Studies on transmission of the *phyllody* of *Parthenium* by *Cuscuta* sp. and different insect-vectors in regard to cultivated plants

T. Henniger[1], C. Ulrichs[2], M. Bandte[1], T. Taye[3], S. von Bargen[1], C. Büttner[1]
Humboldt-Universität zu Berlin, Faculty of Agriculture and Horticulture, Institute of Horticultural Sciences,
[1]Phytomedicine, [2]Urban Horticulture, Lentzeallee 55/57, 14195 Berlin; phytomedizin@agrar.hu-berlin.de
[3]Plant Protection Research Center, Ambo, Ethiopia

Introduction

• *Parthenium hysterophorus* L. is an annual herb of the *Asteraceae* family, originating from the tropical and subtropical America. It has become an **invasive weed** in tropical regions worldwide and is known in Ethiopia since 1980 from the region around Dire Dawa. Since then it has spread **in the middle-high regions** of the Ethiopian highland.

• ***Parthenium* phyllody** (Fig. 2) is an important disease of *P. hysterophorus* L. caused by phytoplasmas, which are thought to be transfered by insect vectors.

Fig. 1
Healthy plant of *P. hysterophorus* (Awash, Ehtiopia)

Aims

•Studies on the transmission of the plant pathogen to cultivated crops (host range) with dodder and insect vectors

• Identification of natural vectors responsible for dispersal of phytoplasmas in Ethiopia

Transmission by leafhoppers

Fig. 6
Transmission studies in cages, each containing 2 healthy *P. hysterophorus* plants and 10 leafhoppers of the species *Orosius cellulosus* Lindberg after aquisition feeding on diseased *P. hysterophorus*

•Experimental vector: *Orosius cellulosus* Lindberg using 5 leafhoppers per plant

•**Acquisition feeding** for 2 days on phyllody diseased *Parthenium hysterophorus*

•**Transfer** to healthy *Parthenium hysterophorus* bait plants and **incubation** for 20 days (Fig. 6)

•**Assessment of phytoplasma infection** in vector insects and bait plants by molecular methods revealed:

Phytoplasmas were aquired by *Orosius cellulosus* Lindberg and could **be transmitted** to *P. hysterophorus* (Fig. 8 plant A and B), but no symptoms were observed on bait plants after 50 days.

Parthenium phyllody

Fig. 2 Inflorescence of a phyllody diseased *P. hysterophorus* L. (Nazreth, Ehtiopia)

Characteristic symptoms of a phytoplasma infection: plant stunting, phyllody (green inflorescences) and reduced seed production

Identification of natural vector insects

Fig. 7
Planthoppers belonging to the *Tettigometridae*, collected from phyllody diseased *P. hysterophorus*, conserved in 70% ethanol

• Putative vector insects collected from phyllody diseased *Parthenium hysterophorus* were conserved in 70% ethanol (Fig. 7) for **species analysis and detection of phytoplasmas** by molecular methods

•**2 different planthopper species** within the family *Tettigometridae* - separated into adults and larvae of different sizes

Transmission by dodder

Fig. 3
Cuscuta sp. shoots with established haustoria curling around a healthy *P. hysterophorus* plant

• Establishment of **dodder** (*Cuscuta sp.*, Fig. 3) as a **cytoplasmatic bridge** between different plant species enables transmission of phytoplasmas

•Dodder got established on healthy as well as on phyllody diseased *P. hysterophorus* (Fig. 4)

Fig. 4
C. campestris connecting a healthy *P. hysterophorus* plant (right) with a phyllody diseased *P. hysterophorus* (left)

• Connection of plants by dodder was more successful by establishment of *Cuscuta sp.* on **leaves and leafstalks** of healthy plants (Fig. 1) than phyllody diseased *P. hysterophorus*

Fig. 5
C. campestris bridge a phyllody diseased *P. hysterophorus* (left) and a healthy *Vicia faba* L. plant (right)

• **Cytoplasmatic bridges** by dodder were sucessfully established between phyllody diseased *P. hysterophorus* and bait plants belonging to the species *Vicia faba* L. (Fig. 5), *Cicer arietinum* L., *Lens culinaris* Medik. and *Phaseolus vulgaris* L.

•No symptoms of an **phyllody infection** in bait plants were visible after 50 days of incubation

Detection of phytoplasmas in vector insects and bait plants of *P. hysterophorus*

•Samples: **DNA preparations** from *P. hysterophorus* L. bait plants and experimental vectors *Orosius cellulosus* Lindberg from leafhopper transmission studies and **natural insect vectors** (planthoppers, *Tettigometridae*)

•**Amplification** of specific phytoplasma 16S rDNA sequence fragments by **nested PCR** using two universal phytoplasma primer combinations (P1/Tint & nested PCR with fU5/rU3; Fig. 8)

•**Detection of phytoplasmas** in 2 planthopper species of the family *Tettigometridae* as well as in the leafhopper species *Orosius cellulosus*, but no phytoplasma detection in aphids

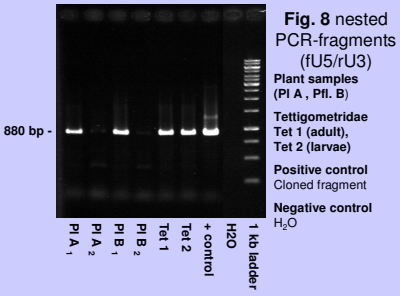

Fig. 8 nested PCR-fragments (fU5/rU3)

•Sequences obtained from **planthopper larvae and adults** of different species within the family *Tettigometridae* as well as from **leafhoppers** of the species *Orosius cellulosus* **were identical** to fragments originating from phytoplasmas found in **phyllody affected *P. hysterophorus*** in Ethiopia

•Sequence analysis covering approx. 300 bp of the 16SrDNA revealed **high nucleotide identities** above 99% to phytoplasmas within the 16SrII species group (Peanut witches´ broom group)

Conclusions

•*Cuscuta sp.* **is suitable** for transmission studies to **determine the host range** of the phyllody disease of *P. hysterophorus* L.

•Detected pathogens are related to phytoplasmas within the **16SRII species group**

•**Close relationship** of phytoplasmas detected in vector insects and *P. hysterophorus*

•The examined planthoppers seem to be **suitable vector insects** for phytoplasmas found in phyllody diseased *P. hysterophorus*.

The research project was financially supported by „Stiftung für tropische Agrarforschung“, DAAD, BHGL-Förderverein, Syngenta, Bayer CropScience, „Freunde und Förderer der LGF“

The identification of vector-insects was supported by Mrs. Hoch (Natural History Museum, Berlin) and Mick Webb (Natural History Museum, London)

Contact: Thomas Henniger (Thomas@Henniger-online.de)