



Growth of *Listeria monocytogenes* on spinach and rocket leaves is affected by cultivation conditions and the vegetable phytobiome

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Overview

- Background, recent outbreaks, experimental setup
- Ability of *L. monocytogenes* to grow on spinach & rocket
 - i) Supermarket produce, ii) Commercial produce, iii) non-commercial produce
- Role of environmental conditions in the field that allows *L. monocytogenes* to grow
 - i) tunnel vs. open field, ii) varieties, iii) growing season
- Phytobiome (phyllosphere bacteriome) on spinach and rocket (tunnel vs. field, variety, season) and its potential effect on *L. monocytogenes* growth potential
- Conclusions on the pre-harvest controls to minimise growth of *L. monocytogenes*



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Background

- FAO – Global 60% Increase in vegetable production
- Ireland and Belgium have high rates of daily consumption of vegetables (Eurostat, 2017)
- Positive relationship between consumption and health status (vitamins, minerals, antioxidants, phytochemicals)
- Most popular raw consumed → Spinach, Rocket and Lettuce
- Largely unprocessed from farm to fork, allowing potential for opportunistic food borne pathogens to be consumed e.g. *Listeria monocytogenes*.



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Listeria monocytogenes: Risks to health

- Healthy adult → mild flu-like symptoms
- Infects **YOPIs**: **Y**oung, **O**ld, **P**regnant or **I**mmunocompromised
 - Listeriosis manifests as **gastroenteritis**, **meningitis**, **encephalitis**, and **septicaemia**, → mortality = 25–30%
 - Mother-to-fetus infections
 - Spontaneous abortion, Stillborn, Meningitis of new-born

Table 28: *Listeria monocytogenes* FSC as described in Regulation (EC) No 2073/2005 for the different RTE categories across the food chain

Sampling stage	RTE foods intended for infants and RTE foods for special medical purposes	Other RTE foods	
		Able to support the growth of <i>Lm</i>	Unable to support the growth of <i>Lm</i>
Processing ^(a)	NA	Based on detection method: <i>Lm</i> not detected in 25 g of sample (n = 5, c = 0) ^(c)	NA
Retail ^(b)	Based on detection method: <i>Lm</i> not detected in 25 g of sample (n = 10, c = 0)	Based on enumeration method: limit of 100 CFU/g (n = 5, c = 0) ^(d)	Based on enumeration method: limit of 100 CFU/g (n = 5, c = 0)

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Recent leafy vegetable related *Listeria* outbreaks

- FSAI Sept 2018: Recall of unwashed spinach leaves and mixed leaves
- USA 2019, 6/284 samples, 6 tested positive for *Listeria monocytogenes* (incl. spinach, lettuce)
- USA 2021 multiple salad recalls
- USA Jan2022, frozen spinach (Lidl), mixed salad (Dole) recall
- Paucity of studies on growth potential (δ) of *L. monocytogenes* on **spinach** and **rocket**;
 - Some reported growth (Sant'Ana *et al.*, 2012), while others did not (Lokerse *et al.* 2016; Söderqvist *et al.*, 2017)

Bags of unwashed spinach and mixed leaves recalled over presence of bug-causing bacteria

Consumers have been warned about the detection of *Listeria monocytogenes* bacteria in two batches of the leaves.

Sep 28th 2018, 11:41 AM 48,916 Views 27 Comments Share 761 Tweet Email 13

THE FOOD SAFETY Authority of Ireland has issued a recall for a range of Irish unwashed spinach products following the discovery of a bug-causing bacteria.

The authority has warned consumers about two batches of spinach and mixed leaves sold in Irish supermarkets following the detection of *Listeria monocytogenes* bacteria.

Batches with the Julienne code 260 and 261 sold in Lidl, Aldi, Dunnes Stores, Tesco, and Supervalu and with 'best before' dates ranging from 22 to 26 September are implicated.

Image: Shutterstock/P3447

Contents lists available at ScienceDirect
Food Control
journal homepage: www.elsevier.com/locate/foodcon

Growth capacity of *Listeria monocytogenes* in ingredients of ready-to-eat salads
R.F.A. Lokerse, K.A. Madowska-Corke, L.C. van de Wierdt, T. Wijzen*

Contents lists available at ScienceDirect
International Journal of Food Microbiology
journal homepage: www.elsevier.com/locate/ijfoodmicro

Growth potential of *Salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life
Anderson S. Sant'Ana^a, Mathews S. Barbosa, Maria Teresa Destro, Marisa Landgraf, Beneditte D.C.M. Franco*

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Experimental setup

➢ Inoculation:

L. monocytogenes 1382: EURL *Lm* reference strain
L. monocytogenes 959: from vegetable sample
L. monocytogenes 6179: persistent strain from food processing plant

➢ Produce:

Lettuce

Spinach

Rocket

10²
CFU/g



Atmosphere: Air or 4% O₂, 8% CO₂

Incubation: 8°C (temp. used in previous studies); 7/12°C (guidance temp. until July 2021)

Produce: A. Retail; B. Commercial; C. Experimental

Analysis: CFU (*L. monocytogenes*, heterotrophs), 16S rRNA gene sequencing

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A. Testing supermarket produce

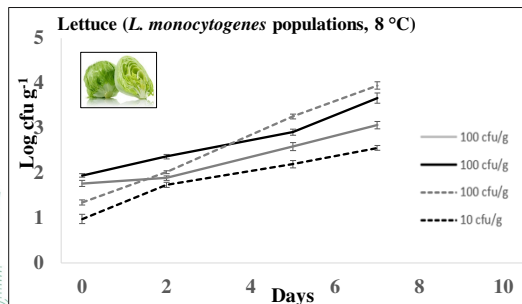
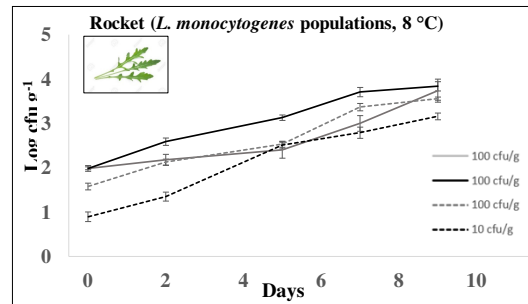
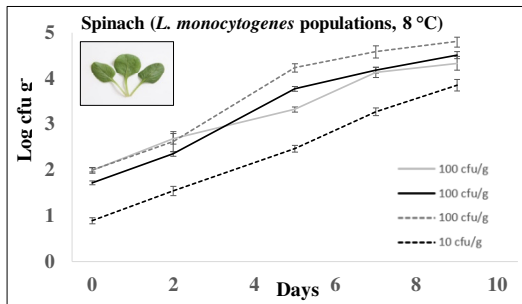
- Growth potential, product appearance



Produce purchased from SuperValu in Castletroy, Limerick
Images for illustration purpose only



Listeria monocytogenes growth potential on supermarket-purchased spinach, rocket & lettuce



***L. monocytogenes* growth potentials (δ):**

Initial inoculum cfu g ⁻¹	Day 7 Spinach	Day 9 Spinach	Day 7 Rocket	Day 9 Rocket	Day 7 Lettuce
100	2.14	2.34	1.72	1.85	1.31
100	2.46	2.79	1.79	1.98	2.60
100	2.58	2.81	1.01	1.75	1.68
10	2.38	2.93	1.89	2.26	1.58

Paul Culliney and Achim Schmalenberger (2020)
Growth Potential of *Listeria monocytogenes* on Refrigerated Spinach and Rocket Leaves in Modified Atmosphere Packaging. *Foods* 9:1211
doi:10.3390/foods9091211



B. Testing produce obtained directly from suppliers

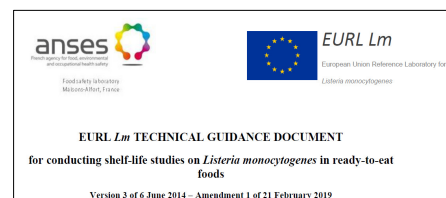
- 2 rocket varieties (open field & polytunnel)
- 1 spinach variety (open field)
- Growth potential, unwashed and washed

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Product	Cultivation Method	Product Washing	Temperature Profile	Growth Potential (δ) Log cfu g ⁻¹
Rocket (Esmee)	Poly-tunnel	Unwashed	8°C 100% (9 days)	1.09
Rocket (Esmee)	Poly-tunnel	Washed	8°C 100% (9 days)	0.92
Rocket (Wild)	Open-fields	Unwashed	8°C 100% (9 days)	1.68
Rocket (Wild)	Open-fields	Washed	8°C 100% (9 days)	1.54
Spinach (Baby)	Open-fields	Unwashed	7°C (2/3) 12°C (1/3) (9 days)	2.57
Spinach (Baby)	Open-fields	Washed	7°C (2/3) 12°C (1/3) (9 days)	2.64

Effect of commercial cultivation method on growth potentials (δ) of *Listeria monocytogenes* on rocket & spinach



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C. Testing produce cultivated in-house (Co. Clare)

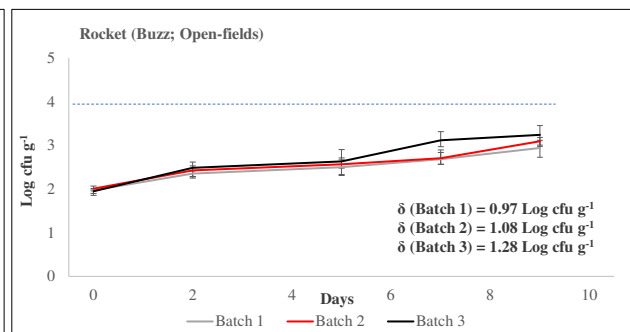
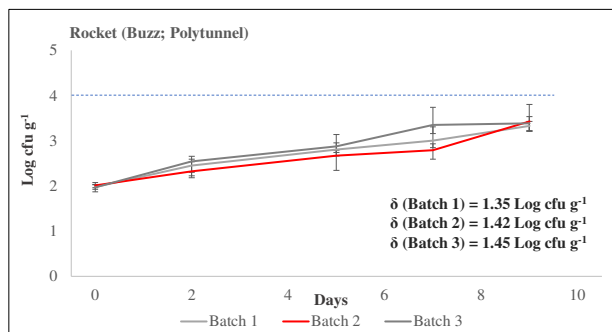
- 2 rocket varieties (open field & polytunnel)
- 2 spinach varieties (open field & polytunnel)
- 1 kale variety

- Different seasons



- I. *L. monocytogenes* growth potential and heterotrophs
- II. Phytobacteriome community structure (*unpublished*)

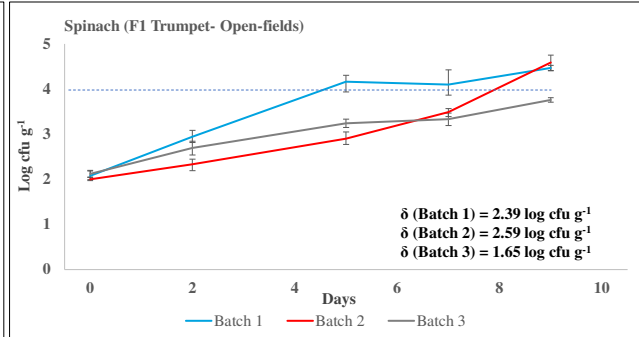
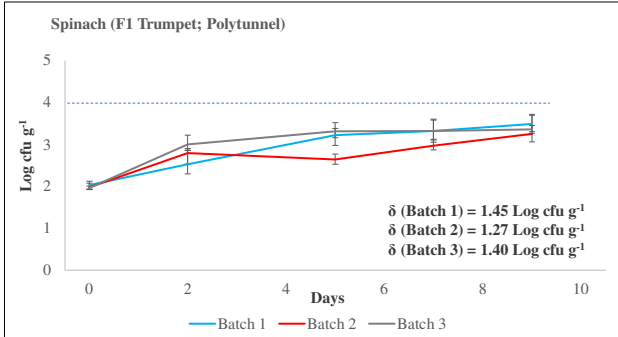
I. *Listeria monocytogenes* growth potential on non-commercial rocket (polytunnel vs open-fields)



7/12°C (6/3 days); Air

doi.org/10.3390/foods11193056

I. *Listeria monocytogenes* growth potential on non-commercial spinach (polytunnel vs open-fields)



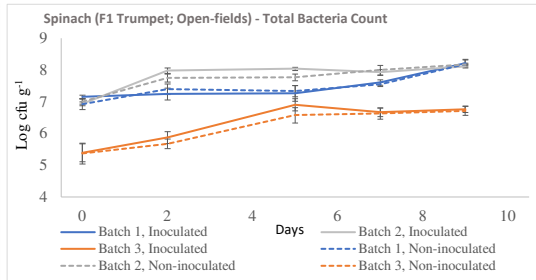
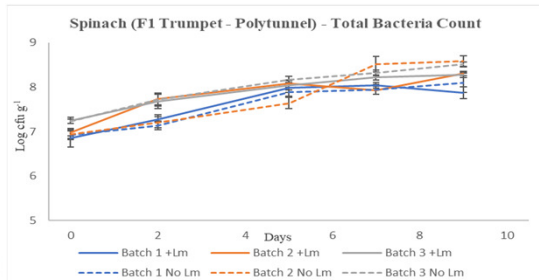
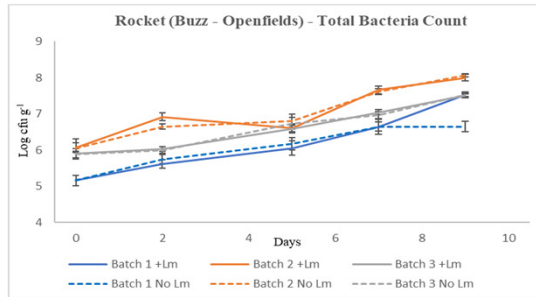
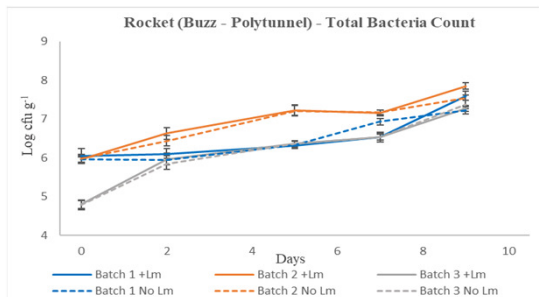
7/12°C (6/3 days); Air

doi.org/10.3390/foods11193056



I. Total heterotrophic bacteria counts on non-commercial rocket & spinach (polytunnel vs open-fields)

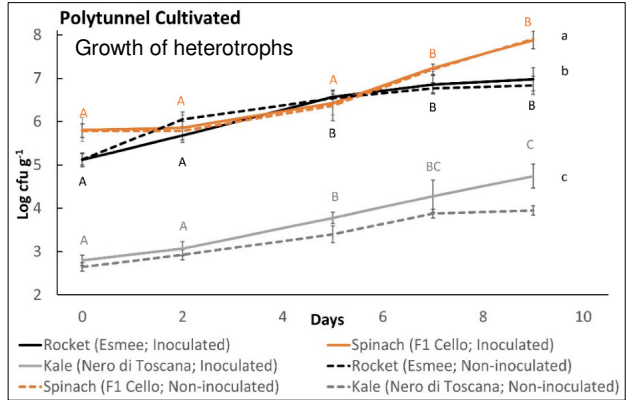
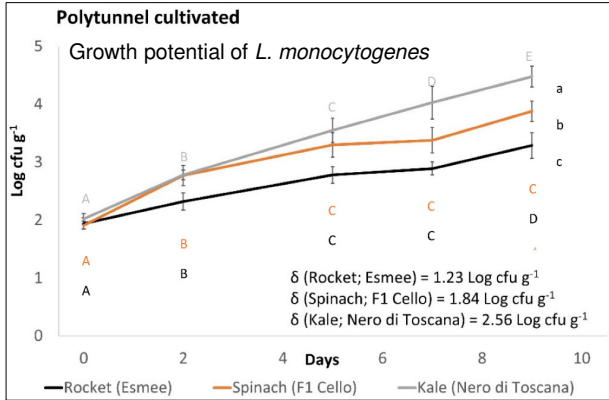
7/12°C (6/3 days); Air



doi.org/10.3390/foods11193056



I. *Listeria monocytogenes* and total bacteria counts of experimental farm rocket (Esmee), spinach (F1 Cello) and kale (Nero di Toscana)



Incubated @ 7°C (6d) and 12°C (3d); (±) error bars indicate standard deviation; δ represents growth potential; Lower and uppercase letters indicate significant differences between groups and over time, respectively. Dashed line = *L. m.* inoculated, solid = non-inoculated.



doi.org/10.3390/foods11193056



II. Open field vs. polytunnel - Comparison of bacterial communities: β -diversity

Polytunnel spinach

- Produce type (i.e., rocket vs spinach)
- Cultivation method (i.e., open field vs polytunnel)

- Significant changes also identified across time

All 4 groups separated highly significantly

Polytunnel rocket

Open field spinach

Open field rocket



II. Open field vs. polytunnel - Comparison of bacterial communities: relative abundances at phyla level between groups

- 34 phyla detected
- *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota* were dominant >89%
- Cultivation method & plant species influenced bacterial community structure

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II. Open field vs. polytunnel - Comparison of bacterial communities: correlation between phyllosphere bacteria and relative abundance and *L. monocytogenes*

Spinach open field ($\delta = 2.59$)			Spinach polytunnel ($\delta = 1.40$)		
	Day 7 (%)	Day 9 (%)		Day 7 (%)	Day 9 (%)
<i>L. monocytogenes</i>	3.48	4.58	<i>L. monocytogenes</i>	3.33	3.36
<i>Pseudomonadaceae</i>	Rel. numbers increased		<i>Pseudomonadaceae</i>	Rel. numbers decreased	
<i>Pectobacteriaceae</i>	Rel. numbers decreased		<i>Pectobacteriaceae</i>	Rel. numbers increased	

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II. Open field spinach (Trumpet F1) summer vs. winter batch - Comparison of bacterial communities: β -diversity

16S rRNA gene
sequencing

Winter produce

Spinach, summer vs winter was significantly different

Summer produce

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II. Open field spinach (Trumpet F1) summer vs. winter batch: correlation between phyllosphere bacteria and relative abundance and *L. monocytogenes*.

Phyllosphere bacteria potentially influencing *L. monocytogenes* on summer (left) and winter (right) open field spinach produce

Summer produce ($\delta = 2.59 \log^{10} \text{ cfu g}^{-1}$) \rightarrow Winter produce ($\delta = 1.65 \log^{10} \text{ cfu g}^{-1}$)

	Total average (%)
<i>Pseudomonadaceae</i>	Unchanged
<i>Pectobacteriaceae</i>	Unchanged
<i>Carnobacteriaceae</i> (LAB)	Not present
Streptococcaceae (LAB)	Increased
Lactobacilales (order)	Unchanged overall but decreased day 9 in summer

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II. Vegetable varieties and species – Comparison of bacterial communities: β -diversity

16S rRNA gene sequencing

Spinach F1 Trumpet

Spinach F1 Cello

Varieties AND vegetable type significantly separated

Rocket Buzz

Rocket Esmee

PCoA (Weighted UniFrac)

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Summary and conclusion:

- All tested vegetables support growth of *L. monocytogenes*
- Washing had only a marginal effect on *L. monocytogenes* abundance/growth potential
- Spinach in open fields had highest growth rates of *L. monocytogenes* while open field rocket had the lowest
 - No “one fits all” solution for vegetable growers
- *L. monocytogenes* growth on kale appears to be linked to abundance of heterotrophic bacteria abundance
 - low heterotrophs = higher *L. monocytogenes* growth rate?
- Phyllosphere bacteriome community structure is affected by environmental conditions (open-field, polytunnel, season), vegetable species and variety
- Members of the phyllosphere bacteriome putatively affect *L. monocytogenes* growth potential
 - Future control via anti-listerial bacteriomes?

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Food and the Marine



Thank you for listening!

