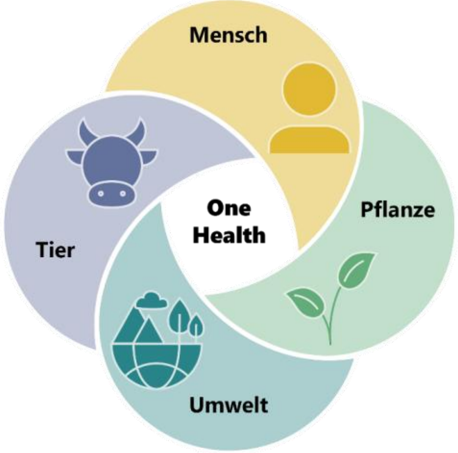
First Report of *Verticillium dahliae* Causing Wilt of Pawpaw (*Asimina triloba*)



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EPPO Conference on Diagnostics of Plant Pests Recent developments and future trends 2026-04-22/24, Vienna (AT)

Background

Verticillium wilt

- A major fungal disease affecting a wide range of crops
  - More than 400 plant species are affected, including vegetables, legumes, fiber crops, fruit crops, trees and ornamentals

- *Verticillium dahliae* is a soil-borne pathogen
  - No effective curative treatment available

- Pawpaw (*Asimina triloba*) is an emerging fruit crop in Austria

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Background

Pawpaw as an emerging crop

- Pawpaw (*Asimina triloba*) native to eastern North America
- Deciduous tree, winter-hardy down to -20°C
- Member of the Annonaceae family (tropical relatives: Cherimoya, Guanábana…)

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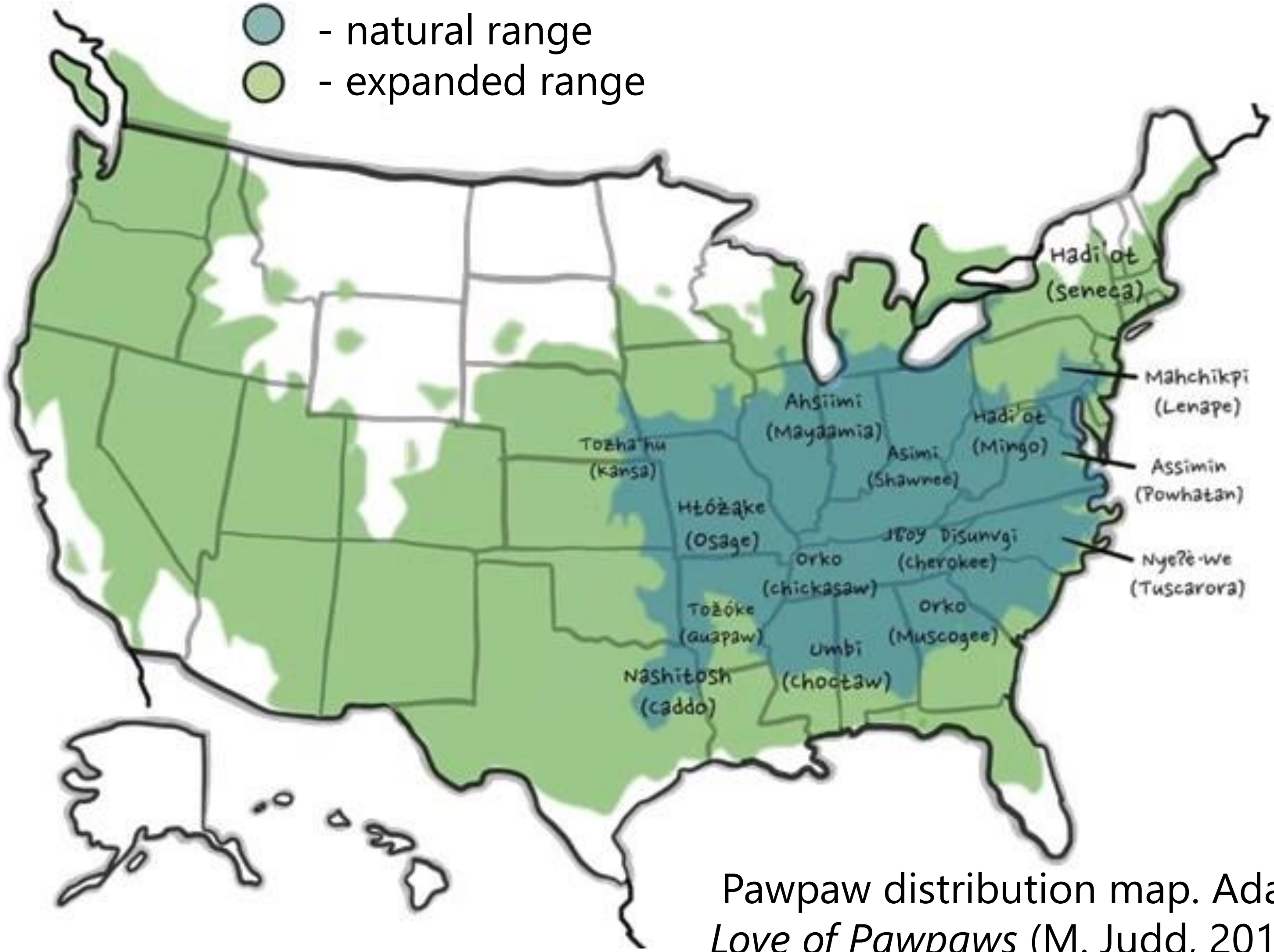
Natural and expanded range of *Asimina triloba*



Pawpaw distribution map. Adapted from *For the Love of Pawpaws* (M. Judd, 2019).

Background

Pawpaw as an emerging crop

- Pawpaw (*Asimina triloba*) native to North America
- Deciduous tree, winter-hardy down to -20°C
- Member of the Annonaceae family (tropical relatives: Cherimoya, Guanábana…)
- Mango-shaped fruits ripen in September-October
- Tropical flavor combining notes of vanilla, banana and mango, with a creamy texture
- Introduced to Austria in the 1990s
- Today it is grown all over Europe (Italy, Germany, Slowenia, Kroatia, Romania…)

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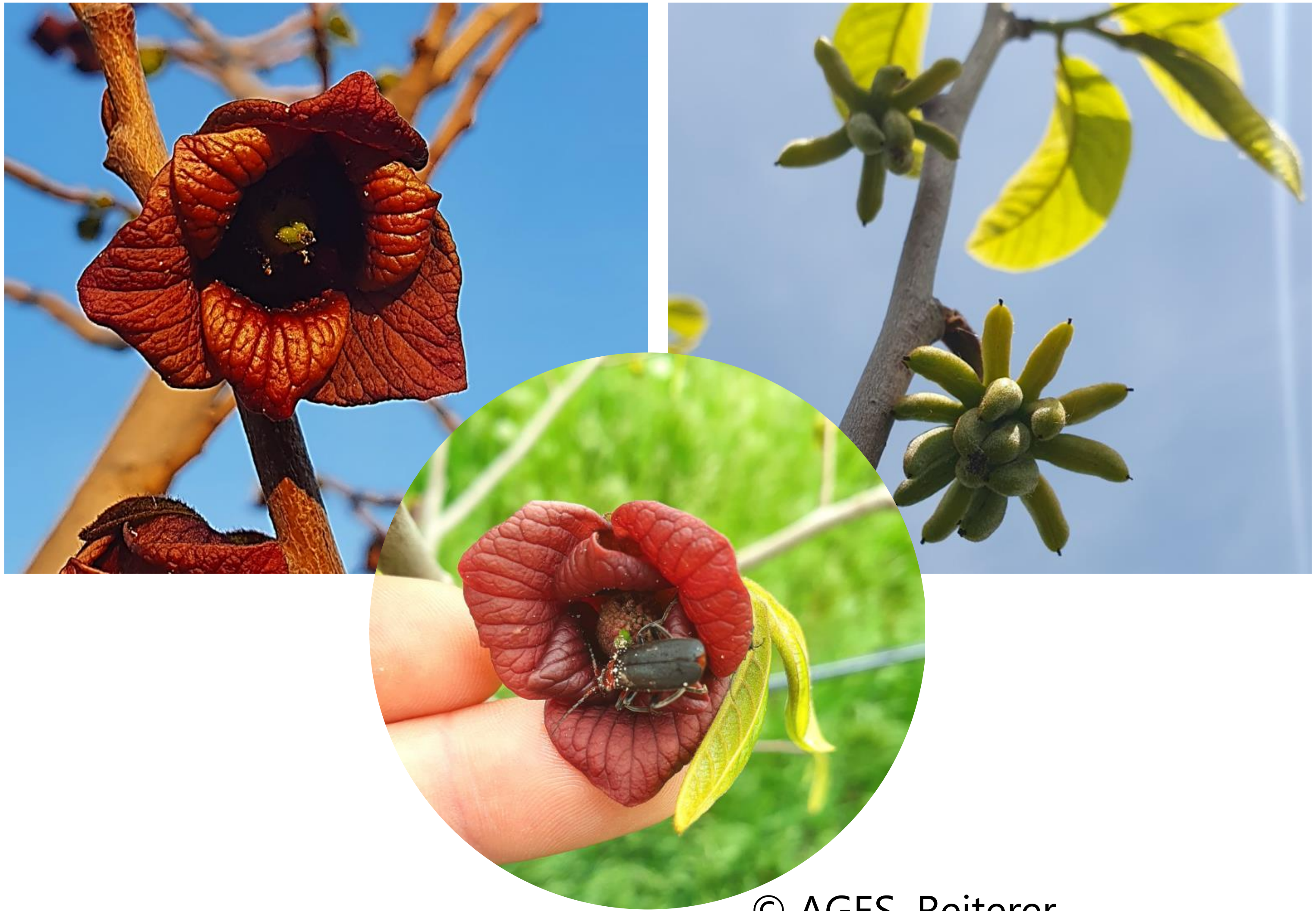
Background

From flowers to fruits



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Background

From flowers to fruits

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Field Observations

Verticillium wilt

- Pawpaw is not known to be susceptible to Verticillium wilt (Gilman and Watson 1993).

- In 2022, first symptoms observed in individual trees in a four-year-old Pawpaw plantation
  - leaf yellowing and wilting
  - vascular browning
  - branch blight
  - complete dieback

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Field Observations

Verticillium wilt – branch blight

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Field Observations

Verticillium wilt – wilting

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Field Observations

Verticillium wilt – complete dieback

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Field Observations

Verticillium wilt – vascular browning

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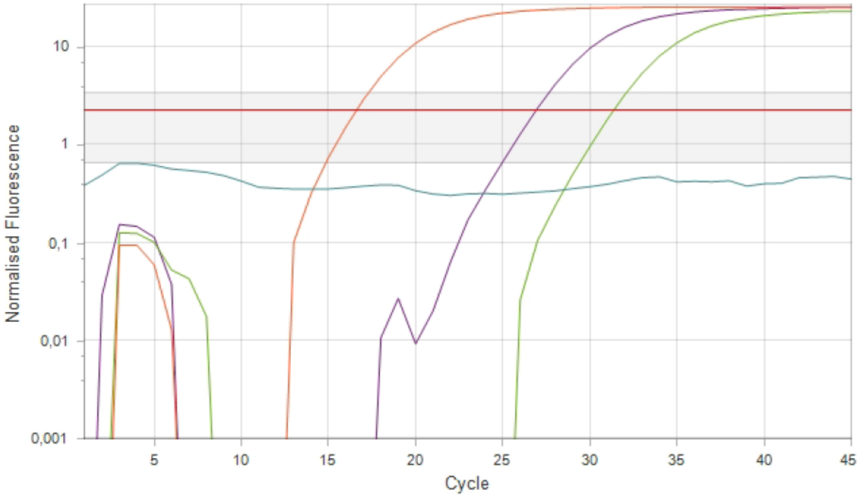
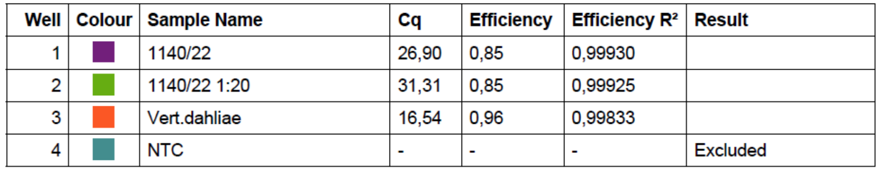
Identification & Methods

From plant material

- DNA extraction from symptomatic branches
  - DNeasy Plant Mini Kit (Qiagen)
- TaqMan real-time PCR specific for *V. dahliae*
  - *Bilodeau et al. 2012*





Well	Colour	Sample Name	Cq	Efficiency	Efficiency R²	Result
1	🟪	1140/22	26,90	0,85	0,99930	
2	🟩	1140/22 1:20	31,31	0,85	0,99925	
3	🟧	Vert.dahliae	16,54	0,96	0,99833	
4	🟦	NTC	-	-	-	Excluded

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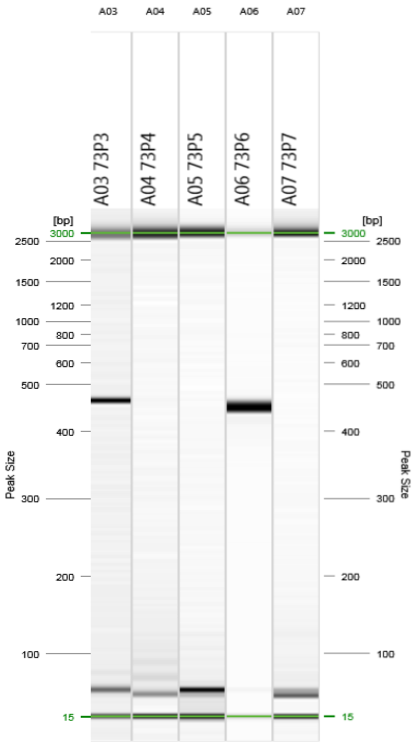
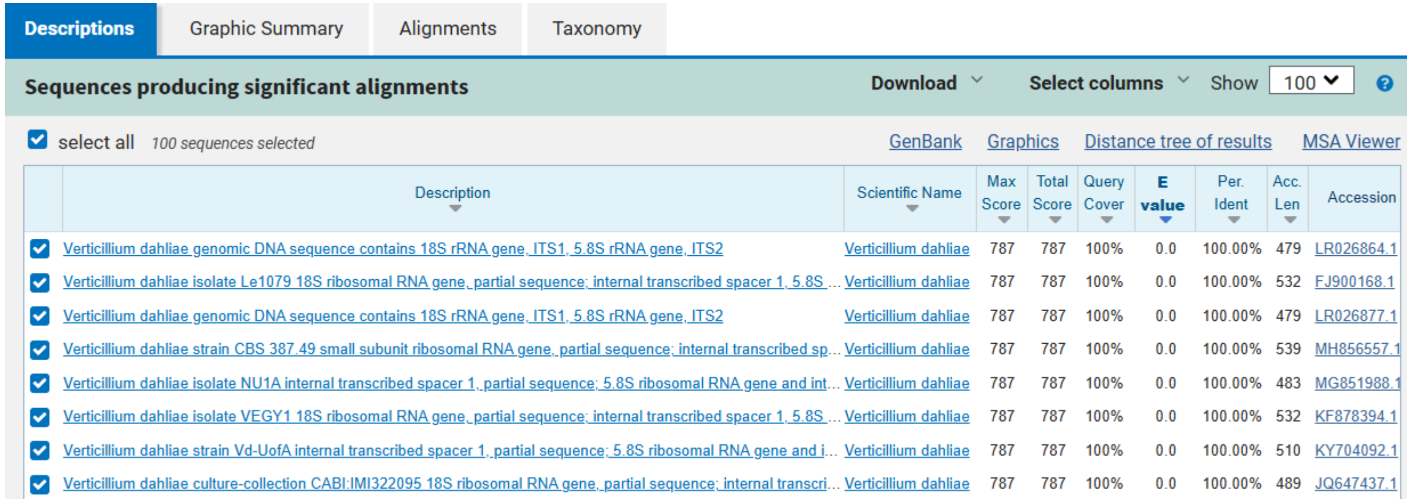
Identification & Methods

From plant material

- Endpoint PCR for *Verticillium* spp., followed by Sequencing
  - *Nazar et al. 1991*





Sequences producing significant alignments

☑ select all *100 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Verticillium dahliae genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2	Verticillium dahliae	787	787	100%	0.0	100.00%	479	LR026864.1
☑	Verticillium dahliae isolate Le1079 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	Verticillium dahliae	787	787	100%	0.0	100.00%	532	FJ900168.1
☑	Verticillium dahliae genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2	Verticillium dahliae	787	787	100%	0.0	100.00%	479	LR026877.1
☑	Verticillium dahliae strain CBS 387.49 small subunit ribosomal RNA gene, partial sequence; internal transcribed sp...	Verticillium dahliae	787	787	100%	0.0	100.00%	539	MH856557.1
☑	Verticillium dahliae isolate NU1A internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Verticillium dahliae	787	787	100%	0.0	100.00%	483	MG851988.1
☑	Verticillium dahliae isolate VEGY1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	Verticillium dahliae	787	787	100%	0.0	100.00%	532	KF878394.1
☑	Verticillium dahliae strain Vd-UofA internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Verticillium dahliae	787	787	100%	0.0	100.00%	510	KY704092.1
☑	Verticillium dahliae culture-collection CABI:IMI322095 18S ribosomal RNA gene, partial sequence; internal transcri...	Verticillium dahliae	787	787	100%	0.0	100.00%	489	JQ647437.1

Identification & Methods

From fungal culture

- Isolation & morphological identification
  - Plating of infected material (surface sterilized) on Potato dextrose agar
  - Morphological features were consistent with *Verticillium dahliae*

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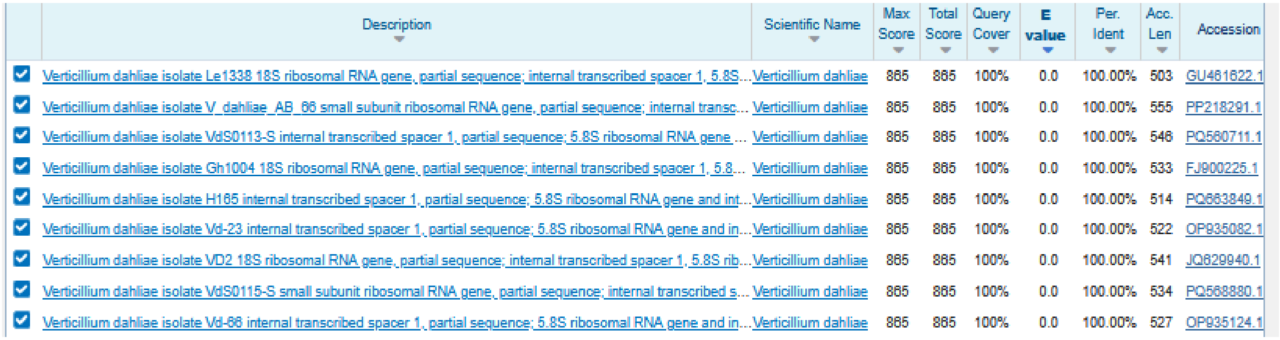
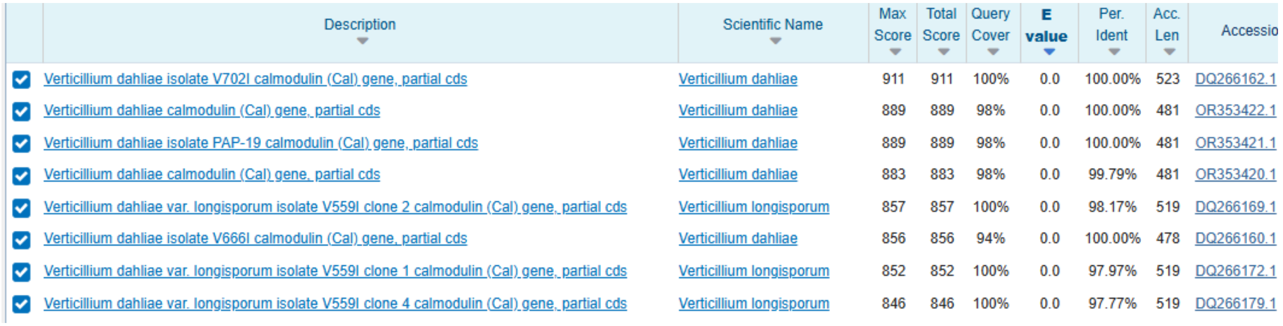
Identification & Methods

From fungal culture

- DNA-Barcoding
  - Internal transcribed spacer (ITS)
  - Calmodulin (CALM) gene



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Verticillium dahliae isolate Le1338 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Verticillium dahliae	885	885	100%	0.0	100.00%	503	GU461622.1
☑	Verticillium dahliae isolate V_dahliae_AB_66 small subunit ribosomal RNA gene, partial sequence; internal transc...	Verticillium dahliae	885	885	100%	0.0	100.00%	555	PP218291.1
☑	Verticillium dahliae isolate VdS0113-S internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Verticillium dahliae	885	885	100%	0.0	100.00%	546	PQ560711.1
☑	Verticillium dahliae isolate Gh1004 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8...	Verticillium dahliae	885	885	100%	0.0	100.00%	533	FJ900225.1
☑	Verticillium dahliae isolate H165 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Verticillium dahliae	885	885	100%	0.0	100.00%	514	PQ663849.1
☑	Verticillium dahliae isolate Vd-23 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in...	Verticillium dahliae	885	885	100%	0.0	100.00%	522	OP935082.1
☑	Verticillium dahliae isolate VD2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S rib...	Verticillium dahliae	885	885	100%	0.0	100.00%	541	JQ829940.1
☑	Verticillium dahliae isolate VdS0115-S small subunit ribosomal RNA gene, partial sequence; internal transcribed s...	Verticillium dahliae	885	885	100%	0.0	100.00%	534	PQ568880.1
☑	Verticillium dahliae isolate Vd-66 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in...	Verticillium dahliae	885	885	100%	0.0	100.00%	527	OP935124.1



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accessio
☑	Verticillium dahliae isolate V702I calmodulin (Cal) gene, partial cds	Verticillium dahliae	911	911	100%	0.0	100.00%	523	DQ266162.1
☑	Verticillium dahliae calmodulin (Cal) gene, partial cds	Verticillium dahliae	889	889	98%	0.0	100.00%	481	OR353422.1
☑	Verticillium dahliae isolate PAP-19 calmodulin (Cal) gene, partial cds	Verticillium dahliae	889	889	98%	0.0	100.00%	481	OR353421.1
☑	Verticillium dahliae calmodulin (Cal) gene, partial cds	Verticillium dahliae	883	883	98%	0.0	99.79%	481	OR353420.1
☑	Verticillium dahliae var. longisporum isolate V559I clone 2 calmodulin (Cal) gene, partial cds	Verticillium longisporum	857	857	100%	0.0	98.17%	519	DQ266169.1
☑	Verticillium dahliae isolate V666I calmodulin (Cal) gene, partial cds	Verticillium dahliae	856	856	94%	0.0	100.00%	478	DQ266160.1
☑	Verticillium dahliae var. longisporum isolate V559I clone 1 calmodulin (Cal) gene, partial cds	Verticillium longisporum	852	852	100%	0.0	97.97%	519	DQ266172.1
☑	Verticillium dahliae var. longisporum isolate V559I clone 4 calmodulin (Cal) gene, partial cds	Verticillium longisporum	846	846	100%	0.0	97.77%	519	DQ266179.1

-> ITS Sequence deposited in GenBank (PX399390)

Koch‘s postulates

Inoculation & Re-isolation of the pathogen

- Two different methods (no experience with *A. triloba*)
- Stem inoculation:
  - T-shaped bark incision *V. dahliae* agar plug (~5 mm) inserted and moist sterile cotton, sealed with Parafilm®
- Root dipping:
  - rootstocks dipped in V. dahliae conidial suspension 1×10^7 cfu/ml (30 min) and transplanted into 2 L pots
  - First symptoms 24 days post inoculation

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Upcoming Questions

Where does the infection come from?

- Plant material
  - many plant species from different sources from AT, CH, SLO, D, I, FR… (Almonds, kaki, kiwi, sweet potatoes, watermelons…)
- Established unnoticed field infestation
  - Before 2017: arable crops

    grain, rapeseed, pumpkins, potatoes…

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Detection Method from Soil

High amounts of microsclerotia detected

- Wet sieving and plating method
  (Neubauer & Heitmann 2011)

- Sampling for soil examination in 3 different spots:
  - next to diseased tree -> **21,6** cfu/g soil **->very high**
  - inter-row vegetation -> **10** cfu/g soil **->high**
  - next to healthy tree -> **2** cfu/g soil

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Orchard management

Possible spread of the pathogen

- Five to ten trees per year die
- Dry soil conditions
- Drip irrigation (roots on surface)
- Regular soil tilling
  - Damaged roots as entry points?
  - use of less invasive machine recommended
- mulching
- Same machines used in different plots

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Conclusions

- First report of *V. dahliae* infecting pawpaw under field conditions
- These finding is relevant for growers as it highlights a new host for a widespread pathogen
- Provide updated details for future growers

Acknowledgements to my colleagues involved:
POWS: Ulla Persen, Eva Lehner, Pepi Altengruber
MOPS: Christina, Claudia, Thomas, Phillip, Chiara, Elena, Richard, Sabine, Helga

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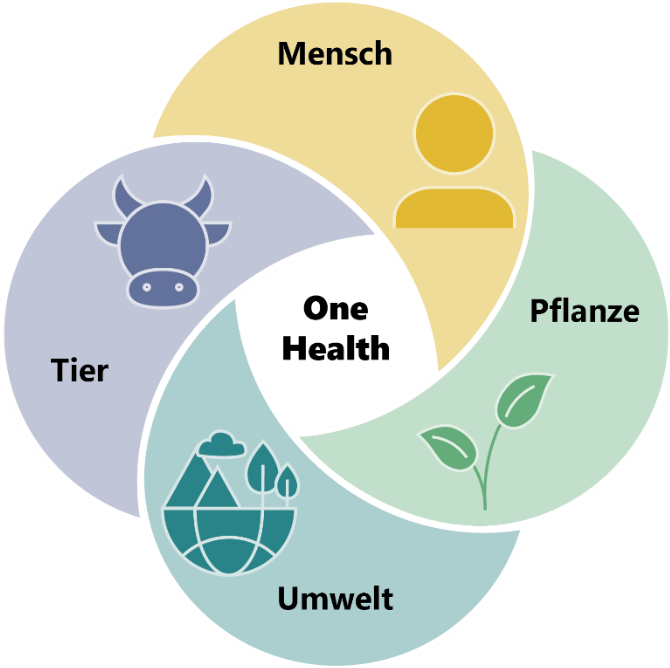
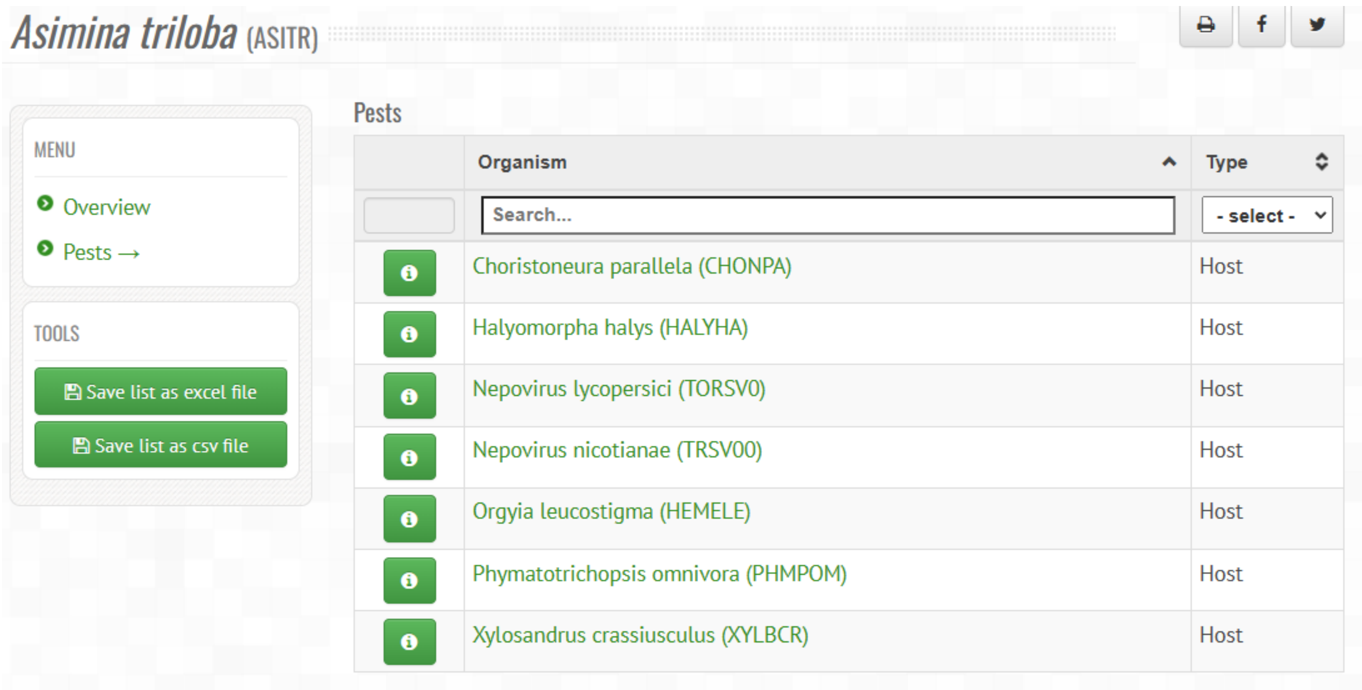


Additional Infos

- EPPO GD:



Asimina triloba (ASITR)

Pests

	Organism	Type
	Choristoneura parallela (CHONPA)	Host
	Halyomorpha halys (HALYHA)	Host
	Nepovirus lycopersici (TORSV0)	Host
	Nepovirus nicotianae (TRSV00)	Host
	Orgyia leucostigma (HEMELE)	Host
	Phymatotrichopsis omnivora (PHMPOM)	Host
	Xylosandrus crassiusculus (XYLBCR)	Host

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