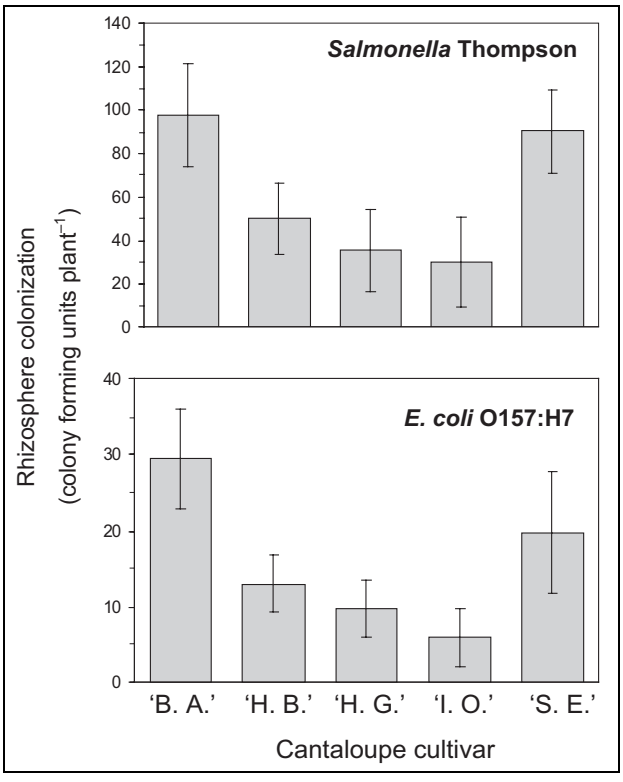


Fig. 1. Rhizosphere populations of human pathogenic *Salmonella enterica* serovar Thompson and *Escherichia coli* O157:H7 on cantaloupe cultivars 'Burpee's Ambrosia' ('B. A.'), 'Hale's Best' ('H. B.'), 'Hearts of Gold' ('H. G.'), 'Israel old original' ('I. O.'), and 'Sweet 'n Early' ('S. E.'). Bars indicate mean standard deviations.

The low pathogen population sizes on root surfaces compared to a recent study with foliar colonisation of cilantro (Brandl and Mandrell 2002) were at first surprising. However, the soil environment contains a greater diversity of potential competitor microbes and this was likely and important factor in our study. The low numbers of bacteria that were recovered, should not be discounted from an epidemiological stand-point because these pathogens have a rapid regrowth potential. On cantaloupe fruit surfaces, small populations surviving at low levels under sub-optimal conditions can rapidly increase in number when conditions become more favourable for pathogen growth (Golden et al. 1993). High temperature is a major environmental variable that favors regrowth of human pathogens on fruit surfaces (Delrosario and Beuchat 1995; Beuchat 2002) and foliage (Brandl and Mandrell 2002). Cantaloupe growing regions typically have such warm conditions. In fact, these low bacterial counts highlight the difficulty that may be encountered in field studies seeking to detect pathogen contaminated produce using real-time assays.

Our findings that cantaloupe cultivars differed in pathogen colonisation is one of the first indications that some degree of plant host specificity occurs in colonisation by human pathogenic bacteria. Root exudate quantity and quality is a key factor in host specificity among non-pathogenic bacteria that are closely associated with plants, and influences bacterial attraction to and growth in the rhizosphere (Pueppke et al. 1998). Another possible factor in host specificity could be varying levels of antimicrobial compounds between cultivars (Magiatis et al. 2001). It may be worthwhile to screen for such compounds in root exudates from cultivars less supportive of pathogen populations. This study introduces the possibility of cultivar selection, and possibly plant breeding, as novel approaches to reduce the risk of human pathogen contamination of plant surfaces and thus to improve food safety. Whereas post-harvest antimicrobial measures have been developed to sanitise cantaloupe fruits (Ukuku and Sapers 2001a), these have limitations and are not adaptable to reduce pathogen populations in non-fruit surfaces during crop production.

Acknowledgments

We thank C. Sarreal for technical assistance, R. Mandrell and M. Brandl for providing pathogen strains, and R. Dias

for providing soil. Financial support for B.D. was partly through CORE Organic ERA-Net Pilot Project 'PathOrganic'.

References

ANONYMOUS 1996: Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice – British Columbia, California, Colorado and Washington, October 1996. Morbid. Mortal. Week. Rep. **45**, 975.

BARAK, J.D., L.C. WHITEHAND and A.O. CHARKOWSKI 2002: Differences in attachment of *Salmonella enterica* serovars and *Escherichia coli* O157:H7 to alfalfa sprouts. Appl. Environ. Microbiol. **68**, 4758–4763.

BERG, G., L. EBERL and A. HARTMANN 2005: The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environ. Microbiol. **7**, 1673–1685.

BEUCHAT, L.R. 2002: Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect. **4**, 413–423.

BRANDL, M.T. and R.E. MANDRELL 2002: Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. Appl. Environ. Microbiol. **68**, 3614–3621.

CALDWELL, K.N., G.L. ANDERSON, P.L. WILLIAMS and L.R. BEUCHAT 2003: Attraction of a free-living nematode, Caenorhabditis elegans, to foodborne pathogenic bacteria and its potential as a vector of Salmonella Poona for preharvest contamination of cantaloupe. J. Food Protect. **66**, 1964–1971.

CAMPBELL, V.J., J. MOHLE-BOETANI, R. REPORTER, S. ABBOTT, J. FARRAR, M.T. BRANDL, R.E. MANDRELL and S.B. WERNER 2001: An outbreak of *Salmonella* serotype Thompson associated with fresh cilantro. J. Infect. Dis. **183**, 984–987.

DELROSARIO, B.A. and L.R. BEUCHAT 1995: Survival and growth of enterohemorrhagic *Escherichia coli* O157:H7 on cantaloupe and watermelon. J. Food Protect. **58**, 105–107.

GOLDEN, D.A., E.J. RHODEHAMEL and D.A. KAUTTER 1993: Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. J. Food Protect. **56**, 194–196.

GUO, X., J. CHEN, R.E. BRACKETT and L.R. BEUCHAT 2001: Survival of Salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Appl. Environ. Microbiol. **67**, 4760–4764.

LATIMER, H.K., L.A. JAYKUS, R.A. MORALES, P. COWEN and D. CRAWFORD-BROWN 2001: A weighted composite dose-response model for human salmonellosis. Risk Analysis **21**, 295–305.

MAGIATIS, P., D. SPANAKIS, S. MITAKU, E. TSITSA, A. MENTIS and C. HARVALA 2001: Verbalactone, a new macrocyclic dimer lactone from the roots of *Verbascum undulatum* with antibacterial activity. J. Nat. Prod. **64**, 1093–1094.

MOHLE-BOETANI, J.C., R. REPORTER, S.B. WERNER, S. ABBOTT, J. FARRAR, S.H. WATERMAN and D.J. VUGIA 1999: An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. J. Infect. Dis. **180**, 1361–1364.

PELL, A.N. 1997: Manure and microbes: Public and animal health problem? J. Dairy Sci. **80**, 2673–2681.

PUEPPKE, S.G., M.C. BOLAÑOS-VÁSQUEZ, D. WERNER, M.-P. BEC-FERTÉ, J.C. PROMÉ and H.B. KRISHNAN 1998: Release of flavonoids by the soybean cultivars McCall and Peking and their perception as signals by the nitrogen-fixing symbiont *Sinorhizobium fredii*. Plant Physiol. **117**, 599–608.

STONE, A., M. SHAFFER and R.L. SAUTTER 1993: *Salmonella Poona* infection and surveillance in a neonatal nursery. Am. J. Infect. Cont. **21**, 270–273.

UKUKU, D.O., V. PILIZOTA and G.M. SAPERS 2001a: Influence of washing treatment on native microflora and *Escherichia coli* population of inoculated cantaloupes. J. Food Safety **21**, 31–47.

UKUKU, D.O. and G.M. SAPERS 2001b: Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. J. Food Protect. **64**, 1286–1291.

WÄCHTEL, M.R., L.C. WHITEHAND and R.E. MANDRELL 2002: Prevalence of *Escherichia coli* associated with a cabbage crop inadvertently irrigated with partially treated sewage wastewater. J. Food Protect. **65**, 471–475.

Received September 24, 2003 / Accepted October 01, 2007

Addresses of authors: Brion Duffy (corresponding author), Agroscope Changins-Wädenswil ACW, Plant Protection Division, CH-8820 Wädenswil, Switzerland, and Subbarao Ravva and Larry Stanker, U.S. Department of Agriculture, Agricultural Research Service, Foodborne Contaminants Research Unit, 800 Buchanan Street, Albany, CA 94710, USA, e-mail: duffy@acw.admin.ch.