Research Papers

Dissemination of esca-related pathogens in German vineyards: do arthropods play roles in vectoring spores?

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Summary. Grapevine Trunk Diseases (GTDs) such as esca challenge viticulture. The main fungal agents of Petri disease or young esca, *Phaeomoniella chlamydospora* (*Pch*), diverse *Phaeoacremonium* species (*Pm* spp.) and *Cadophora luteo-olivacea* (*Clo*), are transmitted to pruning wounds of vines by rain splashes and air currents. Arthropod-mediated dispersal is another possibility for the pathogens to reach pruning wounds. The present study was the first to evaluate possible involvement of arthropods in the dissemination process of esca-related pathogens in German vineyards. Diversity of arthropods on grapevine trunks was determined in 2019 and 2020, using cardboard traps mounted on vine trunks. Captured arthropods were surveyed for the presence of esca-related pathogens on their exoskeletons by using a nested multiplex PCR. In total, 2099 arthropods were examined, of which 35% were positive for *Phaeomoniella chlamydospora* (*Pch*), 21% for *Phaeoacremonium* spp. (*Pm*), and 7% for *Cadophora luteo-olivacea* (*Clo*). Earwigs and spiders were the most prevalent trapped arthropods; *Pch* was detected on 27% of earwigs and 38% of spiders, *Pm* spp. on 17 and 19%, and *Clo* on 3 and 8% of these arthropods. In both years, arthropods carrying the pathogens were already present in April, and therefore within the presumed susceptibility phase of pruned vines. These results indicate involvement of arthropods in the dispersal of esca-related pathogens in German vineyards. Further research, particularly to determine the infection potential of insect-borne fungi, is needed to confirm transmission risk. These results underline the importance of protecting vine pruning wounds to prevent host invasion by GTD pathogens.

Keywords. Grapevine trunk diseases, *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Cadophora luteo-olivacea*, dispersal, pruning wounds.

INTRODUCTION

Grapevine health is severely affected by Grapevine Trunk Diseases (GTDs). According to symptomatology of leaves, berries and wood, and in relation to a variety of wood-inhabiting fungi, GTDs can be subdivided into different diseases or syndromes (Mugnai *et al.,* 1999; Bertsch *et al.,* 2013; Fontaine *et al.,* 2016; Mondello *et al.,* 2018). All of these cause general deteri-

oration of vine vascular systems eventually reducing the production and longevity of vineyards (Vasquez *et al.,* 2007; Hofstetter *et al.,* 2012; Lecomte *et al.,* 2012).

Esca is one of the most important GTDs, and is a disease complex related to distinct symptoms, pathogens and vine age. This includes the so-called grapevine leaf stripe disease (GLSD, previously known as "young esca"), characterized by "tiger-stripes" on host leaves, which is the most important and widespread GTD (Mugnai *et al.,* 1999; Surico, 2009; Mondello *et al.,* 2018). Others include brown wood streaking, Petri disease (black goo or slow dieback), "vine apoplexy" or "acute esca", and "esca proper" (including white rot, caused by *Fomitiporia mediterranea*; Fischer, 2002). Co-existence of GLSD and "esca proper" is a widespread phenomenon in old vineyards. To complicate matters, all of these diseases may overlap, and also occur with other canker agents such as *Botryosphaeriaceae* and/or *Diatrypaceae* (Rolshausen *et al.,* 2010; Gramaje *et al.,* 2018; Moyo *et al.,* 2018). In addition, these diseases are often cryptic and symptoms may take several years to develop (Surico *et al.,* 2000; Surico *et al.,* 2006; Christen *et al.,* 2007).

Crucial for all early stages of esca are trachaeomycotic fungi, including *Phaeomoniella chlamydospora* (*Pch*) (Crous and Gams, 2000), *Phaeoacremonium* spp. (*Pm* spp.) (Mostert *et al.,* 2006) and, possibly, *Cadophora* spp. (Halleen *et al.,* 2007; Gramaje *et al.,* 2011; Travadon *et al.,* 2014). In German vineyards, besides the frequently occurring *Pch*, diverse *Pm* spp. such as *Pm. fraxinopennsylvanicum*, *Pm. viticola* and, above all, *Pm. minimum* have been isolated from symptomatic grapevines (Fischer and Kassemeyer, 2003; Fischer *et al.,* 2016; Kraus *et al.,* 2019). Among *Cadophora*, *C. luteo-olivacea* (*Clo*) was the most frequent species (Fischer *et al.,* 2016; Haag, 2018; Fischer, 2019; Kraus *et al.,* 2019).

For all vine-growing regions, pruning wounds are considered as major entry points for esca-related pathogens (Larignon and Dubos, 1997; Mugnai *et al.,* 1999; Larignon and Dubos, 2000; van Niekerk *et al.,* 2011). Susceptibility of pruning wounds in California to *Pch* and *Pm. minimum* was found to last for up to 4 months (Eskalen *et al.,* 2007), and in Spain susceptibility to *Pch* was found to last for up to 12 weeks (Elena and Luque, 2016). Sucker wounds caused by vineyard management may also be entry points for the pathogens (Makatini, 2014).

Concerning the inoculum sources, *Pch* was observed abundantly sporulating in cracks of vine bark (Edwards *et al.,* 2001), and fruit bodies of *Togninia minima*, the teleomorph of *Pm. minimum* (Mostert *et al.,* 2003), were found in wood crevices of diseased vine trunks (Rooney-Latham *et al.,* 2005b; Baloyi *et al.,* 2013). Dispersal of all

these fungi is by airborne spores in rain splashes or carried by wind (Larignon and Dubos, 2000; Eskalen and Gubler, 2001), and this is also likely for *Clo* (Gramaje *et al.,* 2011). In German vineyards, the presence of airborne *Pch* inoculum has been repeatedly verified by isolation or molecular detection from spore traps (Haag, 2018; Kraus *et al.,* 2020; Molnar *et al.,* 2020).

Arthropods including spiders, ants, and millipedes have been demonstrated as vectors for *Pch, Pm* spp. and other GTD-related pathogens in South Africa (Moyo *et al.,* 2014). The possible significance of arthropods in the transmission of several plant diseases has long been accepted (Leach, 1940). One prominent example for arthropod-mediated dispersal is the association of bark beetles and *Ophiostoma novo-ulmi*, the cause of the Dutch elm disease (Brasier, 1991).

No previous studies have surveyed the possible involvement of arthropods in the dispersal of esca-related pathogens in German vineyards. With a focus on the relationship between pruning measures and epidemiology of esca-related pathogens, the aims of the present study were to determine: i) the occurrence and diversity of arthropods on vine trunks in Germany; and, specifically, ii) the occurrence and identification of *Pch, Pm* spp. and *Clo* on arthropod exoskeletons.

Results from this study provide new information about the epidemiology of esca-related pathogens and may eventually contribute to enhancing measures to reduce the spread of esca, mostly so with regard to an efficient protection of pruning wounds.

MATERIALS AND METHODS

Sampling sites

Arthropod diversity was assessed in 2019 and 2020 in experimental vineyards located at the Julius Kühn-Institute in Siebeldingen, Germany. Two vineyards of different ages, cultivars and management practices were chosen. Vineyard "A" (49°13'00.2"N; 8°02'53.1"E) was planted in 1996, and vineyard "B" (49°13'08.8"N; 8°02'39.6"E) was planted in 2002. Vineyard "A" contained the fungus-resistant (PIWI-) cultivar *Vitis vinifera* cv. 'Phoenix' and was chosen because of the high incidence of GLSD-symptoms and apoplexy observed in previous years (Molnar *et al.,* 2020). Vineyard "B" comprised four different cultivars (PIWI-cultivars 'Calandro' and 'Regent' and the traditional cultivars 'Pinot Noir' and 'Riesling'), and GLSD-symptoms had been observed only rarely on these vines in previous years. Plant protection measures were integrated in vineyard "A" and organic in "B".

Monitoring of arthropods on vine pruning wounds and trunks

A preliminary survey was conducted in April 2019 to monitor arthropods visiting pruning wounds. One camera (Raspberry Pi 3 Model B V1.2, Raspberry Pi Foundation) was installed next to each pruning wound (pruning had been performed in February) on trunks of four different vines in vineyard "A" (Figure 1A). Images were captured every 5 sec for 24 h, and these were subsequently assessed for visiting arthropods. Visual surveys of vines were also carried out at 2 week intervals during the collection of arthropods from cardboard traps.

Assessment of arthropod diversity on vine trunks using cardboard traps

Traps were made from strips of corrugated cardboard (approx. 25 × 60 cm), and these were each wrapped twice around the trunk of each monitored vine (Moyo *et al.,* 2014; Figure 1B). Twelve traps were placed randomly on trunks in vineyards "A" and "B", with three traps used for each of the four cultivars in vineyard "B". During wintertime (November to March), no arthropods were visible in preliminary inspections, so the traps were mounted by early April when temperatures were moderate and arthropods were first observed. Trapping was then continued until the end of October in 2019, and also to the end of November in 2020. Every second week, traps were each emptied into a large plastic box and arthropods were individually transferred with sterile forceps into sterile 2 mL capacity reaction

Figure 1. Camera focused on pruning wounds (A), and cardboard trap mounted around a grapevine trunk (B), used to monitor insects on grapevines.

tubes. Due to the high number of European earwigs (*Forficula auricularia* L.), five individuals were pooled into a small glass container and were considered as one sample. A maximum of five such samples was taken per trap. Springtails (collembolans) from the same trap were also pooled into 2 mL capacity reaction tubes using a vacuum cup. Arthropods were morphologically identified using an identification guide for German fauna (Schaefer, 2017) and spiders were identified using a field guide for spiders (Roberts, 1995).

Washing of arthropod exoskeletons and DNA extractions

For detection of esca-related pathogens on arthropod exoskeletons, the protocol of Moyo *et al.* (2014) was followed, with modifications. Arthropods transferred into reaction tubes were freeze-killed at -20°C for 1–2 h. 1.5 ml of sterile distilled water was then added to each tube, and the tubes were vortexed for 60 sec. Washing suspensions containing possible fungal material were transferred into sterile 1.5 mL capacity reaction tubes, and arthropods were stored in 80% ethanol for later identification.

DNA extractions from the washing suspensions were carried out using the protocol of Tillet and Neilan (2000), with modifications. Tubes with washing suspensions were centrifuged at 15,000 rpm for 10 min and the supernatant was discarded. Two 3-mm steel beads, 750 µL XS-buffer and 50 µL cold TE-buffer were then added to the tubes. Samples were then placed in a tissue-lyser (Tissue Lyser 2; Qiagen) for 3 min at 30,000 Hz, and were then incubated for 1 h at 70°C in a thermomixer (ThermoMixer comfort; Eppendorf AG) at 350 rpm. Samples were vortexed for 10 sec and then put on ice for 30 min prior to centrifugation at 14,000 rpm for 10 min. Supernatant (700 µL) from each sample was then transferred into a new 1.5 mL capacity reaction tube and 700 µL of ice cold 80% isopropanol was added. For DNA precipitation, samples were incubated at room temperature for 15 min prior to centrifugation at 10,000 rpm for 10 min. The supernatant from each sample was discarded and the DNA pellet was then washed with 250 µL of ice cold ethanol. After another centrifugation step at 10,000 rpm for 10 min, the ethanol was discarded and the DNA pellet was air dried. The DNA was then resuspended in 30 µL of TE-buffer and left overnight at 4°C.

Detection of esca-related pathogens using nested multiplex PCR

DNA extracted from washing suspensions was subjected to a nested PCR. In primary PCR reactions,

primers ITS5 and ITS4 (White *et al.,* 1990) were used to amplify fungus-specific DNA. In secondary PCRs, *Pch*-specific primers Pch1H: 5'-CCC GAT CTC CAA CCC TTT GTT T-3' and Pch2H: 5'-CGG GCC TAT CTT CTA TGA AAG-3' (Haag, 2018), *Pm* spp.-specific primers Pm5H: 5'-GGA GGG CAC AGA CTC TGT ATT-3' and Pm3H: 5'-GTA AAC TAC TGC GCT CGG AG-3' (Haag, 2018) and *Clo*-specific primers CLO1F: 5'-TAC TAG AGC AAA GGA CAG GCA GC-3' (Navarette *et al.,* 2011) and Clo3H: 5'-GAA CCC CAA TAC CAA GCG AGA G-3' (Haag, 2018) were used. All primers had final concentrations of 0.2 µM. PCR reactions were carried out in a SimpliAmp™ Thermal Cycler (Applied Biosystems). The primary reactions were each carried out with SuperHot Taq DNA Polymerase (Genaxxon BioScience GmbH) in a reaction volume of 25 µL, according to the user manual, and 1 µL of the extracted DNA was used as template. The conditions of the primary reactions were as follows (Haag, 2018): initial denaturation at 95°C for 10 min; 25 cycles 30 sec at 95°C, 30 sec at 57°C and 30 sec at 72°C; final extension for 10 min at 72°C. After the primary PCR, the reaction was diluted 1:50 with "BioScience-Grade" water (Roth) and 1 µL of the dilution was used for the secondary PCR reaction conducted with the KAPA2G Fast Multiplex PCR Kit (Kapa Biosystems). Each reaction was carried out in a total volume of 10 µL and was composed as advised in the user manual. Parameters were (Haag, 2018): initial denaturation at 95°C for 3 min; 10 cycles at 95°C for 15 sec, 30 sec at 69°C and 30 sec at 72°C; 25 cycles at 95°C for 15 sec, 30 sec at 60°C and 30 sec at 72°C; final extension at 72°C for 3 min. PCR products were loaded on a 2% agarose gel and run at 6 V/5 cm for 1.5 h. PCR products were visualized under ultraviolet (UV) light using a QUANTUM ST5 gel documentation system (Vilber Lourmat). A 100 bp ladder (New England Biolabs) marked the molecular size of the PCR-

products, which were 433 bp for *Clo*, 381 bp for *Pch* and approx. 330 bp for *Pm* spp.

RESULTS

Monitoring of arthropods on vine pruning wounds and trunks

Monitoring in April 2019 only rarely detected arthropods. In total, six arthropod orders were documented, including spiders, mites, bugs, ants, nocturnal centipedes and woodlice (Figure 2). Visual surveys of vine trunks frequently revealed ants (*Temnothorax; Formicidae: Myrmicinae*, or *Lasius niger* L., *Formicidae: Formicinae*) moving up and down the trunks during sunny weather, as well as red velvet mites (*Allothrombium fuliginosum* Hermann, *Acari: Trombidiidae*). Spider webs were often observed inside vine crevices, and from the end of May onwards, earwigs were sometimes observed within cracks in the bark.

Arthropod diversity on vine trunks assessed with cardboard traps

In 2019 and 2020, and from all sampling sites, a total of 2099 arthropods, assigned to 22 families, were collected from the cardboard traps. Significant families and the abundance of associated taxa are outlined in Table 1. Of all samples, approx. 30% (640) were earwigs (*Dermaptera: Forficulidae*) and 27% (568) were spiders (*Aranea*). Only one earwig species, the European earwig *Forficula auricularia* (L.) (Figure 3A), was found in the traps, and was the prevalent arthropod in vineyard "A". In many cases, more than 40 individuals were found in each cardboard trap. In contrast, spiders were the dominant arthropod order in vineyard "B". As the most

Figure 2. Images of arthropods visiting vine pruning wounds: A, an ant (*Formicidae: Formicinae*); B, a centipede (*Chilopoda: Lithobiidae*); C, a woodlouse (*Isopoda: Armadillidiidae*).

diverse order, trapped spiders were of nine families, with 58% being *Salticidae* (jumping spiders), 10% *Gnaphosidae* (ground spiders), 9% *Linyphiidae* (sheet weavers) and 9% being *Thomisidae* (crab spiders). In total, 329 jumping spiders were trapped, of which 57% were *Marpissa muscosa* (Clerck) (Figure 3B), and 43% were *Synageles venator* (Lucas) (Figure 3C). In vineyard "B", *Raglius alboacuminatus* (Goeze) (*Heteroptera*: *Rhyparochromidae*) (Figure 3D), a dirt-coloured seed bug, was frequently found (165). Only 23 individuals of this species were detected in vineyard "A", giving a total number of 188 (9%) of this species from both vineyards and years. Thus, bugs, mainly *R. alboacuminatus*, were the third most common arthropod order. Woodlice were found irregularly in the traps, with a total number of 107. Cockroaches, ants, springtails, harvestmen, centipedes, mites, millipedes and beetles were all collected in total numbers <100 (for species numbers see Table 1).

Arthropods testing positive for esca-related pathogens

Thirty-five percent of the 2099 arthropod samples tested positive for *Pch*, 21% for *Pm* spp. and 7% for *Clo* (Table 1). For the predominant arthropod, the European earwig (640 samples), 27% carried *Pch*, 17% carried *Pm*. spp. and 3% carried *Clo* on their exoskeletons. However, due to the pooling method applied for this species, these are maximum numbers, because pathogen-free individuals may have occurred side-by-side with positive individuals in a sample. For spiders, as the second most com-

Figure 3. Most commonly found arthropods in cardboard-traps mounted to grapevine trunks in two vineyards: A, *Forficula auricularia* L. (*Dermaptera*: *Forficulidae*); B, *Marpissa muscosa* Clerck (*Aranea*: *Salticidae*); C, *Synageles venator* Lucas (*Aranea*: *Salticidae*); and D, *Raglius alboacuminatus* Goeze (*Heteroptera*: *Rhyparochromidae*).

mon arthropod order (568 samples), 38% were positive for *Pch*, 19% for *Pm* spp., and 8% were positive for *Clo*. *Pch* was detected most frequently on mites (59%) and centipedes (57%), *Pm* spp. most frequently on millipedes (43%) and centipedes (40%), and *Clo* most frequently on woodlice (31%).

The rates of *Pch*-positive arthropods were very similar for vineyards "A" and "B", at, respectively, 36% and 35%. Above average in vineyard "A" were bugs, woodlice and mites (respectively, 56%, 51% and 67%). In vineyard "B", 42% of ants tested positive for *Pch*. The rate of arthropods carrying *Pm* spp. was greater in vineyard "A" (25%) than in "B" (16%). In vineyard "A", greatest rates for *Pm* spp. were found from spiders (30%), bugs (37%), cockroaches (41%), mites (42%) or beetles (30%), while this was greatest for woodlice (36%) in vineyard "B". The proportions of *Clo*-positive arthropods was very similar in both vineyards, at 6% in vineyard "A" and 7% in vineyard "B". Very high numbers of woodlice (48%) and harvestmen (23%) were found in vineyard "B".

Some arthropods tested positive for more than one esca-related pathogen (Table 2). Of 2099 surveyed arthropods, 12% carried both *Pch* and *Pm* spp., 1% carried *Pch* and *Clo*, 1% carried *Clo* and *Pm* spp., and 2% carried all three pathogens. Nine percent of earwigs, 10% of spiders and 11% of bugs tested positive for both *Pch* and *Pm* spp. For arthropods collected in smaller numbers, the greatest rates of detection for the combination *Pch* and *Pm* spp. were from centipedes (36%), mites (30%) and millipedes (20%).

Occurrence of positive arthropods during the period of putative pruning wound susceptibility

Figure 4 presents an overview of arthropods that carried at least one of the esca-related pathogens during the sampling periods of April through June in 2019 and 2020. In both years, first occurrence of arthropods was noted at the beginning of April, with increasing numbers over the following months. In April, cumulated over both years and vineyards, spiders were the predominant arthropod order and 35% (30 of 85) tested positive for esca-related pathogens. Ants, as the second most common arthropods, were positive in 41% (12 out of 29) of their captures. The numbers of samples of all other arthropods was <20. In May, spiders were still the most common arthropod order and 52% (35 out of 67) of the captures tested positive for esca pathogens. The first earwigs were found in the same month and 37% (22 of 59) carried esca-related pathogens. Ants and cockroackes had pathogen-positive proportions of 52% (14 of 27) in May. By June, earwigs had replaced spiders as the pre-

Table 1. Overview of arthropods testing positive for the esca-related pathogens *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium* spp. (*Pm* spp.) and *Cadophora luteo-olivacea* (*Clo*). Species identity only given for most frequently detected arthropods in vineyards "A" and "B".

Order (common names)	Vineyard Family/Genus/Species	Vineyard "A" Sum of arthropods	Vineyard "A" Esca-related pathogens Pch	Pm spp.	Clo	Vineyard "B" Sum of arthropods	Vineyard "B" Esca-related pathogens Pch	Pm spp.	Clo	Total (vineyards "A" and "B") Sum of arthropods	Total Esca-related pathogens Pch	Pm spp.	Clo
Total	**Various taxa**	**1002**	358 (36)	253 (25)	59 (6)	**1097**	384 (35)	178 (16)	89 (8)	**2099**	742 (35)	431 (21)	148 (7)
Dermaptera (earwigs)	**Forficulidae** *Forficula auricularia* (L.)	**440**	124 (28)	82 (19)	14 (3)	**200**	47 (24)	27 (14)	6 (3)	**640**	171 (27)	109 (17)	20 (3)
Aranea (spiders)	**Total**	**176**	67 (38)	53 (30)	17 (10)	**392**	149 (38)	53 (14)	26 (7)	**568**	216 (38)	106 (19)	43 (8)
	Salticidae	**63**	22 (35)	24 (38)	8 (13)	**266**	101 (38)	35 (13)	11 (4)	**329**	123 (37)	59 (18)	19 (6)
	Marpissa muscosa (Clerck)	**57**	18 (32)	21 (37)	7 (12)	**129**	51 (40)	20 (16)	9 (7)	**186**	69 (37)	41 (22)	16 (9)
	Synageles venator (Lucas)	**6**	4 (67)	3 (50)	1 (17)	**137**	50 (37)	14 (10)	2 (2)	**143**	54 (38)	17 (12)	3 (2)
Heteroptera (bugs)	**Total**	**27**	15 (56)	10 (37)	1 (4)	**183**	56 (31)	23 (13)	4 (2)	**210**	71 (34)	33 (16)	5 (2)
	Rhyparochromidae *Raglius alboacuminatus* (Goeze)	**23**	13 (57)	8 (35)	0 (0)	**165**	54 (33)	22 (13)	3 (2)	**188**	67 (36)	30 (16)	3 (2)
Isopoda (woodlice)	Armadillidiidae *Armadillidium* spp.	**49**	25 (51)	14 (29)	5 (10)	**58**	21 (36)	21 (36)	28 (48)	**107**	46 (43)	35 (33)	33 (31)
Blattoptera (cockroaches)	**Ectobiinae** *Ectobius* spp.	**46**	17 (37)	19 (41)	6 (13)	**42**	19 (45)	10 (24)	4 (10)	**88**	36 (41)	29 (33)	10 (11)
Formicidae (ants)	**Total**	**43**	14 (33)	5 (12)	1 (2)	**43**	18 (42)	4 (9)	2 (5)	**86**	32 (37)	9 (11)	3 (4)
	Formicinae *Lasius niger* (L.)	**6**	4 (67)	1 (17)	0 (0)	**39**	15 (38)	3 (8)	2 (5)	**45**	19 (42)	4 (9)	2 (4)
	Myrmicinae *Temnothorax* spp.	**37**	10 (27)	4 (11)	1 (3)	**4**	3 (75)	1 (25)	0 (0)	**41**	13 (32)	5 (12)	1 (2)
Collembola (springtails)	Isotomidae	**42**	18 (43)	12 (29)	5 (12)	**37**	15 (41)	3 (8)	5 (14)	**79**	33 (42)	15 (19)	10 (13)
Opiliones (harvestmen)	Phalangiidae	**34**	13 (38)	6 (18)	1 (3)	**35**	13 (37)	8 (23)	8 (23)	**69**	26 (38)	14 (20)	9 (13)
Chilopoda (centipedes)	**Total**	**13**	6 (46)	5 (39)	0 (0)	**42**	25 (60)	17 (41)	2 (5)	**55**	31 (56)	22 (40)	2 (3)
	Linotaeniidae	**5**	2 (40)	1 (20)	0 (0)	**1**	0 (0)	0 (0)	0 (0)	**6**	2 (33)	1 (17)	0 (0)
	Lithobiidae *Lithobius forficatus* (L.)	**8**	4 (50)	4 (50)	0 (0)	**41**	25 (61)	17 (41)	2 (5)	**49**	29 (59)	21 (43)	2 (4)
Diplopoda (millipedes)	Polyxenidae *Polyxenus lagurus* (L.)	**42**	19 (45)	18 (43)	4 (10)	**2**	0 (0)	1 (50)	1 (50)	**44**	19 (43)	19 (43)	5 (11)
Acari (mites)	**Trombidiidae** *Allothrombium fuliginosum* (Hermann)	**24**	16 (67)	10 (42)	0 (0)	**20**	10 (50)	5 (25)	1 (5)	**44**	26 (59)	15 (34)	1 (2)
Coleoptera (beetles)	various families	**20**	7 (35)	6 (30)	0 (0)	**17**	6 (35)	3 (18)	0 (0)	**37**	13 (35)	9 (24)	0 (0)
Unidentified	various taxa	**46**	17 (37)	13 (28)	5 (11)	**26**	5 (19)	3 (12)	2 (8)	**72**	22 (31)	16 (22)	7 (10)

Total numbers of recovered arthropods in **bold** and numbers of arthropods tested positive (%).

Table 2. Overview of arthropods testing positive for more than one of the esca-related pathogens *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium* spp. (*Pm* spp.) and *Cadophora luteo-olivacea* (*Clo*). Species identity only given for most frequent and representative arthropods.

Order (common names)	Family/Genus/Species	Esca-related pathogens (combinations)				
		Sum of arthropods	*Pch + Pm* spp.	*Pch + Clo*	*Clo + Pm* spp.	*Pch + Pm* spp. + *Clo*
Total	Various taxa	**2099**	248 (12)	28 (1)	18 (1)	46 (2)
Dermaptera (earwigs)	**Forficulidae** *Forficula auricularia* (L.)	**640**	58 (9)	6 (1)	2 (0)	6 (1)
Aranea (spiders)	**Total**	**568**	57 (10)	11 (2)	7 (1)	8 (1)
	Salticidae	**329**	31 (9)	5 (2)	4 (1)	4 (1)
	Marpissa muscosa (Clerck)	**186**	22 (12)	4 (2)	2 (1)	4 (2)
	Synageles venator (Lucas)	**143**	8 (6)	1 (1)	2 (1)	0 (0)
Heteroptera (bugs)	**Total** **Rhyparochromidae**	**210**	23 (11)	1 (0)	0 (0)	4 (2)
	Raglius alboacuminatus (Goeze)	**188**	22 (12)	0 (0)	0 (0)	3 (2)
Isopoda (woodlice)	**Armadillidiidae** *Armadillidium* spp.	**107**	16 (15)	1 (1)	3 (3)	10 (9)
Blattoptera (cockroaches)	**Ectobiinae** *Ectobius* spp.	**88**	17 (19)	1 (1)	1 (1)	2 (2)
Formicidae (ants)	**Total** **Formicinae**	**86**	6 (7)	2 (2)	0 (0)	1 (1)
	Lasius niger (L.)	**45**	3 (7)	2 (4)	0 (0)	0 (0)
	Myrmicinae *Temnothorax* spp.	**41**	3 (7)	0 (0)	0 (0)	1 (2)
Collembola (springtails)	**Isotomidae**	**79**	8 (10)	3 (4)	1 (1)	4 (5)
Opiliones (harvestmen)	**Phalangiidae**	**69**	9 (13)	0 (0)	1 (1)	3 (4)
Chilopoda (centipedes)	**Total**	**55**	20 (36)	0 (0)	0 (0)	2 (3)
	Linotaeniidae	**6**	1 (17)	0 (0)	0 (0)	0 (0)
	Lithobiidae	**49**	17 (35)	0 (0)	0 (0)	2 (4)
Diplopoda (millipedes)	**Polyxenidae** *Polyxenus lagurus* (L.)	**44**	9 (20)	0 (0)	1 (2)	4 (9)
Acari (mites)	**Trombidiidae** *Allothrombium fuliginosum* (Hermann)	**44**	13 (30)	1 (2)	0 (0)	0 (0)
Coleoptera (beetles)	Various families	**37**	5 (14)	0 (0)	0 (0)	0 (0)
Unidentified	Various taxa	**72**	7 (10)	2 (3)	2 (3)	2 (3)

Total numbers of recovered arthropods in **bold** and numbers of arthropods tested positive (%).

dominant group. They tested positive in 29% (39 of 134) of the cases, while 47% (24 of 51) of spiders carried esca pathogens. Of the cockroaches, 77% (23 of 30) were pathogen-positive. The rate of positive ants had decreased to 29% (eight of 28).

DISCUSSION

Moyo *et al.* (2014) in South Africa were the first and, to date, the only authors to demonstrate possible involvement of arthropods in the dissemination of GTD-, more precisely, esca-related pathogens. Arthropod mediated dispersal of these pathogens was previously suggested when *T. minima* (teleomorph of *Pm. minimum*) was detected on mites and termites found on symptomatic grapevines (Eskalen *et al.*, unpublished, in Rooney-Latham *et al.,* 2005a), and when *Pm. rubrigenum* was isolated from bark beetles in Czechia (Kubátová *et al.,* 2004). Accumulation of collembolans and mites in grapevine cracks around sporulating mycelium of *Pch* was repeatedly observed by Edwards *et al.* (2001). As esca-related pathogens were frequently documented on arthropod exoskeletons in the present study of Ger-
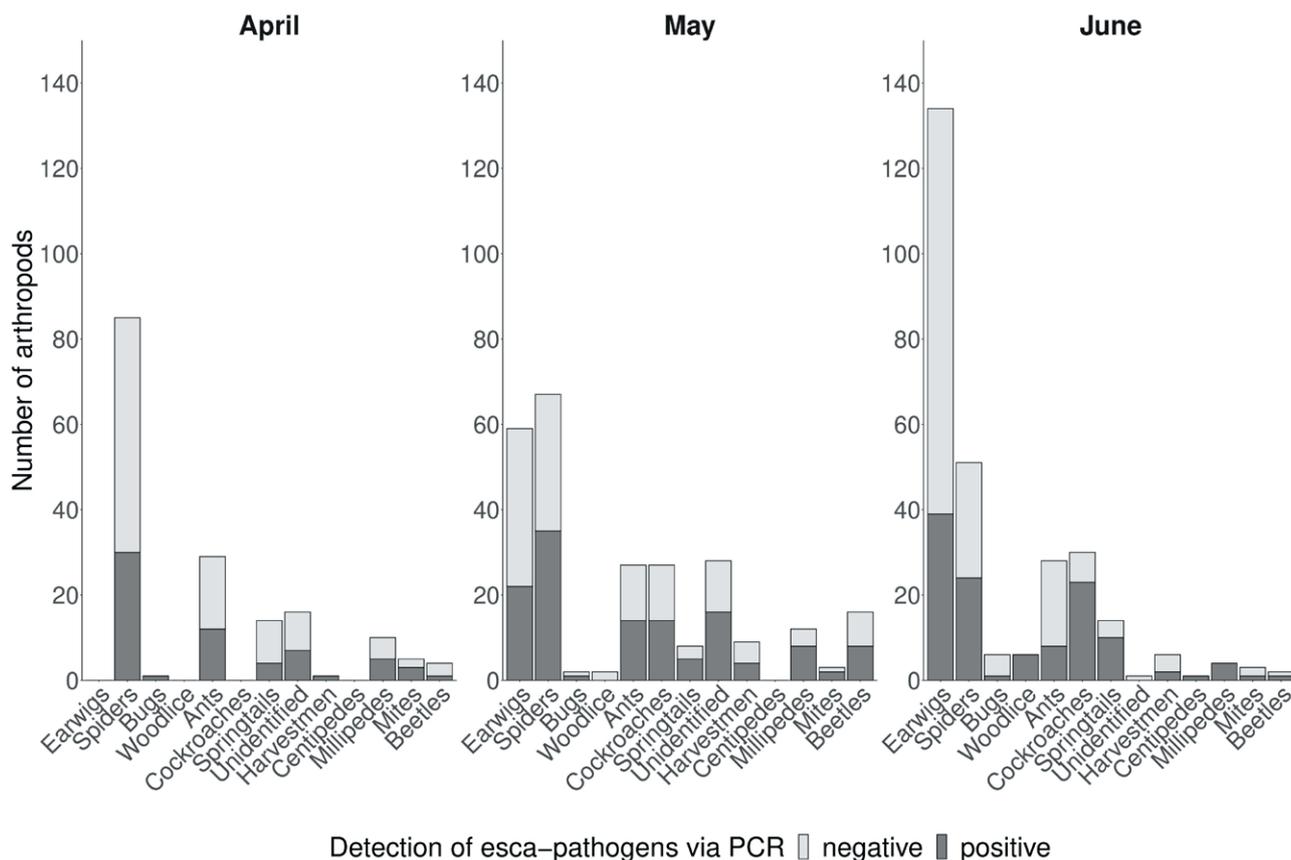
Figure 4. Arthropods testing negative or positive for esca-related pathogens in April, May and June of 2019 and 2020. Arthropods were collected from two different vineyards at 2 week intervals using cardboard traps mounted to grapevine trunks.

man vineyards, this confirms broad geographical indication that arthropods may be vectors for GTD pathogens.

In contrast to the study of Moyo *et al.* (2014) in South Africa, the present study used cardboard traps applied only to grapevine trunks, with no traps directly mounted around pruning wounds, so only arthropods directly collected from trunks were surveyed. Nonetheless, it can be assumed that these arthropods were also active during foraging on pruning wounds. This was confirmed by the camera-mediated monitoring, which showed activity of arthropods on pruning wounds during the putative host susceptibility phase (Figure 2). Particular attraction of arthropods to these wounds, as demonstrated by Moyo *et al.* (2014), was not observed in the present study. While wound sap in German vineyards is usually evident each year for a few weeks from mid March, temperatures during the two survey periods at that time were too low for arthropods to become active.

Moyo *et al.* (2014) in South Africa examined 5,677 arthropods, and 13% of these tested positive for *Pch* and 18% for *Pm* spp. In contrast, the present study of 2,099 arthropods showed greater pathogen-positive proportions of 35% for *Pch* and 21% for *Pm* spp. Possible reasons for this discrepancy could be ages of the studied vineyards, the cultivars, and the overall weather conditions.

A broad range of *Pm* spp. plays roles in GTDs (Mostert *et al.,* 2006; Gramaje *et al.,* 2015; Baloyi *et al.,* 2018). As several of these species, most commonly *Pm. minimum*, occur in German vineyards (Fischer and Kassemeyer, 2003; Fischer *et al.,* 2016; Kraus *et al.,* 2019), a genus-specific primer pair was used to verify their presence on arthropod exoskeletons. Following *Pch* on 35% of the arthropods, *Pm* spp. were detected in 21% of washing suspensions. These results confirm that structures of both *Pch* (Crous and Gams, 2000) and *Pm* spp. (Mostert *et al.,* 2003; Rooney-Latham *et al.,* 2005a; Mostert *et al.,* 2006) are suitable for the adhesion of conidia or mycelium fragments to arthropod exoskeletons. The present study is the first to investigate the roles of arthropods for dissemination of *Clo*. The low frequency of *Clo* on arthropod exoskeletons (7%) cannot be explained, but agrees with previous reports of low *Clo*

isolation rates from previous Germany-based studies (Fischer *et al.,* 2016; Kraus *et al.,* 2019).

In the sampled vineyards, the European earwig *F. auricularia,* was the predominant arthropod followed by different spider species. The increasing numbers of earwigs in German vineyards and beyond, and their putative status as beneficial insects is seen critically (Huth, 2011; Kehrli *et al*., 2012; Mohr, 2012). Our study reveals a possible involvement of earwigs in the dispersal of GTDs, as individuals were found to carry *Pch* (27% of earwigs assayed), *Pm* spp. (17%) and *Clo* (3%). As well, 9% of earwigs tested positive for both *Pch* and *Pm* spp. As the omnivorous earwigs can migrate over considerable distances during foraging, depending on food sources (Lamb, 1975), they are likely to incidentally disperse the pathogens over several vines, also by wandering across pruning wounds. However, the pathogenic relevance may still be low because earwigs were present each year in the surveyed vineyards only from the end of May onwards (see also Huth, 2011). This indicates that earwigs occurred outside the immediate pruning wound susceptibility phase. As the susceptibility of wounds declines with time (Eskalen *et al.,* 2007; Van Niekerk *et al.,* 2011; Elena and Luque, 2016), earwigs are likely to be relevant for late-pruned vines that are potentially susceptible to pathogens until May or June each year.

Besides pruning wounds, three other aspects relate to infection processes and/or disease epidemiology. i) Wounds, caused by vineyard management such as sucker removal, may also be entry points for earwig-mediated pathogen dispersal during the vegetative grapevine phase (Makatini, 2014). ii) Conidia and/or mycelium fragments adhering to the earwig exoskeletons may be spread within and between vines. iii) The faeces of omnivorous earwigs could also be inoculum sources, which is indicated by *Pch* spores maintaining germinability after gut passage of millipedes (Moyo *et al.,* 2014). Whether earwigs feed on esca-related pathogens in the field, and to which amount the resulting faeces may be inoculum sources, remain to be determined.

The majority of spiders found on vine trunks (58%) were *Salticidae* (jumping spiders) with two dominant species, *M. muscosa* and the myrmecomorphic *S. venator* which has been previously identified as a dominant salticid in vineyards (Havlová *et al.,* 2015). In total, 37% of jumping spiders tested positive for *Pch,* 18% were positive for *Pm* spp.*,* and 6% were positive for *Clo,* and 9% of salticids carried both *Pch* and *Pm* spp. on their exoskeletons. Salticids are free-hunting spiders which are very active during warm and sunny weather, and most likely migrate between vines in search of prey (Stresemann, 1992; Roberts, 1995). The small size of the

ant-like *S. venator* (3–4 mm) makes it likely that these spiders, during hunting, encounter fungal pathogens sporulating between cracks and crevices in vine bark (Edwards *et al.,* 2001; Baloyi *et al.,* 2013). In the experimental plots of the present study, spiders were already common in April each year, when late-pruned vines are most likely to still be susceptible to GTD pathogens. Salticids tested positive for esca-related pathogens in April (35%), May (52%) and June (47%), which indicates a possible risk for pathogen dissemination. Moreover, 10% of all spiders tested positive for both *Pch* and *Pm* spp.

All other arthropods, i.e. bugs, woodlice, centipedes, millipedes, cockroaches, ants, springtails, harvestmen, mites and beetles, were not as common as earwigs and spiders. Based on these results, these arthropods are likely to only be of low importance as GTD pathogen dispersal agents. However, woodlice frequently tested positive for all three pathogens, with 43% testing positive for *Pch*, 33% for *Pm* spp. and 31% for *Clo*., and 15% of woodlice were also co-positive for *Pch* and *Pm* spp. As woodlice were not commonly found (a total of 107 over the two seasons), their significance as pathogen vectors remains unclear. Also 36% of the centipedes and 20% of the millipedes carried both *Pch* and *Pm* spp. on their exoskeletons. As for woodlice, few centipedes (55 over the two seasons) and millipedes (44) were found in the traps, and these two arthropods were especially rare during the presumed pruning wound susceptibility phase.

Ants, indicated as important vectors of esca pathogens in South Africa (Moyo *et al.,* 2014), were infrequent during the sampling periods of the present study. However, they were more common during the presumed months of vine susceptibility, and 41% of these insects tested positive for the pathogens in April, 52% were positive in May and 29% were positive in June. Ants were repeatedly observed moving up and down the vines, and were also captured visiting pruning wounds by the cameras in April (Figure 2A). Total numbers were less than 30 per month, and, in contrast to the observations of Moyo *et al.* (2014) in South Africa, no particular attraction by these insects to wound sap was detected.

The present study results, during 2019 and 2020, revealed a possible relationship between many different arthropod orders and esca-related pathogens. The predominant earwigs and spiders, representing 57% of the arthropods caught in traps, may disseminate pathogens in German vineyards, while spiders were already numerous in the presumed host pruning wound susceptibility phase. Furthermore, 12% of all surveyed arthropods tested positive for both *Pch* and *Pm* spp., which indicated possible co-existence of the pathogens on individual vines or within the arthropod migration ranges. No par-

ticular attraction of arthropods to the pruning wounds was noted, which may have been due to the lack of sap during the arthropod activity periods.

Under Central European conditions we therefore conclude that arthropods may be incidental vectors of GTD pathogens. These arthropods could spread pathogens between several vines, as the majority of examined arthropods migrate during foraging.

The present study has broadened understanding of the epidemiology of GTDs. However, accurate assessment of the arthropod contributions to pathogen dissemination remains to be achieved. Besides incidentally vectoring pathogen propagules to vine wounds, the spatial dissemination of esca-causing pathogens on and between grapevines is likely to increase inoculum sources.

Future studies should address several related aspects. i) The actual propagule loads and infection potential carried by frequently occurring arthropods should be evaluated, to assess the significance of these as efficient pathogen vectors. ii) The susceptibility phase of wounds at different periods after grapevine pruning should be evaluated in German vineyards, to determine the risks of arthropod-mediated pathogen spread. iii) The role of earwig faeces as inoculum sources in vineyards needs to be elucidated. As precautionary measures to prevent disease-spread through arthropods, the greening management in vineyards should be adequately adjusted; also, the application of appropriate pruning wound protection should possibly be increased. Usage of insecticides is not encouraged here.

ACKNOWLEDGEMENT

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