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A potential perennial host for *Pseudoperonospora cubensis* in temperate regions

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Abstract *Pseudoperonospora cubensis* causes great losses in cucurbitaceous crops worldwide. In cool temperate climates of northern Europe or North America overwintering as active mycelium is not possible, because all hosts so far reported there are summer annuals. Oospores have not yet been found in these regions under field conditions. The only perennial member of the Cucurbitaceae found naturally in central and northern Europe is *Bryonia dioica*. To date this plant has not been recorded as a host for downy mildews, but our infection trials demonstrate that *P. cubensis* is able to infest this plant. Amplification and sequencing of the ITS rDNA confirmed the observed downy mildew disease on *B. dioica* as *P. cubensis*. From these findings, the possibility that *P. cubensis* may be able to overwinter on this perennial host cannot be excluded. Whether or not *B. dioica* plays a part in the epidemology of *P. cubensis* in Europe requires evaluation by further studies.

Keywords Cucurbit downy mildew · Peronosporaceae · Host range · Internal transcribed spacer · *Bryonia dioica*

Cucumis sativus (cucumber) is a member of the Cucurbitaceae widely grown in the temperate regions of Europe and North America. Many edible species are widely cultivated in the tropical and subtropical areas of the world and can also be found in the Mediterranean region. Due to the climatic conditions in North America and in central and northern Europe, cucumbers, pumpkins and other cucurbit crops can only be grown during the summer months. Downy mildew, caused by *Pseudoperonospora cubensis*, is one of the most important diseases of these crops causing great losses in cucurbit production worldwide (Thomas 1986; Lebeda 1991; Lebeda and Widrlechner 2004). In warm temperate to subtropical regions without frost events, *P. cubensis* overwinters as active mycelium in the tissue of its host (Bains and Jhooty 1976; Holmes et al. 2004). In central and northern Europe, where cucurbit plants die back in autumn, this mode of overwintering is not available to the pathogen. With the notable exception of the report by Bedlan (1989), who found oospores in greenhouse cucumber plants, oospores of *P. cubensis* have so far not been observed in Europe or Northern America. This gives rise to a widely accepted theory, whereby primary infections in northern latitudes are the result of the annual long-range dispersal of spores from subtropical regions (Holmes et al. 2004).

However, there is one perennial member of the Cucurbitaceae that is widely distributed throughout Europe and in the Mediterranean region. It is the climbing perennial, *Bryonia dioica* (bryony or wild hops), known for its medicinal use since the time of the Roman Empire (Janick et al. 2007). *Bryonia dioica* is relatively common throughout southern and central Europe although is more infrequent in north-

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ern Europe. It uses its tendrils to climb fences, hedges and trees, particularly in floodplain forests. *Bryonia* is the only perennial cucurbit that is native to cool temperate Europe and could therefore theoretically serve as a overwintering host, but so far there is no record of any downy mildew disease affecting this species (Waterhouse and Brothers 1981; Lebeda and Widrlechner 2003). It was the aim of this study to determine whether *B. dioica* might be a potential, so far overlooked host for *P. cubensis*, or is truly resistant to this pathogen.

Infection experiments were carried out using the *P. cubensis* strains P.C. 26/01 and P.C. 39/01 from the Olomouc Region of the Czech Republic, kindly supplied by A. Lebeda, as well as one laboratory strain (P.C. Darmstadt) originating from Darmstadt, Germany. All strains were originally isolated from *C. sativus*. The isolates are maintained at the Institute of Botany of the University of Hohenheim in climate chambers (16°C, 14 h light/10 h darkness). Infections for continuous cultivation were done using a dab-off technique. Sporangia present on the lower surface of cucumber leaves from previous rounds of infection were gently dabbed onto the moistened lower surface of healthy, fully mature cucumber leaves (cv. Saladin) taken from plants grown in greenhouses at the University of Hohenheim. Leaves were then transferred to transparent boxes (approximately 30 cm long, 20 cm wide, 5 cm high) on water-soaked paper towels to ensure 100% relative humidity (RH) was maintained within the boxes. For cross-infections the inoculum was dabbed on several fully mature leaves of bryony cut from outdoors (Botanical Garden of the University of Hohenheim) and from greenhouse plants. Bryony leaves were placed in Petri dishes on water-soaked filter paper and the filter paper was

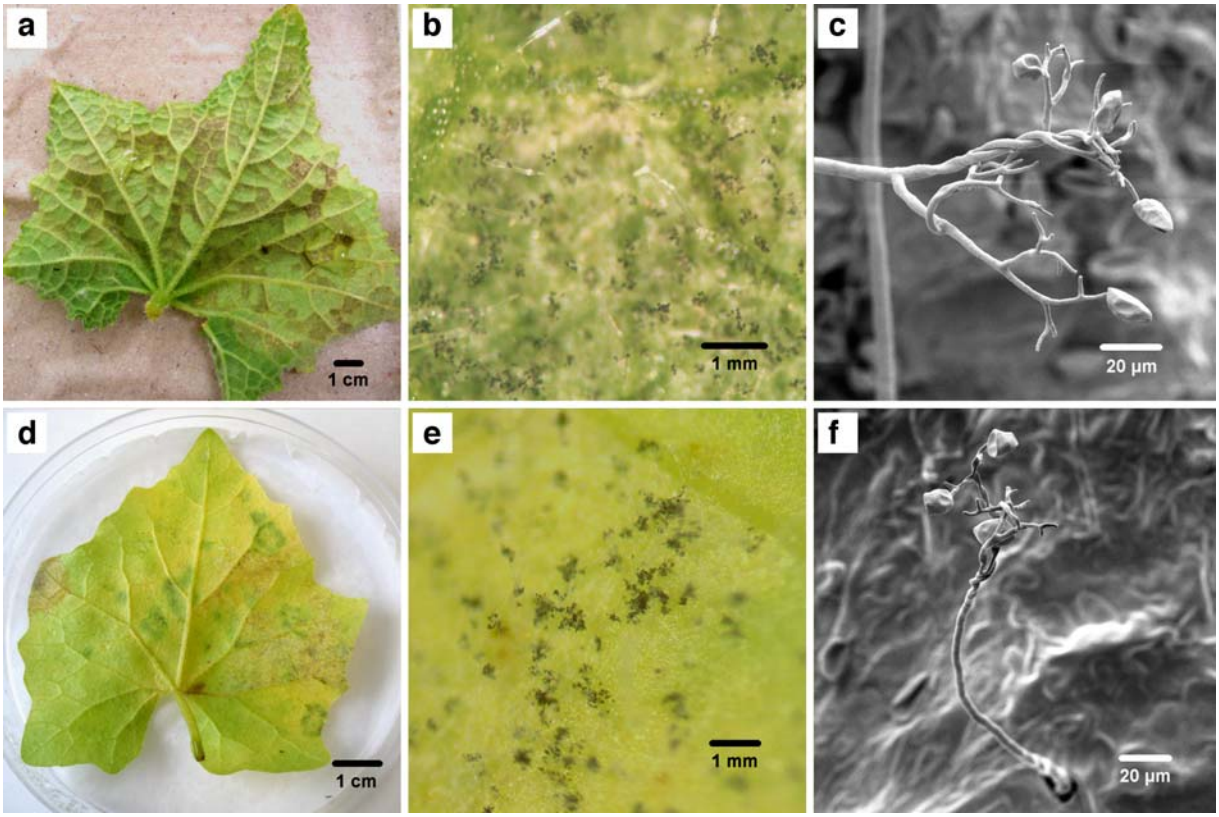

Fig. 1 Downy mildew infected leaves of *C. sativus* (*upper row*) and *B. dioica* (*lower row*). Scanning electron microscopy was carried out as described earlier (Thines 2006). **a** Lower surface of a cucumber leaf showing downy mildew symptoms. **b** Close-up of sporangiophores of *P. cubensis* on cucumber. **c** Sporangiophore of *P. cubensis* on a cucumber leaf. **d** Lower surface of a bryony leaf showing downy mildew symptoms. **e** Close-up of sporangiophores of *P. cubensis* on bryony. **f** Sporangiophore of *P. cubensis* on a bryony leaf

regularly moistened to ensure close to 100% RH within the Petri dishes. Cross-infection experiments were repeated at least four times independently for all strains tested.

Single sporangiophores (Fig. 1 b,e) were picked from the leaf surface of sporulating leaves with precision tweezers and each picked sporangiophore was transferred to a 0.2 ml reaction tube containing 25 µl of PCR reaction mix, with the primers LR-0R (reverse complement to LR-0, Moncalvo et al. 1995) and DC-6F (Cooke et al. 2000). PCR reactions where carried out as described previously (Thines 2007). The amplicons were detected in 1.0% agarose gels after gel electrophoresis and ethidium bromide staining. The fragments were approximately 1200 bp in length and were excised from the agarose gels to be extracted by means of the DNA purification kit from Fermentas (Germany). Sequencing was carried out by a commercial sequencing company (GATC-biotech, Germany).

Five to eight days after inoculation sporulation was observed on the lower surface of the cucumber leaves. Seven to ten days after inoculation bryony leaves showed circular clorotic to necrotic spots (Fig. 1d) with groups of sporangiophores dispersed throughout. All isolates tested showed infectivity to *B. dioica*. While infections and sporulation were regularly observed when using the isolates P.C. 26/01 and P. C. 39/01, the isolate P.C. Darmstadt only sporulated in one out of four cross-infection experiments on bryony. In contrast to the symptoms of the cucumber leaves, where the sporulation is delimited by leaf veins (Fig. 1a), the sporulation was not limited by the leaf veins in *B. dioica* (Fig. 1d).

Sequences of all *P. cubensis* isolates investigated were identical. Sequences obtained from the sporangiophores emerging from bryony leaves were compared with sporangiophores taken from the identical isolates used for the cross-infection experiments, but picked from *C. sativus* leaves and with sequences from the closest relative of *P. cubensis*, *Pseudoperonospora humuli*. The comparison revealed that the sequences of the sporangiophores of *P. cubensis* picked from *B. dioica* were identical with those of *P. cubensis* from *C. sativus* (Fig. 2). Cross-infections from *B. diocia* to *C. sativus* were successfully repeated several times, fulfilling Koch's Postulates. Interestingly, while the *P. cubensis* strains P.C. 26/01 and P.C. 39/01 were regularly found to sporulate on bryony leaves, the strain P.C. Darmstadt sporulated only once on bryony before its detached leaves decayed.

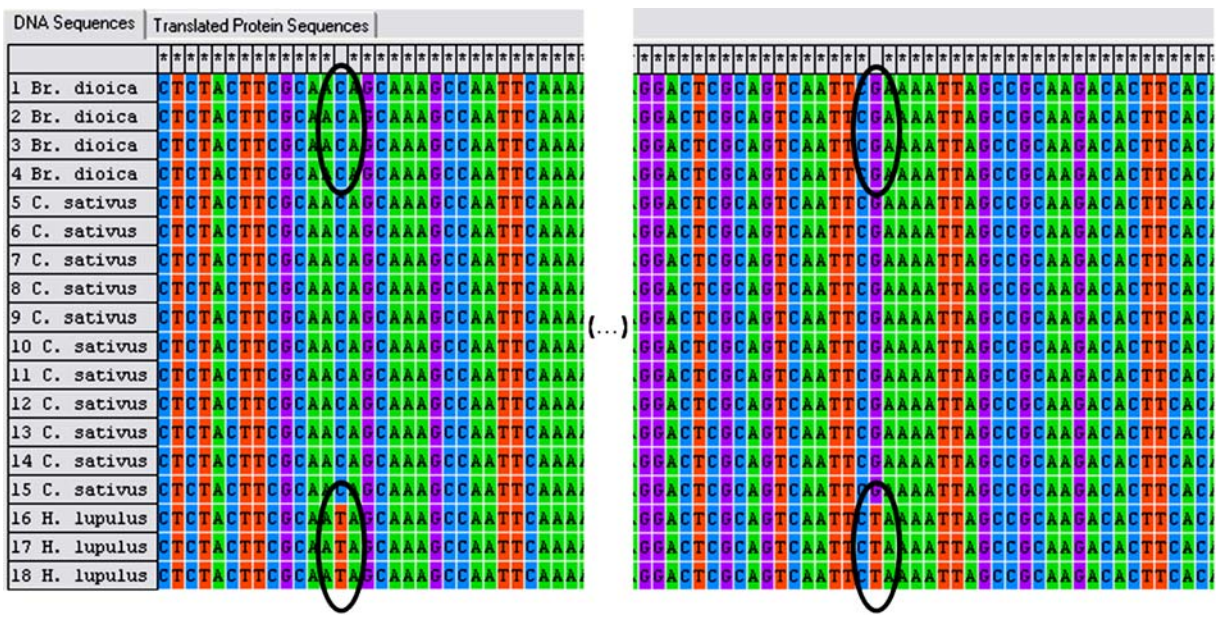

Fig. 2 Sequence alignment of the ITS sequences of *P. cubensis* sporangiophores picked from different hosts. Alignment was done using MEGA (Tamura *et al.* 2007), version 4.0. The names preceding the sequences are abbreviated host names; *Br. Bryonia*, *C. Cucumis*, *H. Humulus*. The closely related *P. humuli* showed only few base differences to *P. cubensis*. Note the complete identity of the ITS sequences of the sporangiophores picked from *B. dioica* and *C. sativus*

Further investigations are necessary to assess whether this result was due to a longer latency time of P.C. Darmstadt on *B. dioica* or related to a lower degree of virulence to *B. dioica*. It will also be interesting to evaluate the ability of *P. cubensis* strains of different pathotype or host origin to colonise and to sporulate on *B. dioica*. Our discovery that *B. dioica* can serve as a host for *P. cubensis* under laboratory conditions supports the hypothesis that *B. dioica*, as a perennial plant, has the potential to function as an intermediary host allowing the overwintering of *P. cubensis* in colder regions of Europe, either in seeds or in tuberous parts below ground. Further investigations screening *B. dioica* populations are warranted to clarify whether *B. dioica* might play a hitherto overlooked part in the epidemiology of *P. cubensis* in Europe.

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