Journal für Kulturpflanzen, 72 (9). S. 479–482, 2020, ISSN 1867-0911, DOI: 10.5073/JfK.2020.09.04 Verlag Eugen Ulmer KG, Stuttgart

Gertraud Michl, Michael Fischer, Christoph Hoffmann

Solanum mealybug *Phenacoccus solani* Ferris, 1918: New in Germany!

Solanum mealybug
Phenacoccus solani Ferris, 1918:
Neu in Deutschland!

Abstract

Scale insects (Insecta, Homoptera, Ord. Sternorrhyncha, Subord. Coccina) are often thermophilic species that, as a result of global warming, are currently expanding their geographic distribution towards the poles. Within the mealybugs (Superfam. Coccoidea, Fam. Pseudococcidae), several species, which are of great importance as virus vectors in viticulture in Southern Europe, attack ornamental plants in greenhouses in Central Europe. It can be expected that these species will also colonise Central European vineyards as global warming progresses.

In this context our newly described finding is remarkable in that the mealybug *Phenacoccus solani*, which is known from grapevines in North America and Asia, has overwintered (2019–2020) outdoors on ornamental plants in the Palatinate wine growing region.

The species cannot be determined with the Central European identification literature and was therefore identified by molecular techniques using bio barcode primers. The diagnosis was generated within the context of the establishment of a national reference laboratory for insects at the Julius Kühn-Institut.

Key words: Solanum mealybug, potential virus vector, climatic change, ornamental plants

Zusammenfassung

Schildläuse (Insecta, Homoptera, Ord. Sternorrhyncha, Uord. Coccina) sind häufig wärmeliebende Arten, die aktuell ihre Verbreitung im Rahmen einer Klimaerwärmung polwärts ausdehnen. Innerhalb der Schmierläuse (Üfam. Coccoidea, Fam. Pseudococcidae) treten in Mitteleuropa mehrere Arten an Zierpflanzen in Gewächshäusern auf, die in Südeuropa als Virusvektoren im Weinbau von großer Bedeutung sind. Von diesen Arten ist zu erwarten, dass sie bei fortschreitender Klimaerwärmung auch mitteleuropäische Weinberge besiedeln.

Vor diesem Hintergrund bemerkenswert ist der hier neu beschriebene Befund, dass die aus Nordamerika und Asien von Weinreben bekannte Pseudococcide *Phenacoccus solani* an Zierpflanzen im Weinbaugebiet Pfalz im Freiland überwintert hat.

Die Art ist mit der mitteleuropäischen Bestimmungsliteratur nicht bestimmbar und wurde hier molekularbiologisch bestimmt. Die Befunde ergaben sich im Rahmen der Etablierung eines nationalen Referenzlabors für Insekten am Julius Kühn-Institut.

Stichwörter: Solanum mealybug, potentieller Virus-Vektor, Klimawandel, Zierpflanzen

Affiliation
Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Geilweilerhof, Siebeldingen, Germany

Correspondence
Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Geilweilerhof, 76833 Siebeldingen, Germany, e-mail: christoph.hoffmann@julius-kuehn.de

Accepted
14 July 2020

Introduction

Climate change and increased plant trade promote the spread of new pests. Greenhouses, apartments and balconies can serve as stepping stones for species previously only found in protected indoor spaces.

Mealybugs (Pseudococcidae) are of particular importance for horticultural crops (e.g. viticulture) because they are vectors of virus related diseases. Due to virus transmission by the native *Phenacoccus aceris* for example, it is becoming increasingly difficult to produce healthy, virus-free vine planting material in Germany. In Southern Europe, this role is taken on by different mealybug species, which in Germany so far have only been found in greenhouses and apartments and which, with advancing global warming, can also colonise the open field, as was recently established for the species *Pseudococcus viburni* (Schmutterer & Schrameyer, 2013). The introduction of new "greenhouse species" of mealybugs into Germany is therefore not only a challenge for ornamental plant cultivation, but with increasing global warming can also become a threat to outdoor perennial crops such as grapevine or stone fruits.

Material und Methods

Origin of the mealybugs

The previously unknown mealybugs were introduced into the home of the first two authors in Landau/Pfalz (Germany) in 2015 with a ponytail palm plant (*Beaucarnea* sp., Asparagaceae) acquired from the ornamental plant trade. Both on the palm plant and on the unprotected balcony of the apartment the species spread further and overwintered outside, especially on succulent plants (*Sempervivum tectorum*, *Echeveria* sp., both members of the Crassulaceae family).

Molecular determination

DNA-extraction and amplification. DNA was extracted from two specimens of *Phenacoccus solani* by using the CTAB-extraction-method according to EPPO PM 7/24(4). Reduced volumes however were applied for all steps of the protocol. DNA was eventually resuspended in 30 µl ddH_2O.

PCR reaction conditions. Used primers are given in Table 1. Amplification of the 28S region was obtained by primers s3360 and 28b, the COI gene was amplified by primers PcoF and LepR1.

In the PCR assay, 1 µl of genomic DNA (2–20ng/µL) in a mix of 0,2mM dNTPs, 0,2 µM of primers each and 2 u of Taq-Polymerase (DreamTaq, ThermoScientific) was filled up with H_2O to a final volume of 15µL. Cycling conditions were according to Sethusa et al. (2014) with the following adaption to the specific properties of the Taq-Polymerase: for both primer pairs a touch down PCR was performed, starting with an initial annealing temperature of 56°C for 1 min, and decreasing to 42°C within 20 cycles. For the remaining 18 cycles, 42°C were kept for 30 sec. After the initial denaturation step of 94°C for 3 min, further amplification cycles included 30 sec at 94°C for denaturation, the appropriate annealing temperatures and times according to the touch down schedule, 30 sec at 72°C for extension and a final extension step of 72°C for 5 min. Cleaned PCR products (SLG, Hi-Yield Clean up) were sent to Mycrosynth SeqLab for bi-directional sequence analyses using the respective PCR primers.

Morphological and microscopical identification

Members of the genus *Phenacoccus* cannot be identified using palaearctic identification literature (Kosztarab & Kozar, 1988, Danzig & Gavrilov-Zimin, 2014), because the insect is not yet mentioned there or it is mentioned under a synonym, with a differing morphospecies, i.e. *P. defectus*. As a further obstacle, macroscopic morphological characters are largely absent in the group of the Pseudococcidae and innerspecific microscopic characters can be variable due to environmental influences (Chatzidimitriou et al., 2016).

Results and Discussion

Throughout our experiments, a 693 bp and 568 bp bi-directional aligned sequence was amplified for the 28S region and the CO1 gene respectively, which was used for DNA barcoding. Sequencing analyses showed 100% consensus with deposited data for *Phenacoccus solani* in GenBank.

For the description of *P. solani* see Danzig & Gavrilov-Zimin (2014) and Chatzidimitriou et al. (2016). The species has 18 short wax thread pairs and appears yellow under the wax layer. There are no egg sacs because the

Table 1. Used primers cited in Sethusa et al. (2014)

Primer	Primer sequence	Length of aligned PCR product in bp	gene of interest	Reference
s3660	GAGAGTTMAASAGTACGTGAAAC	693	28S	Dowton & Austin (1988)
28b	TCGGAAGGAACCAGCTACTA			Whiting et al. (1997)
LepR1	TAAACTTCTGGATGTCCAAAAAATCA	568	COI	Park et al. (2010)
PcoF	CCTTCAACTAATCATAAAAATATYAG			Park et al. (2010)

species is ovoviviparous, i.e. the crawlers hatch more or less immediately after laying eggs (see Fig. 1). No males are present as the species is parthenogenetic.

Since the studied mealy bug colony originates from the plant trade, the species is probably more widespread on ornamental plants in Germany. It was already described from greenhouses under the synonym *P. defectus* in Great Britain (Malumphy, 1997). In the field, the species is known in Europe from Southern France (Germain & Matile-Ferrero, 2006), Italy (Chatzidimitriou et al., 2016), greenhouses in England (Malumphy, 1997, as *P. defectus*) and Spain (Beltrà & Soto, 2011). From USA, Iran (Chatzidimitriou et al., 2016) and South Africa (Walton & Pringle, 2004) the species has been reported from vineyards. Pseudococcids occurring on grapevine, *Vitis vinifera*, usually transmit leaf roll virus and grapevine virus (Herrbach et al., 2017). The German wine-growing regions are located in climatically favoured areas. With advancing global warming, it is therefore to be expected that original greenhouse species will make the leap into the open field. This has already been described by Schmutterer & Schrameyer (2013) for the greenhouse mealybug *Pseudococcus viburni*. According to Schmutterer & Hoffmann (2016), the greenhouse scale insect *Coccus hesperidum* has been able to overwinter in the Southern Palatinate outdoors since 2013. With *P. solani*, this is now also true for a species which outdoors has only been found in the Mediterranean region. The species overwintered on *Sempervivum tectorum* on an unprotected south-facing balcony in Landau in the Palatinate (winter of 2019/2020). This makes the new record even more remarkable for Germany.

Possible control strategies

Control strategies of mealybugs include biological control by predators and parasitoids as well as chemical control by insecticides; both strategies are reviewed by Mani et al. (2014) for the viticulture sector. Chemical control of mealybugs is difficult since the highly effective active ingredients are not longer registered in Europe (Paranjape et al., 2015). The chances of establishing a natural balance between *P. solani* and its natural enemies in the field are relatively good. García Morales et al. (2016) list seven different parasitoids from the hymenopteran families of Aphelinidae and Encyrtidae. Out of these the species *Leptomastix epona* is native in Germany. Hayon et al. (2016) report on predatory gall midge larvae that can feed on this mealybug. It is also to be expected that introduced ladybirds such as *Harmonia axyridis* will accept *P. solani* as food resource as long as the latter does not exclusively occur in specifically sheltered places, such as below the bark.

A

B C

Fig. 1. *Phenacoccus solani*. A) Infestation on *Sempervivum tectorum*. B) Adult female with 18 pairs of wax filaments (~ 6 mm long). C) Adult females in the middle (ovoviviparous) with newly hatched crawler (right).

Literatur

Beltrà, A., A. Soto, 2011: New records of mealybugs (Hemiptera: Pseudococcidae) from Spain. Phytoparasitica **39**, 385–387, DOI: 10.1007/s12600-011-0178-0.

Chatzidimitriou, E., M. Simonato, G.W. Watson, I. Martinez-Sañudo, H. Tanaka, J. Zhao, G. Pellizzari, 2016: Are *Phenacoccus solani* Ferris and *P. defectus* Ferris (Hemiptera: Pseudococcidae) distinct species? Zootaxa **4093**, 539-551, DOI: 10.11646/zootaxa.4093.4.5.

Danzig, E.M., I.A. Gavrilov-Zimin, 2014: Palaearctic mealybugs (Homoptera: Coccinea, Pseudococcidae), Part 1: Subfamily Phenacoccinae. Russian Academy of Sciences, St. Petersburg, Zoological Institute, 678 pp.

Dowton, M., A.D. Austin, 1988: Phylogenetic relationships among the microgastroid wasps (Hymenoptera: Braconidae): combined analysis of 16S and 28S rDNA genes and morphological data. Molecular Phylogenetic Evolution **10**, 354-366.

EPPO, 2018: PM 7/24(4) *Xylella fastidiosa*, Bulletin OEPP/EPPO Bulletin **48** (2), 175–218, DOI: 10.1111/epp.12469.

García Morales, M., B.D. Denno, D.R. Miller, G.L. Miller, Y. Ben-Dov, N.B. Hardy, 2016: ScaleNet: A literature-based model of scale insect biology and systematics. Database. DOI: 10.1093/database/bav118. http://scalenet.info.

Germain, J.-F., D. Matile-Ferrero, 2006: *Comstockiella sabalis* (Comstock), *Crisicoccus pini* (Kuwana) et *Phenacoccus defectus* Ferris, cochenilles nouvelles pour la France (Hem., Diaspididae et Pseudococcidae). Bulletin de la Société Entomologique de France **111** (3), 395-401.

Gwiazdowski, R.A., R.G. Foottit, H.E.L. Maw, P.D.N. Hebert, 2015: The Hemiptera (Insecta) of Canada: Constructing a reference library of DNA barcodes. PLOS ONE **10** (4), e0125635, DOI: 10.1371/journal.pone.0125635.

Hayon, I., Z. Mendel, N. Dorchin, 2016: Predatory gall midges on mealybug pests – diversity, life history, and feeding behavior in diverse agricultural settings. Biological Control **99**, 19-27.

Herrbach, E., A. Alliaume, C.A. Prator, K.M. Daane, M.L. Cooper, R.P.P. Almeida, 2017: Vector transmission of grapevine leafroll-associated viruses. In: Meng, B., G.P. Martelli, D.A. Golino, M. Fuchs (eds) Grapevine viruses: molecular biology, diagnostics and management. Springer International Publishing, New York, pp 483-503.

Kosztarab, M., Kozar, F., 1988: Scale Insects of Central Europe. Dr. W. Junk Publishers, Dordrecht, Boston, Lancaster, 456 pp.

Mani, M., C. Shivaraju, S. Narendra Kulkarni, 2014: The Grape Entomology, Springer India, 202 pp.

Malumphy, C., 1997: Imperfect mealybug, *Phenacoccus defectus* Ferris (Homoptera: Coccoidea, Pseudococcidae), a pest of succulent ornamental plants, new to Britain. Entomologist's Gazette **48**, 285-288.

Mazzeo, G., S. Longo, G. Pellizzari, F. Porcelli, P. Suma, A. Russo, 2014: Exotic scale insects (Coccoidea) on ornamental plants in Italy: a never-ending story. Acta Zoologica Bulgarica Suppl. **6**, 55-61.

Paranjape, K., V.R. Gowariker, V.N. Krishnamurthy, S. Gowariker, 2015: The Pesticide Encyclopedia. CABI, 2015, 703 pp., ISBN 1785392158, 9781785392153.

Park, D.S., Y.J. Leem, K.W. Hahn, S.J. Suh, K.J. Hong, H.W. Oh, 2010: Molecular identification of mealybugs (Hemiptera: Pseudococcidae) found on Korean pears. Journal of Economic Entomology **103**, 25-33.

Schmutterer, H., C. Hoffmann, 2016: Die wild lebenden Schildläuse Deutschlands (Sternorhyncha, Coccina) [The Outdoors Living Scale Insects of Germany]. Entomologische Nachrichten und Berichte **104,** 7(20), Bernhard Klausnitzer, Dresden, 104 pp.

Schmutterer, H., K. Schrameyer, 2013: *Pseudococcus viburni* (Signoret), die Gemeine Gewächshausschmierlaus (obscure mealybug) – Erstmaliges Auftreten im Freiland in Mitteleuropa. Journal für Kulturpflanzen **65** (5), 211-216.

Sethusa, M.T., I.M. Millar, K. Yessoufou, A. Jacobs, M. van der Bank, H. van der Bank, 2014: DNA barcode efficacy for the identification of economically important scale insects (Hemiptera: Coccoidea) in South Africa. African Entomology **22** (2), 257-266.

Walton, V.M., K.L. Pringle, 2004: A survey of mealybugs and associated natural enemies in vineyards in the Western Cape Province, South Africa. South African Journal of Enology and Viticulture **25** (1), 23-25.

Whiting, M.F., J.C. Carpenter, W.D. Wheeler, W.C. Wheeler, 1997: The Strepsiptera problem: phylogeny of holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Systematic Biology **46**, 1-68.