Sub-theme:

PEST SURVEILLANCE IN PHYTOSANITARY SYSTEMS

Title:

OCCURRENCE OF PECTOBACTERIUM SPECIES IN SOIL COLLECTED FROM SIX MAJOR POTATO GROWING REGIONS OF KENYA

Presented by:

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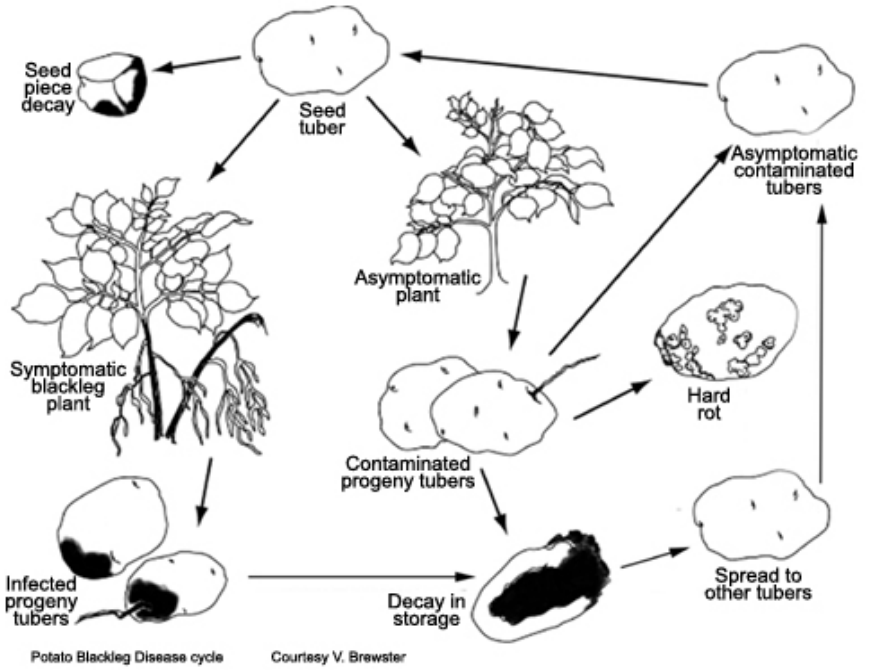
Introduction

Why *Pectobacterium*?

- It is the primary pathogen associated with blackleg infection in the field and soft rot damage during transportation and storage.
- Belongs to the Soft rot *Enterobacteriaceae (*SREs*)* family.
- Infects through production of cell wall degrading enzymes especially cellulases, pectinases and proteases facilitating rotting
- *Pectobacterium* is present in soil as a saprophyte, water and in the plant root biosphere when not causing disease.
- *Pectobacterium* species have a world-wide distribution.
- In Africa, *Pectobacterium* species especially *P. carotovora*, *brasiliense, P. parmentieri* have been reported.

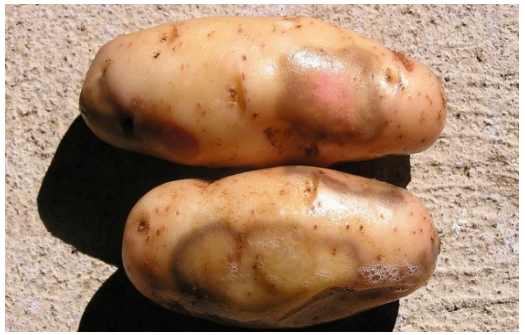
Disease mechanism- Blackleg and soft rot



Source: V. Brewstar in (De Boer and Rubio, 2004)

Introduction cont

- Exposure of seed tubers to high inoculum -soil or contaminated irrigation water.
- *Pectobacterium* species require conducive growth conditions and a susceptible host.
- Presence of infected plant debris, infected volunteer crops, insect vectors, contaminated tools/seeds amongst other sources.
- Poor agronomic practices such as use of disease-infested seed. Most of such seed originates from the informal sector.
- Injury on tubers during harvesting allows for entry of the pathogen.



Problem Statement

- Pest and diseases are one of the major challenges faced by potato farmers in Kenya.
- They contribute an estimated 80% reduction in production resulting to shortage of clean seed, low yields and a threat to food security.
- Shortage of clean seed results to recycling of own seed (with unknown disease status) by farmers hence spread of diseases to most of the traditional potato producing regions.
- Since *Pectobacterium* is know to occur in soil, growing seed in fields with unknown disease status is a risk to the potato sector.
- Unlike *Ralstonia Solanacearum*, there is limited information on the occurrence of *Pectobacterium* species in soil in Kenya.
- Without such knowledge, it is not possible to delineate disease-free zones for production of disease-free seed.

Justification

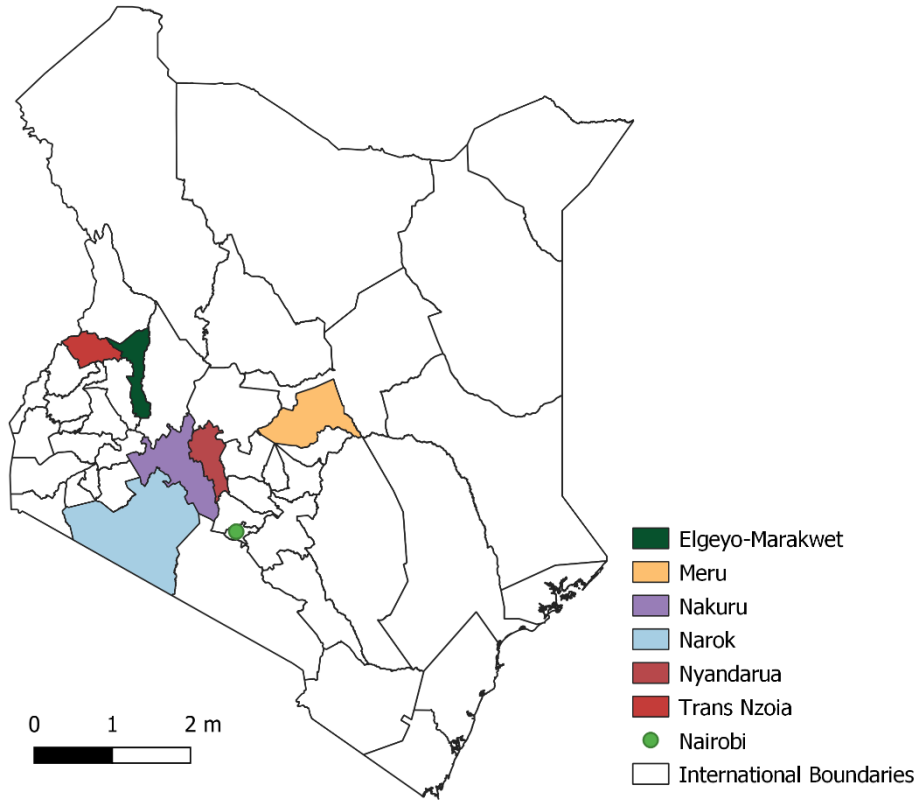
- Blackleg and soft rot diseases causes substantial losses in yield in potato in the field, storage and transit hence great economic losses along the potato value chain.
- It is thought that the pathogen remains latent in soil for sometime, infecting tubers and impacting potato yield in subsequent seasons.
- There is lack of knowledge on their status of SREs in Kenya's major potato producing counties.
- Farmers majorly depend on the informal sector for potato planting materials resulting in distribution of diseases.
- It is necessary to establish if SREs are present in smallholder farmer fields and determine the key main subspecies.
- This is essential in refining extension messages on disease management, raise awareness on the importance of using certified seed, and reduce dependence on the informal sector in smallholder potato production systems.

Objectives

- To evaluate the presence of SRE; as causative agents of blackleg and soft-rot diseases in the major potato growing counties in Kenya (Elgeyo Marakwet, Meru, Nakuru, Narok, Nyandarua and Trans Nzoia).
- Isolate, identify and characterize pectolytic bacteria from soils collected from the six major potato growing counties of Kenya.

Methodology

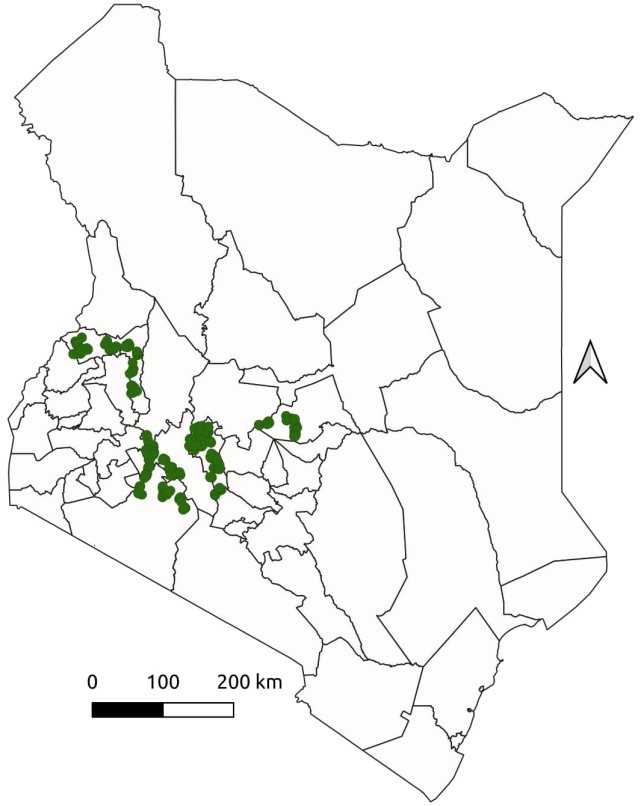
- Extensive surveillance was conducted in six major potato growing counties based on selective or targeted sampling (in ISPM 6 and ISPM 31).
- The farmers were selected by the Ward Agricultural Officers based on; 1) a long history of potato production and 2) knowledge of previous cases of blackleg and soft rot.
- Samples were collected from plants showing symptoms associated with blackleg and soft rots.
- Whole plants (leaves, stems and tubers) samples were collected, put in Khaki paper bags and placed in insulated containers.
- Soil samples were placed in plastic bags which were then placed within paper bags.



Methodology

- Samples were given unique identifiers, shipped to KEPHIS Laboratories at HQ within 24 h of collection and placed in cold storage (4^{o}C) until processing.
- Plant samples were portioned into leaves, stems, roots and tubers while soil was measured in 50 gram portions. All processed portions were placed well labelled 50-mL-falcon tubes and kept at -20^{o}C until needed for isolation
- Information such as potato variety and GPS was documented. Additional socioeconomic information was obtained from farmers using a structured questionnaire programmed on the ODK platform.
- SREs were isolated from soil using established procedures first by enriching the pathogens in an enrichment media (D-PEM) for 48 hr followed by isolation on selective Crystal Violate Pectate media at 28^{o}C for 5-7 days.
- Presumptive colonies were cleaned and subjected to PCR using primer sets that confirm *Pectobacterium* species and other sets that confirm specific subspecies.

Results cont'



Samples were collected from 6 counties, 25 Sub Counties and 55 Wards.

Number of farm households surveyed

County	Number of samples
Elgeyo Marakwet	91
Meru	122
Nakuru	268
Narok	94
Nyandarua	317
Trans Nzoia	110
Total	**1,002**

Number of all samples collected

County	Number of samples
Elgeyo Marakwet	125
Meru	144
Nakuru	320
Narok	125
Nyandarua	368
Trans Nzoia	109
Total	1,191

Source of seed for farmers in the six counties

County	Own seed	Friends	Market	Seed distributor	Others
Elgeyo Marakwet	16	67	2	6	1
Meru	45	44	10	64	2
Nakuru	170	161	2	36	6
Narok	48	58	5	26	
Nyandarua	233	147	6	28	5
Trans Nzoia	9	64	26	12	
Total	**521**	**541**	**51**	**172**	**14**

Number of samples processed

Sample type	Number
Soil	803
Stem	1,334
Tuber	697
Total	**2,834**

Results

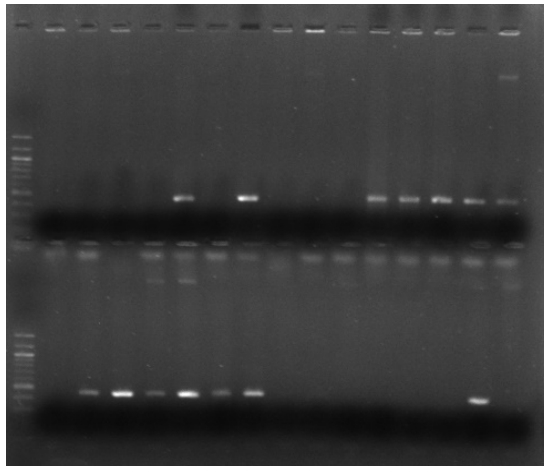
Positive Controls of *Pectobacterium* species used in this study

NCPPB Number	Name of Organism	Country
4642	*P. brasiliense*	Brazil
4609	*P. brasiliense*	Brazil
3427	*P. carotovorum*	Canada
3398	*P. carotovorum*	Netherlands
4645	*P. parmentieri*	United Kingdom

Primer sets used in this study

Primer set	Primer sequence	Product size (bp)	Species
Y1 Y2	TTACCGGACGCCGAGCTGTGGCGT CAGGAAGATGTCGTTATCGCGAGT	434	*Pectobacterium* species
SR3F SR1CR	GGTGCAAGCGTTAATCGGAATG AGACTCTAGCCTGTCAGTTTT	119	*Pectobacterium* species
BR1 L1	GCGTGCCGGGTTTATGACCT CA(A/G)GGCATCCACCGT	322	*P. Brasiliense* (*Pb*)
EXPCCF EXPCCR	GAACTTCGCACCGCCGACCTTCTA GCCGTAATTGCCTACCTGCTTAAG	550	*P. Carotovorum* (*Pc*)
PW7011F PW7011R	CTATGACGCTCGCGGGTTGCTGTT CGGCGGCGTCGTAGTGGAAAGTC	140	*P. Parmentieri* (*Pw*)

Image of samples that tested positive



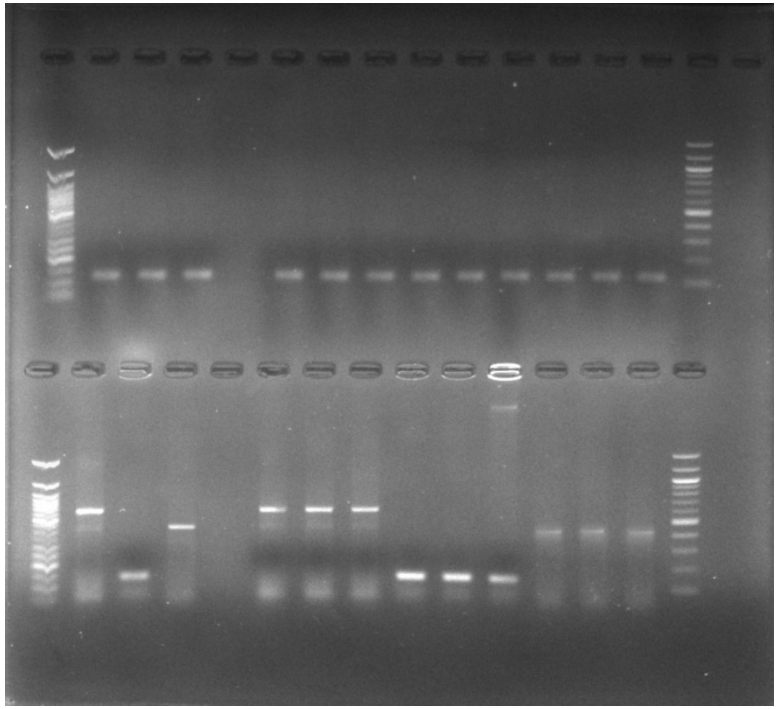
- The extreme left is the 100 bp ladder.
- Samples that tested positive with Y1/Y2 primer set are presented.
- Y1/Y2 confirms the *Pectobacterium* genera .
- The expected product size of xx bp was obtained.

County	Samples that turned positive
Elgeyo Marakwet	1
Meru	6
Nakuru	6
Narok	21
Nyandarua	56
Trans Nzoia	2
Grand Total	**92**

- In total, *Pectobacterium* species were confirmed in 292 samples
  - 183 were stem samples (result not shown)
  - 17 tuber samples (result not shown)
  - 92 Soil samples
- More samples were collected from Nyandarua hence the high positives but also indicates high contamination.
- Narok and Elgeyo Marakwet were the lowest but Narok recorded relatively higher positives than even Nakuru which had the second largest number of samples.

Results cont’

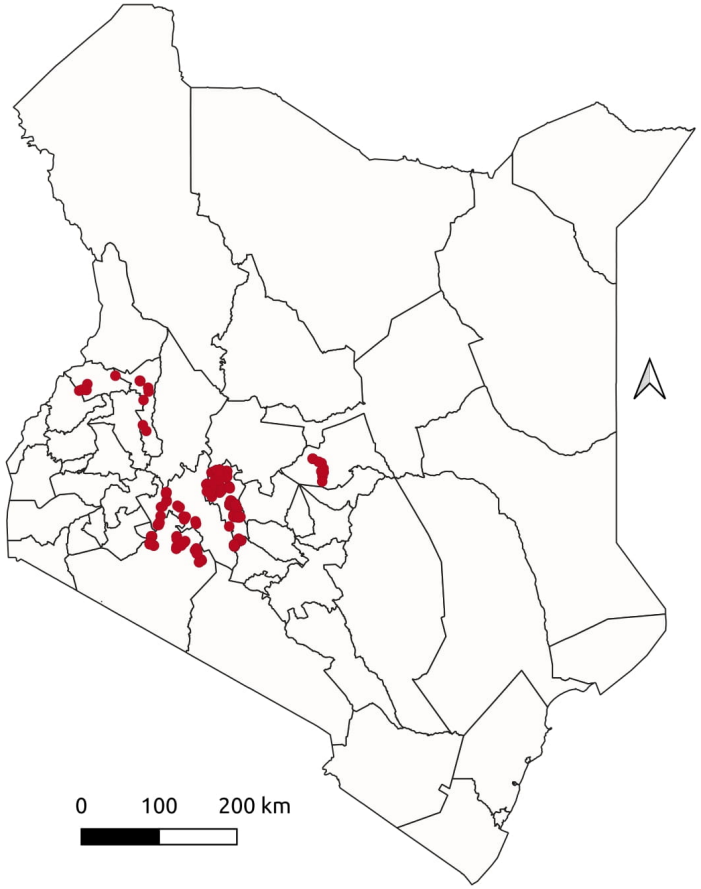
Image of samples that tested positive with the general primer and species-specific primers



- The extreme left and right is the 50 bp and 100 bp ladders respectively.
- Top lanes are samples originally confirmed with Y1/Y2 but reconfirmed with SR3F/SR1CR (general *Pectobacterium* primer set).
- Lane 2, 3 and 4 (above) are positive controls in the order of *Pc, Pw* and *Pb* respectively
- Lane 5 (above and below) is a no template (negative) control.
- Lanes 6-8 above is there test samples confirmed as *P. carotovorum* below. The expected product size of 550 bp which corresponds with the positive control (Lane 2) was obtained.
- Lanes 9-11 above is there test samples confirmed as *P.* parmentieri, below. The expected product size of 140 bp which corresponds with the positive control (Lane 3) was obtained.
- Lanes 10 -12 above is there test samples confirmed as *P. brasiliense*, below. The expected product size of 322 bp which corresponds with the positive control (Lane 4) was obtained.

Conclusion

- Potato is the major value chain in the counties surveyed
- The majority of farmers depend on the informal seed sector for potato planting materials.
- *Pectobacterium* species were widely distributed in all the counties surveyed.
- One third of the samples that confirmed positive for *Pectobacterium* species were soil.
- Most of the samples were from Nyandarua, a county that leads in potato production in Kenya besides Nakuru.
- Although Narok had one of the lowest number of samples, it recorded relatively high numbers of positives for .
- SREs can infect tubers through different ways but water and soil are one of them. This has implications production of disease-free seed for the potato industry



Recommendations

- There is need to raise awareness amongst smallholder potato farmers about blackleg and soft rots.
  - This could be achieved through developing fit-for-purpose information and communication materials in the form of Pest Management Decision Guides (PMDG)s, illustrative factsheets and photo guides.
- Train extension officers in field identification of blackleg and soft rots as well as sampling and hygiene, and appropriate actionable management strategies.
- Support interventions that increase availability of certified seed. Farmers are over-relying on seed from the informal sector which is resulting in spread diseases. This affects quality and yield.
  - Address issues related to access and affordability of the certified seed.
- In situations where farmers can't afford certified seed, they should be supported by extension systems to adopt technologies that can enable production of better quality disease-free seed on-farm.
  - Positive seed selection
  - Seed plot technology

Acknowledgements

Theme: *"Enhancing Phytosanitary Systems for Healthy Plants, Safe & Sustainable Trade"*
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