Initial assessment of the population structure of *Phytophthora infestans* in the Venezuelan Andes

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An urban society (ca. 92%) that needs to guarantee food security
A fast growing agroproductive frontier challenges natural ecosystems and threatens biodiversity.
### Main agricultural products in Venezuela (FAO 2004): the top ten (in tons)

<table>
<thead>
<tr>
<th></th>
<th>Product</th>
<th>Tonnes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sugar cane</td>
<td>8,814,248</td>
</tr>
<tr>
<td>2</td>
<td>Maize</td>
<td>2,176,160</td>
</tr>
<tr>
<td>3</td>
<td>Rice</td>
<td>974,071</td>
</tr>
<tr>
<td>4</td>
<td>Citrus fruit, total</td>
<td>584,722</td>
</tr>
<tr>
<td>5</td>
<td>Sorghum</td>
<td>563,345</td>
</tr>
<tr>
<td>6</td>
<td>Cassava</td>
<td>511,444</td>
</tr>
<tr>
<td>7</td>
<td>Bananas</td>
<td>463,047</td>
</tr>
<tr>
<td>8</td>
<td>Plantains</td>
<td>426,298</td>
</tr>
<tr>
<td>9</td>
<td>Potatoes</td>
<td>350,063</td>
</tr>
<tr>
<td>10</td>
<td>Pineapples</td>
<td>322,768</td>
</tr>
</tbody>
</table>
Production (X 1000 Ton)

Year

Potato
Cassava

Yield (kg X1000 per Ha)

- **Potato**
- **Cassava**

Year:
- 1992
- 1996
- 2001
- 2006
Potato producing areas in Venezuela along history...
Potato production in Venezuela

<table>
<thead>
<tr>
<th>STATE</th>
<th>CULTIVATION AREA (ha)</th>
<th>YIELD Kg/ ha</th>
<th>PRODUCTION (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARAGUA</td>
<td>800</td>
<td>16,500</td>
<td>13,200</td>
</tr>
<tr>
<td>CARABOBO</td>
<td>1500</td>
<td>16,500</td>
<td>24,750</td>
</tr>
<tr>
<td>LARA</td>
<td>3500</td>
<td>18,000</td>
<td>63,000</td>
</tr>
<tr>
<td>MÉRIDA</td>
<td>6500</td>
<td>22,300</td>
<td>144,950</td>
</tr>
<tr>
<td>TÁCHIRA</td>
<td>3200</td>
<td>14,800</td>
<td>47,360</td>
</tr>
<tr>
<td>TRUJILLO</td>
<td>4800</td>
<td>16,400</td>
<td>78,720</td>
</tr>
<tr>
<td>Totals</td>
<td>20,300</td>
<td>18,324</td>
<td>371,980</td>
</tr>
</tbody>
</table>

71.43% 72.86%
Años 90: Concentración de la producción de papa en la región andina

1987 1991 1995

Venezuela  Lara  Mérida  Táchira  Trujillo  Aragua  Carabobo

0 50.000 100.000 150.000 200.000 250.000 300.000 350.000

Producción (T)
Lower Páramo

*(2000-3300 masl)*

Very productive agriculture on very fertile soils and with technological advances
Higher Páramo
(3300-3800 masl)

Mesa Julian

Low income agriculture with “rest”

Industrial agriculture (garlic-potato)

El Churao

Extensive cattle exploitation

Grass cover on valleys; soils thicker and more humid

Llano del Hato
A way of living
Phytophthora infestans in Venezuela

• It is reported for the first time (at least in a scientific report) in 1939 by Müller (but we are looking back in history…)

• By 1949 late blight is considered to be the main disease of potato in producing areas in the country

• It is ubiquitous in the Venezuelan Andes. If not (chemically) controlled 100% losses have been reported.

• However, no alternate hosts have been studied in the country. Almost never is potato cultivated along with tomato, or other solanaceae.

• In the late sixties it starts what is considered to be the second boom in the use of the imported seeds.

• In the nineties, races 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 1.3, 1.4, 2.3, 2.4, y 1.2.3, were reported in the central states, but no screening was preformed in the Andean states
**Phytophthora infestans**

- **Native potatoes**

**Project ULA**

- 2003:
  - **Sampling, characterization, storage, mating**

**Project BID**

- 2004:
  - **Mitochondrial Haplotype, SSR**
- 2005:
  - **GPI**

**Project FUNDACITE**

- 2006:
  - **Nuclear and mitochondrial genes**
- 2007:
  - **All the above**
The Strategy

Collection I: >500 isolates
- Isolation, growth & storage
- Observation/microscope

1 per production unit

Collection II: 84 isolates
- PCR validation of identity
- Mating type
- Polymorphism by SSR
- Polymorphism by GPI
- Metalaxyl susceptibility

Collection III: 12 isolates
- Sequencing of nuclear & mitochondrial genes
- Infection assays
UNIDADES ECOLOGICAS DEL ESTADO MERIDA
Autores: Michele Ataroff y Lina Sarmiento

- Arbustal espinoso
- Bosque caducifolio seco
- Bosque siempreverde seco montano alto
- Bosque siempreverde seco montano bajo
- Páramo altiandino
- Páramo andino
- Sabana montana
- Selva húmeda tropical
- Selva nublada montana alta
- Selva nublada montana baja
- Selva submontana
- Selva semicaducifolia montana
Processing I: Sampling

- Collected, generally, before 10 am
- Information gathered directly from producer
- Mean: ten samples per unit, leaflets still alive
- Samples kept cold
- Samples processed no later than two days after collection
Processing I: The media

We had to try what was available to us...

The “faster”

How the same isolate looked like after 21 days growing in different media
Processing I: isolation and growing

- Disinfection with chlorine
- Leaflets discs taken from line of advance of infection
- Transferred to growth media, M3, supplemented with antibiotics and fungicides
- Incubation at 16°C, for 15 days in the dark
- Staining with basic lacto-fuchsin & observation under the microscope
Processing I: Storage

- Collection is frequently replicated
- Kept cold
- A replica of the collection is being maintained at -80°C

The place for the manipulation of *P.infestans* is different to the molecular lab!
## Sampling Results

<table>
<thead>
<tr>
<th>Name of Potato Variety</th>
<th>Mérida</th>
<th>Táchira</th>
<th>Trujillo</th>
<th>Totals (%)</th>
<th>Totals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Units</td>
<td>Samples</td>
<td>Units</td>
<td>Samples</td>
</tr>
<tr>
<td>Granola</td>
<td>170</td>
<td>24</td>
<td>24</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Única</td>
<td>82</td>
<td>14</td>
<td>15</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>R12</td>
<td>48</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Roja</td>
<td>26</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caribay</td>
<td>22</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Andina</td>
<td>8</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Idrafvin</td>
<td>8</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Andinita</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sin nombre</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tibisay</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Merengue</td>
<td>9</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Revoltijo</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Reinosa</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td>35</td>
<td>5</td>
<td>53</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Totals (%)</td>
<td>431 (76.4)</td>
<td>61 (72.6)</td>
<td>105 (18.6)</td>
<td>19 (22.6)</td>
<td>28 (5.0)</td>
</tr>
</tbody>
</table>
Processing II: Susceptibility to metalaxyl

Although resistance was variable, no single isolate was “highly resistant”
Processing II: mating type

- Mating with reference strains (control)
- Mating of each reference (A1 and A2) with one isolate per unit
- Observation under the microscope
- Additionally, PCR amplification of specific products for A1 (and absence for A2)

All isolates were A1, except for one self-fertile isolate
Processing II: Mitochondrial haplotype

Amplification

Digestion

Purify → Sequence → Compare

MspI
Processing II: Mitochondrial haplotype

All tested isolates were la, except only one that turned out to be IIa
### Processing II: Microsatellites

- Four different pair of primers were used (Knapova et al, 2001), and amplification products run on Urea-PAGE, 6%.

- Three SSR were monomorphic. One pair of primers allowed the detection of a single isolate with a differential pattern; and this one turned out to be:

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Haplotype</th>
<th>MT</th>
<th>SSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>Santa Rosa, Mérida</td>
<td>Ia</td>
<td>Self-fertile</td>
<td>=</td>
</tr>
<tr>
<td>V2</td>
<td>Las Porqueras, Táchira</td>
<td>IIa</td>
<td>A1</td>
<td>≠</td>
</tr>
<tr>
<td>V3</td>
<td>El Valle, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V4</td>
<td>Pueblo Llano, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V5</td>
<td>Pueblo Llano, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V6</td>
<td>Pueblo Llano, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V7</td>
<td>El Cobre, Táchira</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V8</td>
<td>Valle del Chama, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V9</td>
<td>Valle del Mocotíes, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V10</td>
<td>Valle del Tuñame, Trujillo</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V11</td>
<td>El Trompillo, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
<tr>
<td>V12</td>
<td>Santo Domingo, Mérida</td>
<td>Ia</td>
<td>A1</td>
<td>=</td>
</tr>
</tbody>
</table>
Processing III: the basics

1st boom of imported seeds

Post WWII immigration

2nd boom of imported seeds

Black, pink and white potatoes

Almost only white potatoes

*curtilobum?*

*andigena*

Black, pink and white potatoes

*vs*

*tuberosum*

Almost only white potatoes
Processing III: Search

Páramo de Gavidia, Mérida
Cladogram obtained using 12 different pairs of SSR primers in selected potatoes
Processing III: controlled infection assays

Esquema general del procedimiento

Observación microscópica de Zoosporangios de *P. infestans*
Processing III: controlled infection assays

• “Granola”, as expected, was susceptible to all 12 different assayed isolates
• “Andinita”, regarded as resistant, showed resistance to some isolates, while it was susceptible to others
• Referentials allowed us to differentiate better among the 12 isolates used (isolates tested may vary in virulence)
• Additionally, from all nuclear and mitochondrial genes amplified and sequenced, only $avr3a$ reveals polymorphism and relates Venezuelan samples to Ecuadorian ones
• Some “native” potatoes showed a promising response in terms of resistance (experiments currently being replicated)
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1 per production unit

PCR validation of identity
Mating type
Polimorphism by SSR
Polimorphism by GPI
Metalaxyl susceptibility

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Sequencing of nuclear & mitochondrial genes
Infection assays
Over the centuries...

- **XV**
- **XVI**
- **XVII**
- **XVIII**
- **XIX**
- **XX**
- **XXI**

- Wheat
- Relictual
- Recovering
- Cacao
- Coffee
- Back and forth
- Potato
- Increasing

Back and forth
Conclusions and recommendations

• Sampling might have introduced a bias for Ia isolates. Analyze whole collection later.

• However, our collection seems to be composed mainly by Ia isolates of mating type A1. The only self-fertile isolate (very aggressive) coexisted with A1 isolates in the same production unit.

• GPI analysis reveals a low level of heterosis, but also localized patterns apparently related with monocrop cultivation.

• Few alternate hosts have been found, but a more extensive search is needed before the advance of the agroproductive frontier makes more difficult their very presence.

• For the same reason, it is very important to continue efforts to locate, identify and recover other native potatoes and wild tuber-bearing solanaceous species.

• An increase of SSR in the analysis might help elucidate better the true structure of the P. infestans population in Venezuela.

• To better understand the relationship established between populations of the pathogen in Venezuela and Colombia, a more comprehensive sampling must be performed, specially on the Colombian side…

• There seems to be a wide variability in terms of virulence, which study might be helped with the inclusion of more native potatoes.
The People...

Prof. Luis Cedeño (IIAP)

MSc Maira Moreno

Johanna and Katherina

MSc. Mario San Román
The People...

TSU K. Quintero  Lic. J. Moreno  TSU H. Pino  MSc. A. Briceño  Lic. K. Boscán
The Financing...