World Journal of Microbiology
Vol. 4(1), pp. 064-071, February, 2017. © www.premierpublishers.org. **ISSN**: XXXX-XXXX

WJM

Research Article

Salmonella spp. dynamics in wild blueberry, *Vaccinium angustifolium* Aiton

Vivian CH Wu[1], Frank Drummond[2,5,*], Shravani Tadepalli[3], ME Camire[3], Katherine Davis-Dentici[3], Alfred Bushway[4], David E. Yarborough[3,5]

[1]Produce Safety and Microbiology Research Unit, USDA-ARS Western Regional Research Center, Albany, CA.
[2]University of Maine, School of Biology and Ecology, Orono, ME, USA.
[3]University of Maine, School of Food and Agriculture, Orono, ME, USA.
[4]University of Maine, Professor Emeritus, School of Food and Agriculture, Orono, ME, USA.
[5]University of Maine, Cooperative Extension, Orono, ME, USA.

A six-year field study (2012-2015) was conducted in the two major wild blueberry growing regions in Maine USA, Midcoast and Downeast. This study used data from two cropping cycles (four years) to model the dynamics of *Salmonella* spp. prevalence in wild blueberry fields (*Vaccinium angustifolium* Aiton). A path diagram based upon structural equation modeling suggests that beyond annual variation, the type of cropping system determined wild blueberry production methods of fertilization and fungicide applications for control of plant pathogens that then both affect the prevalence of *Salmonella* spp. Fungicide applications have a direct negative effect on *Salmonella* spp. prevalence and the microbial community on the fruit that positively affects *Salmonella* spp. prevalence. Fertilizer application has an indirect effect on the presence of *Salmonella* spp. by determining soil fertility that then determines the blueberry plant nutrient profile. This then determines specific nutrient levels in the plant, especially Cu, K, Mg, Mn, and K. These nutrients (especially Ca, K, and Mg and to a lesser extent Cu, Mn, and Zn) directly affect *Salmonella* spp. prevalence in a complex mix of indirect and direct, and negative and positive interactions, including the regulation of sugars in the fruit that appears to have a negative effect on *Salmonella* spp. prevalence. The conceptual model presented in this study generates several new hypotheses to test regarding the ecology of *Salmonella* spp. in commercial wild blueberry fields in Maine, USA.

Keywords: Low bush blueberry, wild blueberry, *Vaccinium angustifolium*, foodborne pathogens, food safety, *Salmonella*, ecology, agricultural management practices.

INTRODUCTION

The wild blueberry, *Vaccinium angustifolium* Aiton, is a North American native plant that is managed as a food crop (Munson, 1901; Hall et al., 1979; Jones et al., 2014). This fruit's wild, non-planted production system is similar in many respects to the European bilberry (V. myrtillus L.), the Western huckleberry (V. membranaceum Douglas ex Torr.) and the bog bilberry (V. uliginosum L.) in China. However, wild blueberry is subjected to more intense management (Yarborough, 2013). This species has a native range extending from Newfoundland Canada south to North Carolina and west to Manitoba (Vander Kloet, 1988; Bell et al., 2009) and grown as a fruit crop in Maine, and Quebec and the Maritime Provinces in Canada (Yarborough, 2015).Wild blueberries represent about 31% of the total North American blueberry production, that also includes the highbush blueberry, V. corymbosum L. and the rabbit-eye blueberry, Vaccinium virgatum Aiton (Syn. Vaccinium ashei) (Brazleton, 2015).

***Corresponding author**: Frank Drummond, University of Maine, School of Biology and Ecology, Orono, ME, USA. E-mail: fdrummond@maine.edu

Wild blueberries are managed on a two-year cropping cycle with plants burned or mowed to the ground every other year. This production method results in a non-flowering vegetative growth year that disrupts many insect frugivore and fruit pathogen life cycles (DeGomez et al., 1990; Drummond et al., 2001). Despite the disruptive two-year cropping cycle, as well as production tactics represented by fertilization, irrigation, and pesticide application; the wild blueberry agroecosystem is characterized by a high diversity of arthropod and vertebrate wildlife (Eaton, 1957; Maloney et al., 2005; Karem et al., 2006; Huebner, 2009; Drummond et al.,2010; Choate and Drummond, 2012; Jones et al., 2014; Bushmann and Drummond, 2015; Jones et al., 2016; Groff et al., 2016).

The presence of wildlife in wild blueberry fields has been identified as the primary potential source of some human pathogens, specifically *Escherichia coli* O157:H7 (Jones et al., 2015) and *Salmonella* spp. (Sullivan, 2012). However, there is little known on the dynamics of *Salmonella* spp. or other foodborne pathogens on wild or cultivated highbush blueberry. Ecological studies conducted on *Salmonella* spp. have focused on ecology of livestock production or within animal gastrointestinal environments (Foltz, 1969; Dunkley et al., 2009). Most current research on *Salmonella* spp. contamination and blueberries has focused on decontamination of blueberry fruit and not on dynamic field ecology (Sy et al., 2005, Bialka and Demirci, 2007). Although some research has been conducted concerning the ecology of *Salmonella* spp. in vegetable production (Franz and van Bruggen, 2008). Contamination of blueberries by *Salmonella* spp. in the marketplace does occur (Miller et al., 2013) and threats to U.S. food safety due to foodborne/human pathogens have affected farm operations in many states including Maine (Loader and Hobbs, 1999; Ropkins and Beck, 2000; Burlingame and Pineiro, 2007; Martinez et al., 2007; Powell et al., 2011). The U.S. Food and Drug Administration (FDA) has implemented the Food Safety Modernization Act to minimize consumer exposure to foodborne pathogens from food crops (Food and Drug Administration, 2015). In order to develop best management practices for food safety, there has been much recent research aimed at enhanced detection and monitoring of foodborne pathogens (Alocilja and Radke, 2003; Wang et al., 2006; Mandal et al., 2011; Singh et al., 2013) and the ecology of food pathogens in the farm landscape (Beuchat, 2002; Nightingale et al., 2004; Critzer and Doyle, 2010; Strawn et al., 2013; Jones et al., 2015; 2016).

The objective of the present study was to quantify the major farm management and ecological factors that influence *Salmonella* spp. associated with wild blueberry fruit. Structural equation modeling was applied to field data characterizing the prevalence of Salmonella spp. and associated system measures collected during the first four years of a six-year cropping systems study in Maine wild blueberries (2010-2015).

MATERIALS AND METHODS

Sample Collections

A multi-disciplinary geographic-scale study of four cropping input systems in wild blueberry in Maine - Organic, Low input, Medium input and High input was conducted. These blueberry production cropping systems are practiced by more than 500 blueberry growers farming 17,600 ha in Maine (Yarborough and Cote, 2014). The systems represent gradients of capital inputs and potential environmental effects that affect yield, fruit quality, plant and animal communities, soil, ground and surface waters, and grower profits. Research was conducted on commercial blueberry fields that represented the four cropping systems. Fields were located in Washington, Hancock, Knox and Lincoln counties, Maine USA. The geographic strata provided fields with a wide range of soil types, forest edge (Acadian mixed forest to boreal forest), animal and plant diversity, and weather conditions. In the 2010 to 2011 two-year cycle, two fields per cropping input system were established for a total of eight fields. In 2012 to 2013 four fields per cropping system for a total of 16 were provided for a better geographic distribution by including more sites in Knox and Lincoln counties and increasing to four fields per cropping system. At each field, data on capital inputs such as pesticides, fertilizer, pruning method, the number of honey bee hives rented, etc. were obtained from the grower. Block size was 0.4 ha within a field for the insect, disease and weed sampling (both years of the crop cycle), leaf and soil samples (prune year) were obtained from meter square quadrats on four 15 m transects. These samples were processed by drying in a laboratory walk-in drying facility (maintained at 35º C) at the Maine Agricultural and Forestry Experiment Station Analytical Laboratory and Maine Soil Testing Service (University of Maine, Orono, Maine, USA). Samples were then assessed for soil morphological characteristics and soil and leaf nutrient content using standard laboratory procedures (https://umaine.edu /soiltestinglab/). Mechanical (low – high input systems) and hand-rake harvesting (organic) was conducted long the four 15 m transects to obtain yield samples. Low input and organic berries were harvested by researchers and stored in plastic Ziploc® (S.C. Johnson & Son Inc., Racine, WI) containers; farm crews harvested the berries from Medium and High input sites. Berries were winnowed then sorted manually to remove leaves and other debris, and unripe and damaged fruit, then were stored at 4°C overnight.

Food Quality Measures.

Subsamples of harvested yield (two each of 2.0 kg samples) from each block were taken to the laboratory for the measurement of fruit quality and the detection of foodborne pathogens. Random 50-g berry samples were counted to estimate berry size (no./g), and surface color was made with a Hunter Lab Lab Scan XE colorimeter™ (Hunter Associates Laboratory, Inc., Reston, VA), which proved CIE L, a and b values.

Texture was calculated as newtons of force (kg m/s^2) required to compress individual berries oriented with their calyx end pointed upward by using a Brookfield LFRA Texture Analyzer (Brookfield Engineering, Middleboro, MA) with a 2mm cylindrical probe. Berries were ground by mortar and pestle and extracted with a solution of acetone (Fisher Scientific, Fair Lawn, New Jersey) and 30% reverse osmosis (RO) water acidified with 0.1% HCl(Fisher Scientific, Fair Lawn, New Jersey); then analyzed to total anthocyanins (Lee et al. 2005) and total free phenolics (Velioglu et al., 1998). Pureed berries were used in other assays. The percent dissolved solids (largely glucose and fructose) (°Brix) was measured with an Atago Palette PR-101 (0-45%) refractometer™ (ATAGO Co., Ltd., Tokyo, Japan); pH was determined using an Orion 410 pH meter (Thermo Fisher Scientific, Waltham, MA). AOAC method 942.25b (2005) was used to quantify titratable acidity. Total aerobic plate counts, as well as, yeast and mold counts were measured according to FDA recommended procedures (Food and Drug Administration, 1998). Details of these methods are described by Kerames (2012) and Chadbourne (2014).

Detection of *Salmonella* spp.

A total of 40 blueberry samples from four management systems were evaluated for the 2010-2011 crop cycle and for the 2012-2013 crop cycle, a total of 32 samples were evaluated. Isolation and detection of Salmonella spp. was conducted using culture methods and PCR screening. For culture methods, isolation and detection were done based on the methods recommended by the U.S Food and Drug Administration with few modifications (Andrews et al., 2016).Three blueberry subsamples of 25g each were aseptically weighed and subjecting to a sequence of steps including pre-enrichment using lactose broth, selective enrichment using selenite cysteine broth and tetrathionate broth, selective-differential plating using Bismuth sulfate agar, Xylose lysine deoxycolate agar and Hektoen enteric agar, and biochemical characterization including urea, phenol red lactose broth, methyl red, voges-proskauer reagents, and simmons citrate tests. Salmonella culture positives were further screened using an enterotube test. All positives were confirmed using serological testing for *Salmonella* somatic (O) antigen and Salmonella flagella (H) antigen. For PCR screening, DNA was extracted from the overnight enrichment broth and later screened for *Salmonella* spp. using specific primers ST-11 (5'-AGC CAA CCA TTG CTA AAT TGG CGC A-3') & ST-15 (5'-GGT AGA AAT TCC CAG CGG GTA CTG-3') (amplified a 429 bp fragment of a cryptic 2.3kb chromosomal fragment of *Salmonella*).

Statistical Modeling

Structural equation modeling was used to produce a "path analysis" of the dynamics. AMOS software (Arbuckle, 2006) was used to estimate the beta coefficients for each of the relationships in our apriori-hypothesized relationships among state variables. AMOS software allows both categorical independent variable structure and dependent binary variables. Year was a categorical independent variable in our model and presence of *Salmonella* spp. was the dichotomous dependent variable. No latent variables were used. Initial hypothesized models were based upon the literature and previous observations. Additional possible causal factors were added to the model as proxies when some of the initial hypothesized relationships were not significant. Prior to modeling, a hierarchical nested analysis of variance was used to decompose the nested expected variance structure of the data, cropping system, fields within cropping system, and blocks within fields; so that cropping system, field, and block variances could be estimated (Cochran, 1977). Variance among fields was compared to variance among blocks using an F test to determine if variance among blocks differed from variance among fields. Since there was no difference in the two variances ($F_{(23,65)}$ = 1.536, P=0.785), block was used as the experimental unit to increase the power of the analyses. Relationships are described by standardized Betaor correlation coefficients with the following symbols:†, *, **, and ***; representing P value intervals of: < 0.10,<0.05, < 0.01, < 0.0001; respectively.

Additionally, in 2010, peat mulch was added to 10m x 10m plots within each block. The mulch was added to a depth of 10 cm before blueberry shoots emerged in the spring. In the following year (2011) fruit was harvested both within the mulched plots and an adjacent non-mulched plot in each of 8 fields (paired treatments). Nominal logistic regression (JMP® 2015) was used to determine if mulch affected the prevalence of *Salmonella* spp. associated with the blueberry fruit harvested from the two treatments, mulch and no mulch.

RESULTS AND DISCUSSION

During the four years of the study, *Salmonella* spp. was detected in 18% of fields. In 2011, a study was conducted to determine if *Salmonella* spp. prevalence was affected by peat mulch applied to the fields in the previous year. There was no evidence to suggest that mulch application had any effect on the prevalence of Salmonella spp. on wild blueberry fruit at harvest (P = 0.518). However, several factors affected the prevalence of *Salmonella* spp. on wild blueberry fruit in fields were found. Figure 1 shows the results of our statistical modeling (structural equation modeling) and illustrates the statistical coefficient estimates by delineating the direction (positive or negative interactions) and the significance and strength (magnitude of the beta or partial regression coefficient) of the interactions that drive the dynamics of Salmonella spp. occurrence on blueberry fruit by using graph theory. Production system management techniques affected *Salmonella* spp. prevalence both directly and indirectly. The production cycle year can be seen (as year) to determine the number of fungicide and fertilizer applications that were made during the study. This mainly suggests that annual variation is significant in determining farming inputs. In the case of

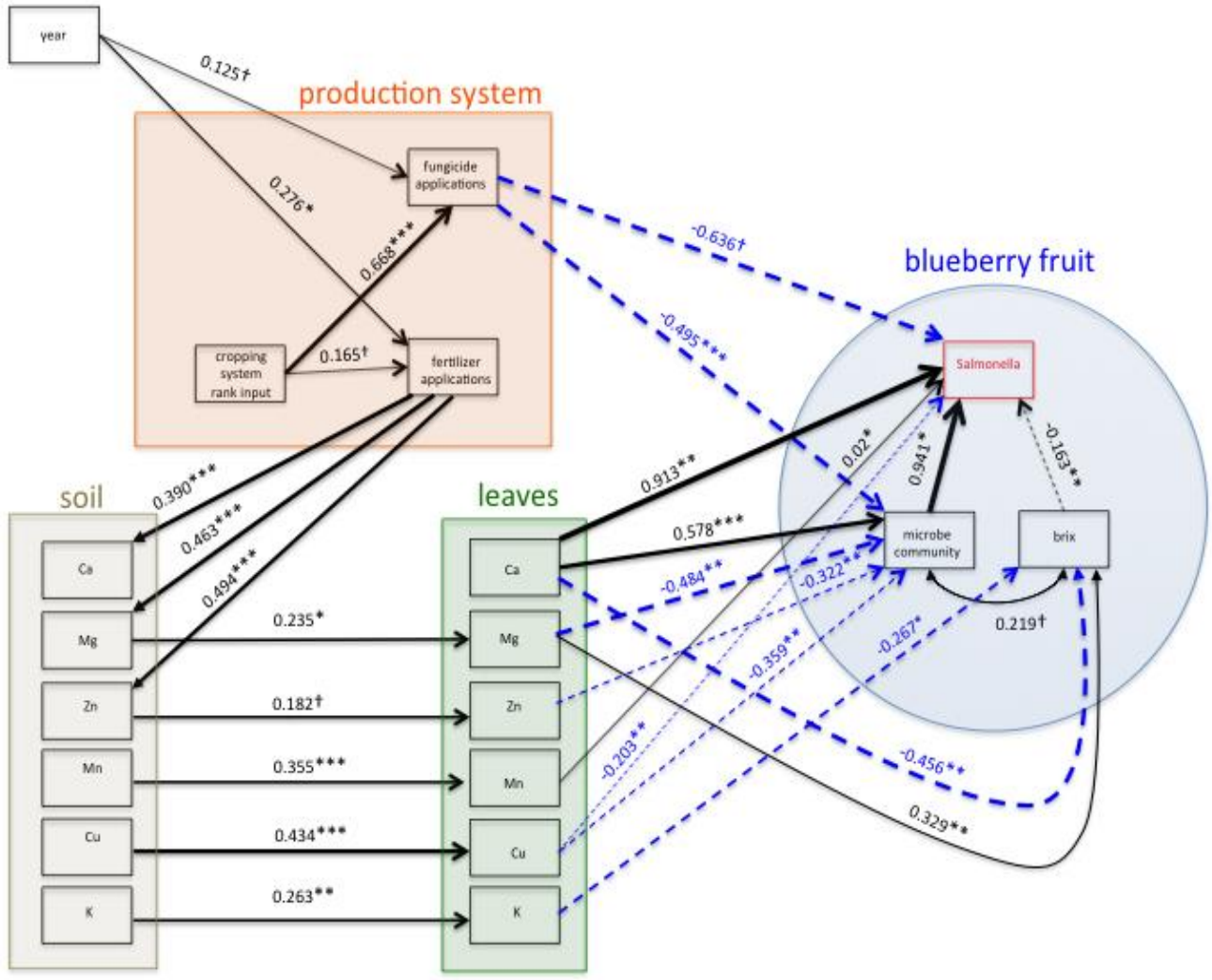

Figure 1. Conceptual model of *Salmonella* spp. dynamics based upon a structural equation model. Beta coefficients are deemed significant using the following symbols: †, *, **, and ***; representing P value intervals of: < 0.10, <0.05, < 0.01, < 0.0001; respectively. The strength of the association is represented also by the line thickness, solid black lines are positive relationships, blue dashed lines are negative relationships and double-headed arrows are correlations.

the present study, we can only speculate at the reason for the increased inputs over the course of the two production cycles. However, since 2005 the yields in Maine continuously increased after a five-year period (2000-2005) of continuous decrease (Yarborough, 2016). Some of this overall trend in yield reduction may have been due to inclement weather cycles over this period. However, the continuous increase in yield after this period of decline suggests that many growers increased their frequency and rates of inputs in order to increase yields after the decline.

Overall, the path from weather affirms that variance between years in production practices is significant but small to moderate.

Fungicide and fertilizer applications not only varied by year but also were determined by the production system. It can be seen that as the production system gradient increased along the input gradient from organic to the high input conventional production system, a concomitant increase in fungicide and fertilizer applications was observed. This relationship is much stronger than the production cycle (year) trend and expected since the overall definition of the production systems is based upon inputs (Yarborough and Cote, 2014). The significance of increased fungicide applications is that there appears to be a direct negative effect of fungicide application on the presence of Salmonella spp. on fruit in blueberry fields. A negative effect, of slightly less strength, was also observed on the overall background microbial community. We suspect that this fungicide effect may be due to toxicity of the fungicide on these microorganisms. There are numerous reports of fungicides being acutely toxic or mutagenic to Salmonella spp. (Ruiz and Marzin, 1997; Niewolak et al., 2002; Shukla et al., 2004; Dobhal et al., 2014)2014), although few studies reported the survival of *Salmonella* spp. in a fungicide solution (Guan el al., 2005). The effect of fungicides on *Salmonella* spp. could be due to a dynamic between fungicide applications and potential bird vectors. Several

fungicides have been reported repellent to birds (Sandhu et al., 1987; Avery and Decker, 1991; Oruc, 2010). Turkeys have been shown in Maine to harbor *Salmonella* spp. and to deposit this foodborne/human pathogen in their feces (Sullivan, 2012) and this bird species is a common resident of Maine blueberry fields (Huebner, 2009). It was also reported by Sullivan (2012) that 18% of turkey feces can harbor Salmonella spp. therefore, this wild bird species is a likely reservoir for this foodborne pathogen in this ecosystem.

Fertilizer applications were shown to directly affect soil fertility, specifically increasing soil levels of Ca, K, Mg, and Zn. However, there appears to be much correlation among soil nutrients and so effects of fertilization on individual minerals are difficult to establish. Soil fertility affects the blueberry plant profile of nutrients in leaves, especially Cu, K, Mg, Mn, and Zn. The plant leaf nutrient profile appears to be both positively and negatively, and both directly and indirectly, associated with *Salmonella* spp. and the background microbial community on the fruit. Calcium in the plant has a very complex relationship with *Salmonella* spp. on the fruit. Calcium positively affects the presence of *Salmonella* spp. on fruit in a direct manner, but it also affects Salmonella spp. in an indirect manner by positively affecting the background microbial community that in turn enhances the presence of *Salmonella* spp. The enhancement of the microbial community also is correlated with higher levels of sugars in the blueberry fruit (measured in degrees Brix). Often it has been found that endophytic bacteria colonizing fruit will negatively affect fruit pathogens (Rosenblueth and Martínez-Romero, 2006), but the research on the microbial phylloplane and its interaction with foodborne pathogens is inconclusive and contradictory (Beuchat, 2002; Heaton and Jones, 2008) and therefore, it is not possible to evaluate our finding in wild blueberry. The state of our minimal understanding of the phylloplane microbial community and foodborne pathogens may be due to the findings of Leff and Fierer (2013) demonstrating that the microbial community species diversity on fruits and vegetables is highly dependent upon the specific fruit or vegetable.

Higher levels of glucose and fructose in the fruit negatively affect *Salmonella* spp. presence, although this is seen to be a weak relationship. The sugar content of the blueberry, being correlated with the background microbial community was observed toimpart a negative influence on the presence of *Salmonella* spp. through the Ca and K content in the plant, but a positive influence due to the levels of Mg in the plant (as measured in the leaves). Sugar content of the fruit while being correlated with the background microbial community and demonstrating a negative effect on *Salmonella* spp. was also weakly correlated with anthocyanin levels in the fruit ($r = + 0.300$, $P = 0.016$), although anthocyanin concentrations in fruit were not a good predictor of *Salmonella* spp. contamination on fruit. The anthocyanin levels in fruit were strongly correlated with titratable-acidity ($r = + 0.736$, $P< 0.0001$). Therefore, it may be that sugar levels in fruit were not the cause of decreased *Salmonella* spp. contamination, but may be a correlate of another fruit quality / chemistry factor that was not measured in or studied. Han and Micallef (2016) reported that sugars, sugar alcohols, and organic acids of tomato surface compounds were associated with increased *S. enterica* growth, while fatty acids, including palmitic and oleic acids, were negatively correlated. There are several studies that suggest complex nutrient and phytochemicals are involved in regulating plantphyllosphere microbial communities (Janisiewicz and Bors, 1995; Ruppel et al., 2008). This dynamic in wild blueberry may be difficult to investigate due to the additional complexity of the association of Ericoid mycorrhizal fungi in the wild blueberry plant (Smagula and Litten, 1988; Dalpe, 1989; Glass et al., 2005) that are also known to regulate the associated microbial community and at the same time be influenced by nutrients (Andrews and Harris, 2000; Porras-Alfaro and Bayman, 2011).

CONCLUSION

The present study has shown that *Salmonella* spp. contamination in a wild blueberry field is a dynamic phenomenon, dependent upon management practices implemented by growers and the resulting soil and plant nutrient profile, as well as, fruit quality and the background microbial community associated with fruit colonization. This research provides new hypotheses to test of *Salmonella* spp. ecology in the wild blueberry agro-ecosystem. Knowledge of *Salmonella* spp. dynamics will provide insight into minimizing consumer exposure to this foodborne pathogen.

ACKNOWLEDGEMENTS

We would like to thank Ms. Judy Collins and Ms. Jennifer Cote for assisting in data collection and database management. Thanks also go to Michael Dougherty, Stephanie Kerames and Jennifer Chadbourne for performing some of the laboratory analyses. Thirty-nine undergraduate students also helped in this study, especially with harvesting the crop. We would also like to thank two anonymous reviewers from providing recommendations for improvement. This project was funded in part by USDA/NSFA SCRI Grant No. 2009-51181-05845, Systems Approach to Improving Sustainability of Wild Blueberry Production. This is publication number 3502 of the Maine Agricultural and Forestry Experiment Station.

REFERENCES

Alocilja EC, Radke SM (2003). Market analysis of biosensors for food safety. Biosens.andBioelec.18(5): 841-846.

Andrews JH, Harris RF (2000). The ecology and biogeography of microorganisms on plant surfaces. Ann. Rev. Phytopath. 38(1): 145-180.

Andrews WH, Wang H, Jacobson A, Hammack T (2016).

Salmonella. Food and Drug Administration. Bacteriological Analytical Manual. Ch. 5.
AOAC (2005). Official methods of analysis. 18th ed. AOAC International, Rockvill, MD. Method No. 942.25b.
Arbuckle JL (2006). Amos, Version 7.0, statistical software. Chicago: SPSS.
Avery ML, Decker DG (1991). Repellency of fungicidal rice seed treatments to red-winged blackbirds. The J. Wildlife Mgmt. 15: 327-334.
Beuchat LR (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Micro. Infect.4(4): 413-423.
Bell DJ, Rowland LJ, Smagula J, Drummond F (2009). Recent Advances in the biology and genetics of lowbush blueberry. Maine Agric. and Forest Exp. Stn. Tech. Bull. 203, Univ. Maine, Orono, ME.
Brazelton C (2015). World Blueberry Statistics & Global Market Analysis. U. S. High-bush Blueberry Council, Folsom, CA. 105 pp.
Burlingame B, Pineiro M (2007). The essential balance: Risks and benefits in food safety and quality. J. Food Comp. Anal. 20(3):139-146.
Bushmann S, Drummond FA (2015). Abundance and diversity of wild bees (Hymenoptera: Apoidea) found in lowbush blueberry growing regions of Downeast Maine. Environ. Entomol. 43: 1 -15.
Chadbourne J (2012). Agricultural input effects on sensory quality and chemical composition of wild blueberries. Electronic Theses and Dissertations. Paper 2100. http://digitalcommons.library.umaine.edu/etd/2100
Choate BA, Drummond FA (2012). Ant diversity and distribution (Hymenoptera: Formicidae) throughout Maine lowbush blueberry fields in Hancock and Washington counties. Environ. Entomol. 41(2): 222-232.
Cochran, WG (1977). Sampling Techniques, 3rd ed. John Wiley & Sons, Inc. N.Y., N.Y. 448 pp.
Critzer FJ, Doyle MP (2010). Microbial ecology of foodborne pathogens associated with produce. Current Opin. Biotech.21(2): 125-130.
Dalpe Y (1989). Ericoid Mycorrhizal Fungi in the Myxotrichaceae and Gymnoascaceae. The New Phytologist113(4): 523-527.
DeGomez T, Lambert DH, Forsythe HY, Collins JA (1990). The influence of pruning method on disease and insect control. Univ. Maine Coop. Ext. Fact Sheet no. 218.
Dobhal S, Zhang G, Royer T, Damicone J, Ma LM (2014). Survival and growth of foodborne pathogens in pesticide solutions routinely used in leafy green vegetables and tomato production. J. Sci. Food and Agric.94(14): 2958-2964.
Drummond FA, Dill J, Yarborough DE (2001). Monitoring for the blueberry maggot fly, *Rhagoletis mendax* Curran. Wild Blueberry Fact Sheet 201. http://umaine.edu/blueberries/factsheets/insects/201-monitoring-for-the-blueberry-maggot/
Drummond FA, Collins JA, Choate B, Woodman D, Jennings DT, Forsythe HY, Cokendolpher JC (2010). Harvestman (Opiliones) fauna associated with Maine lowbush blueberry fields in the major production areas of Washington and Hancock Counties. Environ. Entomol. 39(5): 1428-1440.
Dunkley KD, Callaway TR, Chalova VI, McReynolds JL, Hume ME, Dunkley CS, Kubena LF, Nisbet DJ, Ricke SC (2009). Foodborne Salmonella ecology in the avian gastrointestinal tract. Anaerobe. 15(1):26-35.
Eaton EL (1957). The spread of blueberry seed through manure and by migrating robins. Proceed. Amer. Soc. for Hort. Sci.69: 293.
Foltz VD (1969). Salmonella ecology. J. Amer. Oil Chem. Soc. 46(5): 222-4.
Food and Drug Administration (2015). Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. 21 CFR Parts 11, 16, and 112. Document Number FDA-2011-N-0921. https://www.federalregister.gov/documents/2015/11/27/2015-28159/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption (Accessed 09/24/2016)
Food and Drug Administration (1998). Bacteriological Analytical Manual, Edition 8, Revision A. Ch. 3 Aerobic Plate Count and Ch. 18 Yeasts, Molds and Mycotoxins.
Franz E, van Bruggen AH (2008). Ecology of *E. coli* O157: H7 and *Salmonella* enterica in the primary vegetable production chain. Crit. Rev. Microbiol. 34(3-4):143-61.
Glass VM, Percival DC, Proctor JTA (2005). Tolerance of lowbush blueberries (*Vaccinium angustifolium* Ait.) to drought stress. I. Soil water and yield component analysis. Can.J. Plant Sci.85(4): 911-917.
Groff SC, Loftin CS, Drummond FA, Bushmann S, McGill B (2016). Spatial prediction of lowbush blueberry native bee pollinators in Maine, USA. Environ. Modelling & Software 79: 1-9.
Guan TTY, Gregory B, Holley RA (2005). Survival of pathogenic bacteria in pesticide solutions and on treated tomato plants. J Food Prot.68(2): 296-304.
Hall IV, Aalders LE, Nickerson NL, Vander Kloet SP (1979). The biological flora of Canada. 1. *Vaccinium angustifolium* Ait., sweet lowbush blueberry. Can. Field-Nat.93: 415–430.
Han S, Micallef SA (2016). Environmental metabolomics of the tomato plant surface provides insights on Salmonella enterica colonization. Appl. Environ.Microbiol.82(10): 3131-3142.
Heaton JC, Jones K(2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. J. Appl. Microbiol.104(3): 613-626.
Huebner, JA(2009). Wild turkey foraging behavior and crop depredation on wild blueberry farms in Maine. M.S. thesis, University of Maine, Orono.
Janisiewicz WJ, Bors B (1995). Development of a microbial community of bacterial and yeast antagonists to control wound-invading postharvest pathogens of fruits. Appl. Environ.Microbiol.61(9): 3261-3267.
JMP®(2015). Version 12. SAS Institute Inc., Cary, NC, 1989-2007.
Jones MS, Vanhanen H, Peltola R, Drummond FA (2014).

A global review of arthropod-mediated ecosystem-services in *Vaccinium* berry agroecosystems. Terr. Arthro. Rev. 7: 41-78.
Jones MS, Tadepalli S, Bridges DF, Wu VCH, Drummond F (2015). Suppression of *Escherichia coli* O157:h7 by dung beetles (Coleoptera: Scarabaeidae) using the lowbush blueberry agroecosystem as a model system. PLoS ONE 10(4): e0120904. doi:10.1371/journal. pone.0120904.
Jones MSH, Halteman W, Drummond FA (2016). Predator- and scavenger-mediated ecosystem services determined by distance to field-forest interface in the Maine lowbush blueberry agroecosystem. Environ. Entomol. doi: 10.1093/ee/nvw082.
Karem J, Woods SA, Drummond FA, Stubbs CS (2006). Sampling native wasps along both vertical and horizontal gradients in the Maine lowbush blueberry landscape. Environ. Entomol. 35(4): 1083-1093.
Kerames SK (2012). The effects of different agronomic practices on the composition and quality of wild blueberries. Electronic Theses and Dissertations. Paper 1723. http://digitalcommons.library.umaine.edu/etd/1723
Lee J, Wrolstad RE, Durst RW (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int. 88(5):1269-1278.
Leff JW, Fierer N (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. PloS One 8.3: e59310.
Loader R, Hobbs JE (1999). Strategic responses to food safety legislation. Food Policy24 (6): 685-706.
Maloney D, Drummond FA, Alford AR (2005). Predation of blueberry insect pests by ground spiders in Maine. Trends in Entomol. 4: 43-57.
Mandal PK, Biswas AK, Choi K, Pal UK (2011). Methods for rapid detection of foodborne pathogens: an overview. Am. J. Food Tech. 6(2): 87-102.
Martinez MG, Fearne A, Caswell JA, Henson S (2007). Co-regulation as a possible model for food safety governance: Opportunities for public–private partnerships. Food Policy, 32(3): 299-314.
Miller BD, Rigdon CE, Robinson TJ, Hedberg C, Smith KE (2013). Use of global trade item numbers in the investigation of a Salmonella Newport outbreak associated with blueberries in Minnesota, 2010. J. Food Protect. 76(5):762-9.
Munson WM (1901). The blueberry in Maine. Me. Agr. Expt. Sta. 14th Ann. Rpt. Orono, ME: 164-172.
Niewolak S, Komar M, Golas I (2002). Effect of fungicide Topsin M70 WP (tiophanate-methyl) on the survival of some indicator bacteria and enteric pathogens in lake water. Polish J. Nat. Sci. 112: 23-35.
Nightingale KK, Schukken YH, Nightingale CR, Fortes ED, Ho AJ, Her Z, Grohn, YT, McDonough PL, Wiedmann M (2004). Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl. Environ. Microbiol.70(8): 4458-4467.
Oruc, HH (2010). Fungicides and their effects on animals. Fungicides. InTech 12: 349-362.
Porras-Alfaro A, Bayman P (2011). Hidden fungi, emergent properties: endophytes and microbiomes. Phytopath.49(1): 291.
Powell DA, Jacob CJ, Chapman BJ (2011). Enhancing food safety culture to reduce rates of foodborne illness. Food Control 22(6): 817-822.
Ropkins K, Beck AJ (2000). Evaluation of worldwide approaches to the use of HACCP to control food safety. Trends in Food Science & Technol.11(1): 10-21.
Rosenblueth M, Martínez-Romero E (2006). Bacterial endophytes and their interactions with hosts. Mol. Plant-microbe Inter.19(8): 827-837.
Ruiz MJ, Marzin D (1997). Genotoxicity of six pesticides by Salmonella mutagenicity test and SOS chromotest. Mutation Research/Genetic Toxicol. Environ. Mutagen. 390(3): 245-255.
Ruppel S, Krumbein A, Schreiner M (2008). Composition of the phyllospheric microbial populations on vegetable plants with different glucosinolate and carotenoid compositions. Micro. Ecol. 56(2): 364-372.
Sandhu PS, Dhindsa MS, Toor HS (1987). Evaluation of methiocarb and thiram as seed treatments for protecting sprouting maize from birds in Punjab (India). Inter. J. Pest Mngt. 33(4): 370-372.
Singh A, Poshtiban S, Evoy S (2013). Recent advances in bacteriophage based biosensors for food-borne pathogen detection. Sensors 13(2): 1763-1786.
Shukla Y, Taneja P, Arora A, Sinha N (2004). Mutagenic potential of mancozeb in Salmonella typhimurium. J.Environ. Path., Tox., Oncol. 23(4): 1-13.
Smagula JM, Litten W (1988). Effect of ericoid mycorrhizae isolates on growth and development of lowbush blueberry tissue culture plantlets. IV Intl. Symp.onVaccinium Cult. 241: 110-114.
Strawn LK, Fortes ED, Bihn EA, Nightingale KK, Gröhn YT, Worobo RW, Wiedmann M, Bergholz PW (2013). Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Appl. Environ. Micro.79(2): 588-600.
Sullivan K (2012). Wild turkey management – Maine.gov. www.maine.gov/ifw/wildlife/ reports/species.../wildturkeymanagement.pdf
Sy KV, McWatters KH, Beuchat LR (2005). Efficacy of gaseous chlorine dioxide as a sanitizer for killing Salmonella, yeasts, and molds on blueberries, strawberries, and raspberries. J. Food Protect. 68(6):1165-75.
Vander Kloet SP (1988). The Genus *Vaccinium* in North America. Research Branch, Agriculture Canada. Ottawa, Canada.
Velioglu YS, Oomah BD, Gao L, Mazza G (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46(10):4113-4117.
Wang N, Zhang N, Wang M (2006). Wireless sensors in *Salmonella* spp. dynamics in wild blueberry, *Acciniu-*

mangustifolium Aiton agriculture and food industry—Recent development and future perspective. Comp. Electron.Agric.50(1): 1-14.
Yarborough DE (2013). Production – improving your wild blueberry yields. Univ. Maine Coop. Ext. Fact Sheet. https://extension.umaine.edu/blueberries/factsheets/production/improving-your-wild-blueberry-yields/
Yarborough DE, Cote JD (2014). Evaluating field inputs for productivity and profitability in wild blueberry fields in Maine. Proc. North Amer. Blueberry Res. and Ext. Workers Conf. RUCore http://dx.doi.org/doi:10.7282/T3SQ9215
Yarborough DE (2015). Wild blueberry culture in Maine. Univ. Maine Coop. Ext. Fact Sheet No. 220.
Yarborough DE (2016).Wild Blueberry Crop Statistics https://extension.umaine.edu/blueberries/factsheets/statistics-2/statistics/

Accepted 25 January, 2017.

Citation: Wu VCH, Drummond F, Tadepalli S, Camire ME, Davis-Dentici K, Bushway A, Yarborough DE (2017). *Salmonella* spp. dynamics in wild blueberry, *Vaccinium angustifolium* Aiton, World Journal of Microbiology, 4(1): 064-071.