NATURAL PEST SUPPRESSION IN VINEYARDS UNDER INNOVATIVE MANAGEMENT

by

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from Pirmasens

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Abstract

Grapevine growers have struggled with defending their crops against pests and diseases since the domestication of grapevine over 6000 years ago. Since then, new growing methods paired with a better understanding of the ecological processes in the vineyard ecosystem continue to improve quality and quantity of grape harvests. In this thesis I am describing the effects of two recent innovations in viticulture on pest and beneficial arthropods in vineyards; Fungus-resistant grapevine cultivars (PIWIs) and the pruning system semi-minimal pruned hedge (SMPH). The SMPH pruning system allows for a drastic reduction of manual labor in the vineyard, and PIWIs are resistant to two of the most common fungal diseases of grapevine and therefore allow a drastic reduction of fungicide applications compared to conventional varieties. Heavy use of pesticides is linked to a number of problems, including pollution of waterways, negative effects on human health, and biodiversity loss. Here, I studied the effects of fungicide reduction and minimal pruning on arthropods that are beneficial for natural pest suppression in the vineyard ecosystem such as predatory mites, spiders, ants, earwigs, and lacewings. All of these groups either benefitted from the reduction of fungicide sprayings or were not significantly affected. Structural changes in the canopy of SMPH grapevines altered the microclimate in the canopy which in turn influenced some of the arthropods living in it. Overall, my findings suggest that PIWIs and SMPH, both in combination or separately, improve conditions for natural pest control. This adds to other advantages of these innovative management practices such as a reduction in production cost and a smaller impact on the environment.

Zusammenfassung

Seit der Domestizierung von Wein vor über 6000 Jahren haben Weinbauern mit Krankheiten und Schädlingen ihrer Pflanzen zu kämpfen. Seitdem führen neue Anbaumethoden und ein besseres Verständnis der ökologischen Prozesse im Weinberg zu wachsenden Erträgen und steigender Traubenqualität. In dieser Arbeit beschreibe ich die Effekte zweier innovativer Anbaumethoden auf Schädlinge und Nützlinge im Weinbau; Pilzwiderstandsfähige Sorten (PIWIs) und das Reberziehungssystem Minimalschnitt im Spalier (SMPH). SMPH erlaubt eine drastische Reduktion des Arbeitsaufwands im Weinberg. PIWIs sind resistent gegenüber zwei der destruktivsten Pilzkrankheiten der Rebe und bleiben daher bei deutlich weniger Pflanzenschutzbehandlungen als herkömmliche Sorten gesund. Übermäßiger Gebrauch von Pestiziden wird mit einer Reihe von Problemen wie Gewässerverschmutzung, Gesundheitsfolgen beim Menschen, und Biodiversitätsverlust in Verbindung gebracht. In dieser Arbeit wurden Effekte von reduzierten Fungizid Spritzungen auf Nützlinge wie Raubmilben, Spinnen, Ameisen, Ohrwürmer und Florfliegen untersucht. Diese Gruppen profitierten entweder von den reduzierten Fungizidmengen, oder sie wurden nicht signifikant beeinflusst. Strukturelle Unterschiede in der SMPH Laubwand beeinflussten das Mikroklima im Vergleich zur Spaliererziehung. Sowohl strukturelle als auch mikroklimatische Veränderungen beeinflussten einige Arthropodengruppen im Wein. Insgesamt lässt sich Schlussfolgern, dass sowohl PIWI Sorten als auch das Schnittsystem SMPH ein großes Potential haben, die Bedingungen für natürliche Schädlingskontrolle zu verbessern. Dies reiht sich in eine Liste anderer Vorteile dieser Managementmethoden, wie zum Beispiel eine Reduktion der Produktionskosten und verbesserte Nachhaltigkeit.

Chapter I

General Introduction

Theresa Pennington

Since the 14th Century, the human world population has experienced incessant growth, from approximately 370 million in 1350 to almost 7.6 billion in 2018. Agricultural goods have sponsored this explosion, and as the number of people on the planet continues to increase, so too does the demand for food, shelter, and other products that the Earth can provide. In Germany, agriculture accounts for over half of the country's surface area, and while much of this agriculture is for the creation of foodstuffs, it has a tremendous impact on the environment, to include water, soil, air, biodiversity and climate (Geiger et al. 2010). Unmanaged ecosystems are converted into fields, and natural landscape elements of agroecosystems such as hedgerows and meadows make way for more intensive agriculture. Many high-yielding crop varieties demand the use of agricultural chemicals to protect them from pests and diseases. Viticulture, for example, relies on very frequent fungicide applications both in conventional and organic cultures. In fact, grapevine is the most fungicide-intensive culture in Europe. Even though it takes up only five per cent of agricultural areas in the European Union, it receives 58 per cent of all fungicides used in farming (Eurostat 2007).

While these practices may produce favorable commercial results, they have alarming environmental consequences. One of the most important concerns is arthropod biodiversity and abundance, which has been drastically reduced through landscape simplification and pesticide overuse (Hallmann et al. 2017). Arthropods play important roles in terrestrial ecosystems, especially in agro-ecosystems, and their dwindling numbers will engender serious problems in ecosystem functioning (Geiger et al. 2010). Vineyards in particular are important for species conservation. Because of their location in particularly warm areas they can host many rare and threatened species, for example the praying mantis (*Mantis religiosa (L.)*) or the sand lizard (*Lacerta agilis).* Though managed to optimize productivity, agricultural ecosystems still depend on supporting and regulating ecosystem services such as soil fertilization and bioturbation, pollination, and pest control. On the other hand, agro-ecosystems also receive ecosystem disservices, most notably through herbivory or competition for water and other resources (Zhang et al. 2007), and these services and disservices require careful management in order to maintain a productive agro-ecosystem.

High amounts of fungicides are needed to control the three most destructive fungal diseases in grapevine: Botrytis bunch rot (*Botrytis cinerea*), downy mildew (*Plasmopara viticola*), and powdery mildew (*Erysiphe necator*). Yet fungicide applications also affect non-target organisms in the vineyard and beyond. Even though synthetic fungicides are prohibited in organic viticulture, the commonly used Copper Hydroxide and Sulphur products still have a negative impact on vineyard fauna. For example, these fungicides have been shown to reduce the number of *Trichogramma* wasps, which are egg parasitoids of *Lepidoptera,* and the most commonly used biological control agents in the world (Castaneda-Samayoa et al. 1993; Smith 1996; Hassan et al. 1998; Knutson 1998; Thomson et al. 2000). In viticulture, *Trichogramma* wasps function as biological control of The European Grapevine Moth, *Lobesia botrana* (Denis & Schiffermüller, 1775) and the Vine Moth *Eupoecilia ambiguella* (Hübner) (Lepidoptera, Tortricidae), both of which are major pests in the grape-growing regions of Europe. Other beneficial arthropods in the grapevine canopy are predatory mites, especially those belonging to the family *Phytoseiidae*. Their ability to survive on alternative food sources when prey is scarce makes them very efficient at controlling pest mite populations (e.g., Eriophyidae, Tetranychidae) even when pest numbers are low (Flechtmann and McMurtry 1992; Duso et al. 2003, 2012; Gerson et al. 2008; Pozzebon and Duso 2008). Effects on many other arthropods that are relevant for pest control in vineyards such as spiders and ants are probable but have previously not been described extensively. Beyond their effects on arthropods, Copper and Sulphur products are also known to negatively affect earthworms and their ecosystem services (Eijsackers et al. 2005; Lemtiri et al. 2014), and fungicide runoff even impacts aquatic ecosystems (Bundschuh et al. 2016).

Two recent innovations in viticulture aim to reduce these negative impacts on the environment and make winegrowing more sustainable: Fungus resistant grapevine cultivars (PIWIs - from German: "Pilzwiderstandsfähige Sorten") and the pruning system semi-minimal pruned hedge (SMPH). Fungus resistant grapevines have been bred by crossing high quality though highly susceptible grapevine cultivars with more resistant wild grapevine species. At first, these crosses did not meet the quality standards favored by growers and consumers, but careful selection and back crossing produced new cultivars with fine taste and excellent fungus resistance. These resistant varieties allow the production of high quality grapes while reducing fungicide use by up to 85 per cent (Töpfer et al. 2011).

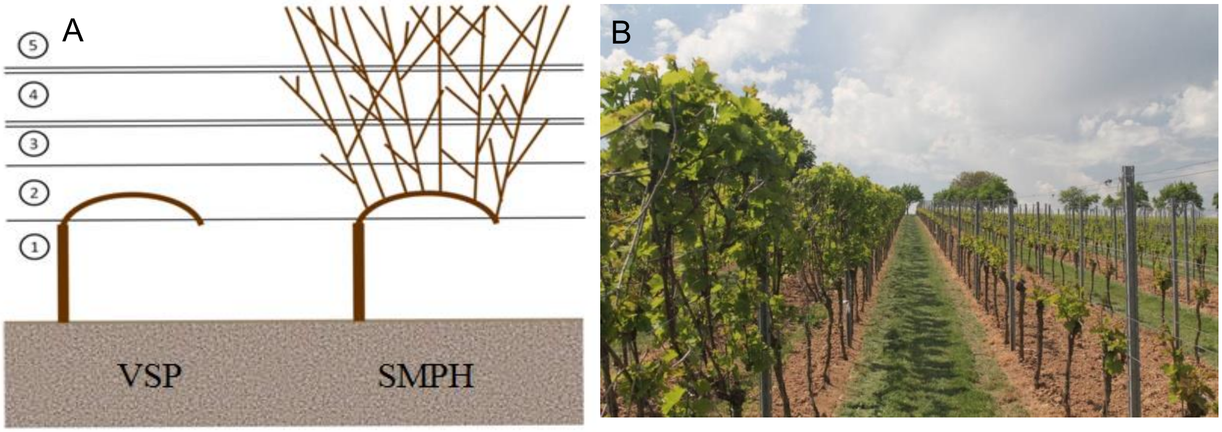

Fig. 1: A) Schematic comparison between vertical shoot positioned (VSP) and semi-minimal pruned hedge (SMPH) grapevines in the dormancy period. B) SMPH (left) and VSP (right) pruned grapevines in the early season. Note the much larger and almost closed canopy in the SMPH grapevines compared to VSP.

Growing grapevines in the SMPH pruning system leads to number of changes in physiology and morphology of the plants, especially when compared to the traditional trellis pruning system (VSP - vertical shoot positioning). In SMPH grapevines, the branches remain in the trellis after the growing season, and the plant is not pruned back to just one cane as in VSP (Figure 1). This produces a more voluminous canopy with older wood and smaller leaves that close earlier in the spring than the VSP canopy. SMPH pruning also changes the architecture of the grape clusters themselves, generating smaller berries that sit on longer stalks in smaller clusters. This makes the looser grape clusters less susceptible to fungal disease than the tight, large clusters in VSP grown grapevines (Walg 2011, 2012).

Both PIWIs and the SMPH growing system are recent innovations in viticulture and their advantages and disadvantages are still a matter of investigation. The underlying research question of this project is: How does minimal pruning and reducing fungicide sprayings affect biodiversity and natural pest suppression in vineyards?

Chapter II analyzes phytoseiid mite numbers, assessing and comparing innovative and traditional management methods. High densities of these generalist predators are effective at preventing outbreaks of pest mites and other small arthropod pests that may inflict considerable damage on grapevines. Both minimal pruning and reduced fungicide applications led to a higher abundance of predatory mites, though pest mite numbers were low independent of management.

Chapter III illustrates the use of artificial inoculation of grape bunches, egg baits, camera surveillance, and beat sheet sampling to investigate the natural control of *Lobesia botrana.* Reducing fungicide applications enhanced densities of *L. botrana* predators, increased egg predation, and reduced feeding damage of artificially inoculated grape bunches. There was no clear effect of minimal pruning on predators of *L. botrana*.

Chapter IV addresses structural and microclimatic differences between the canopy of SMPH and VSP trained grapevines. SMPH canopies were more humid and slightly colder than VSP canopies. The berry skins in the two pruning systems displayed no significant differences. Both pruning system and berry skin have the potential to influence the incidence of fungal diseases as well as damage by the invasive fruit fly *Drosophila suzukii* (Matsumura, Diptera: Drosophilidae). SMPH trained grapevines were more susceptible to Downy Mildew and Powdery Mildew than VSP trained grapevines, yet Botrytis Bunch Rot incidence was higher in VSP. The number of adult *D. suzukii* was higher in SMPH, however this increase in abundance did not correspond with increased oviposition on the grape berries.

While all experiments in chapters I - IV were conducted in the same experimental vineyard, Chapter V compares spider communities between pairs of larger vineyards planted with either PIWIs or traditional grapevine varieties, as well as between pairs of SMPH and VSP vineyards. Spiders are generalist predators and often very abundant in vineyards which makes them excellent pest control agents. Some spider families were affected by the different management methods, but neither pruning method nor the reduced fungicide use in PIWI vineyards had significant effects on overall spider abundance.

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Chapter II

Minimal pruning and reduced plant protection promote predatory mites in grapevine

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Abstract

Improving natural pest control by promoting high densities of predatory mites (Acari: Phytoseiidae) is an effective way to prevent damage by pest mites (e.g., Eriophyidae, Tetranychidae) and other arthropod taxa that can cause serious damage to vineyards. Here, we investigate the influence of innovative management on predatory mite densities. We compare (i) full versus reduced fungicide applications and (ii) minimal pruning versus a traditional trellis pruning system in four fungus-resistant grapevine varieties. As predatory mites also feed on fungus mycelium, we assessed fungal infection of grapevine leaves in the experimental vineyard. Predatory mites were significantly more abundant in both minimal pruning and under reduced plant protection. Increases in predatory mites appeared to be independent of fungal infection, suggesting mostly direct effects of reduced fungicides and minimal pruning. In contrast to predatory mites, pest mites did not increase under innovative management. Thus, conditions for natural pest control are improved in fungus-resistant grapevines and under minimal pruning, which adds to other advantages such as environmental safety and reduced production cost.

Keywords

Viticulture, beneficial arthropods, Typhlodromus pyri, fungicide, fungus-resistant cultivars, sustainable agriculture, ecosystem services, natural pest control

1. Introduction

Growing consumer demand urges agriculture to adopt more sustainable and environmentally friendly practices [1,2]. At the same time, farmers need to secure productivity to satisfy the market. Intensification of farming should take advantage of natural regulation and promote ecosystem services to be sustainable [3]. Viticulture sets a positive example for sustainable farming with the innovation and adaption of cultivars that are resistant to fungal pathogens. They can be a valuable tool to allow for a sustainable intensification, especially when combined with minimal pruning. Planting of these new cultivars can reduce the amounts of sprayed fungicides significantly [4] and minimal pruning increases the yield while still producing high quality grapes [5]. The use of resistant cultivars and minimal pruning may affect phytoseiid mites that play a major role in the biocontrol of pest mites and other arthropods in grape vineyards [6-8]. These predatory mites are generalists that can sustain stable populations even when prey numbers are low by surviving and reproducing on alternative food sources such as pollen and fungi [7,9-11]. This is an advantage of generalist predators over specialists, whose populations fluctuate depending on prey availability [12]. In addition to food availability, the presence of these beneficial predatory mites in grape vineyards is limited by their susceptibility to pesticides [13-16]. Once their importance for the agroecosystem was understood, many plant protection chemicals were selected to be less harmful for predatory mites. Despite these measures, some plant protection chemicals are still damaging to mite populations, including the combination of sulfur and copper [17,18] that are both intensively used to control plant pathogenic fungi in organic vineyards. In addition to the plant protection regime, canopy management is another vital part of grapevine production. The pruning system has an important impact on canopy architecture and microclimate [19] and consequently on the arthropod fauna. The traditional vertical shoot positioning (VSP) system and semi-minimal pruned hedge (SMPH) differ in their structural diversity with higher percentage of old wood and more but smaller leaves in SMPH than the VSP grapevines [19]. More structurally complex ecosystems have been shown to enable higher densities of predatory arthropods in both natural and agricultural systems [20] but may impede the host or prey finding success of parasitoids and predators [21-23].

We hypothesize that the populations of predatory mites will benefit from a reduced plant protection regime. We expect a higher density of phytoseiids in plots with reduced plant protection compared to those plots that were treated with the fungicide regime typical of fungus susceptible varieties. While pest mites themselves may also benefit from reduced fungicide treatments [17], they should be suppressed by higher densities of phytoseiids. Correspondingly, we expect no benefit or even decreased abundance of pest mites under reduced plant protection. We also hypothesize that the SMPH grapevines offer a more favorable habitat to many arthropods including phytoseiid mites because there is more shelter, better overwintering conditions and a larger, more heterogenous habitat. We investigate these two hypotheses in a controlled field experiment comparing VSP and SMPH pruning regimes under three levels of crop protection intensity.

2. Materials and Methods

To analyze the effects of pruning system and plant protection on mite populations in grape vineyards, we used a randomized block design. All data were collected during the two years, 2015 and 2016, in a 15-year-old experimental vineyard of Geilweilerhof located in Siebeldingen, Germany (N 49°13'13.7", E 8°02'43.0"). It was planted with four fungus-resistant *Vitis vinifera* cultivars; “Reberger”, “Villaris”, “Felicia” and “Gf 84-58-988”. Interrow distance was 2 m and grapevine spacing was 1 m. We treated the four varieties as our four experimental blocks. Each variety was cultivated in six to ten rows, half of which were VSP and half were SMPH trained. For practical reasons, the pruning treatment was not randomly applied but the two pruning treatments were applied to half of each cultivar block. Each treatment block was again divided into three parts that received one of three randomly assigned organic plant protection regimes.

This resulted in six combinations of plant protection intensity and pruning system. Each of those treatment combinations was replicated in the four different varieties, resulting in 24 different treatment plots.

An organic spraying regime consisting of two ("low"), four ("reduced") or twelve (2016) and thirteen (2015) ("standard") sprayings of Funguran progress® (Spiess-Urania Chemicals GmbH, Hamburg, Germany) (350 g copper per kg), Wettable Sulfur Stulln (agrostulln GmbH, Stulln, Germany) (796 g sulfur per kg) and VitiSan® (BioFa AG, Münsingen, Germany) (995 g potassium bicarbonate per kg) per season (Table S1). This allowed us to apply standardized amounts of active ingredients in the organic spray treatments. Twelve organic spray treatments equal a frequency of plant protection which is commonly applied to vineyards planted with fungus susceptible grape varieties. Although none of the varieties in our experimental vineyards needed the standard spraying regime, we included the schedule for susceptible cultivars as a reference point to be able to compare the mite populations in susceptible cultivars with a standard spraying regime to the mite populations in fungus-resistant cultivars that need less protection.

2.1. Grapevine Leaf Fauna

To assess the mite fauna on grapevine leaves, we followed the protocol introduced by Hill and Schlamp [24]. Twenty-five mature, randomly selected leaves from vines in each of the 24 treatment plots were collected and washed onto a filter paper, where all mites could be counted and identified using a stereomicroscope (Stemi 2000, Zeiss, Jena, Germany). We counted predatory mites as well as the pest mites *Colomerus vitis* (Pagenstecher) and *Calepitrimerus vitis* (Nalepa) (Acari: Eriophyidae). After the mites were washed off, the leaf area was determined using a leaf area meter (Li-COR, Modell 3100 area meter, Lincoln, NE USA).

We randomly selected 436 adult predatory mites from the filter paper to determine the species using the preparation method described by Krantz [25] and determined them using a microscope with phase contrast (Leica DM 4000 B, Leica Microsystems, Wetzlar, Germany). We took nine overall samples, two in 2015 (one in July and one in September) and seven in 2016 (one per month in June, July and October and two samples per month in August and September). All samples of one year were combined by averaging mite densities across dates. Data were analysed using R [26]. We fitted a linear mixed model with "grapevine variety" as a random effect and "pruning method" and "plant protection intensity" as fixed effects using the nlme package and the multcomp package for multiple comparisons [27,28].

2.2. Fungal Disease

Since the availability of fungal material might be a major factor in regulating phytoseiid populations [7], we monitored the fungus affected leaf area (infection level) as well as the percentage of affected leaves (incidence rate). Monitoring of fungal grapevine diseases was done according to the European and Mediterranean Plant Protection Organization (EPPO) guidelines: *Plasmopara viticola* ([Berk. and M.A. Curtis] Berl. and De Toni, 1888) (PP 1/31(3)), *Erysiphe necator* (Schw.) (PP 1/4(4)), *Botrytis cinerea* (Pers.) (PP 1/17(3)). For each training system and variety, 100 grapevine leaves were screened and rated for disease symptoms of the particular fungal pathogen. The score ranged from 0% (no symptoms) to 100% (symptoms on the whole leaf) with a scaling interval of 10%. Additionally, a scoring of 5% was added to the ranking for the assessment of minimal symptoms. Data were analysed using R [26]. We fitted a linear mixed model with "grapevine variety" as random and "pruning method" and "plant protection intensity" as fixed effects using the nlme package and the multcomp package for multiple comparisons [27,28].

3. Results

Typhlodromus pyri (Scheuten) was by far the most common predatory mite species in our sample with 89%, followed by *Paraseiulus soleiger* (Ribaga) (10%) and *Euseius finlandicus* (Oudemans) (1%).

3.1. Effects of Reduced Plant Protection

In both years, reduced plant protection increased the density of predatory mites significantly (Figure 1; 2015: $t_{18} = -3.80$, $p = 0.0013$, 2016: $t_{18} = -2.39$, $p = 0.028$). A post hoc Tukey test showed that in 2015 both lower plant protection treatments differed significantly from the high plant protection treatment at $p < 0.05$. In 2016, the same test revealed significant differences only between 2 and 12 treatments ($p < 0.05$). The difference between the treatments was more pronounced in 2015 than in 2016. In 2015, there were 81% more predatory mites under reduced plant protection than under full plant protection. In 2016, this difference was 34%.

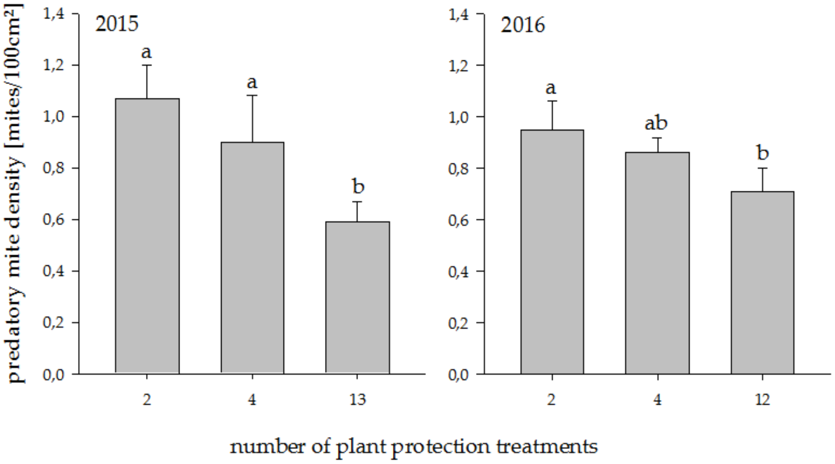

Figure 1. Predatory mite density in plots with increasing plant protection intensities in the years 2015 and 2016. Different letters indicate significant differences between groups ($p < 0.05$). Values represent means + standard error (N = 24).

The densities of the pest mites *Co. vitis* and *Ca. vitis* were low in both years. Plant protection intensity had no significant effect on either pest mite species (Figure 2; 2015: *Co. vitis:* $t_{18} = -0.51$, $p = 0.61$ *Ca. vitis*: $t_{18} = -0.84$, $p = 0.41$, 2016: *Co. vitis:* $t_{18} = -1.39$, $p = 0.18$, *Ca. vitis*: $t_{18} = -1.28$, $p = 0.22$).

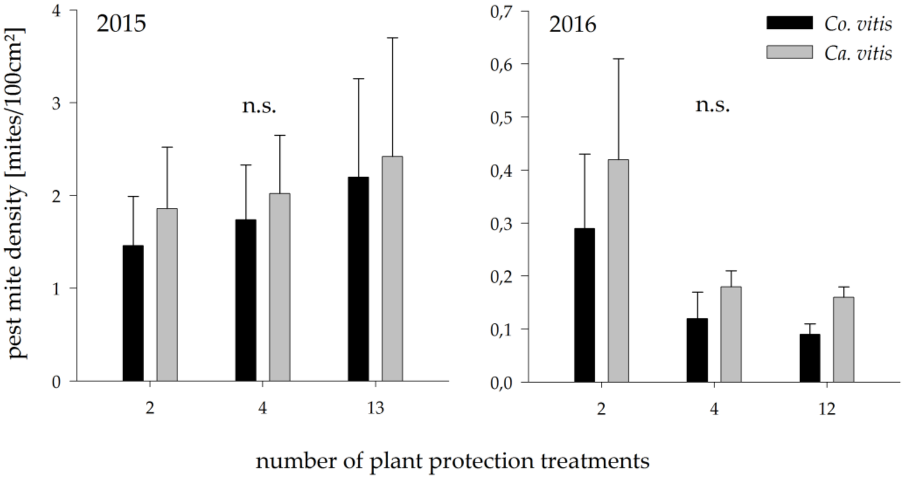

Figure 2. Pest mite density in plots with increasing plant protection intensities in the years 2015 and 2016. n.s. indicates a non-significant difference between groups ($p > 0.05$). Values represent means + standard error (N = 24). Co. = *Colomerus*; Ca. = *Calepitrimerus*.

3.2. *Effects of Minimal Pruning*

The predatory mite density was almost two times higher in SMPH compared to VSP grapevines in the year 2015. In the year 2016, the difference was less pronounced but still statistically significant (Figure 3; 2015: $t_{18} = -4.70$, $p = 0.0002$, 2016: $t_{18} = -3.07$, $p = 0.007$).

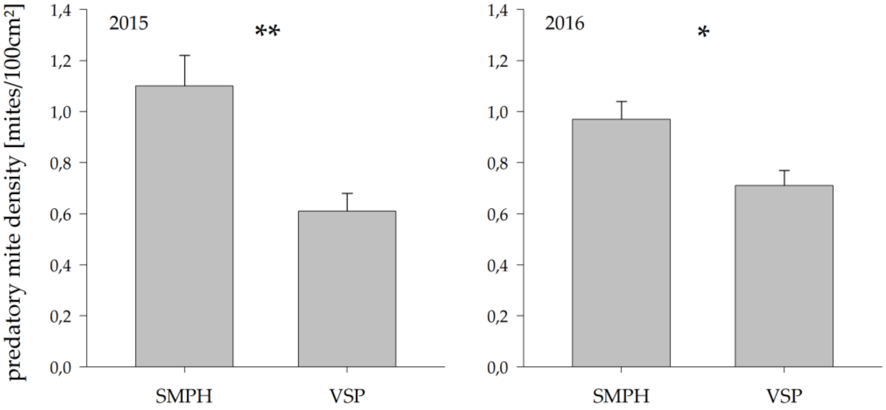

Figure 3. Predatory mite density in minimally pruned versus trellis-trained plots in the years 2015 and 2016. * indicate significant differences between groups (*$p < 0.05$, **$p < 0.005$). Values represent means + standard errors (N = 24). SMPH: semi-minimal pruned hedge, VSP: vertical shoot positioning.

Both pruning systems harbored only low numbers of both observed pest mite species and there was no significant effect of minimal pruning on their density (Figure 4; 2015: *Co. vitis:* t_{18} = 1.28, $p = 0.21$ *Ca. vitis*: t_{18} = 1.50, $p = 0.15$, 2016: *Co. vitis:* t_{18} = −1.69, $p = 0.11$, *Ca. vitis*: t_{18} = −1.07, $p =$ 0.30).

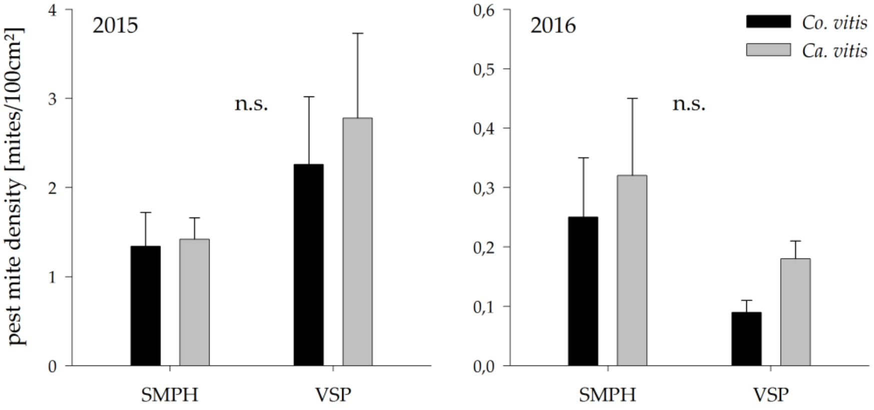

Figure 4. Pest mite density in minimally pruned versus trellis-trained plots in the years 2015 and 2016. n.s. indicates a non-significant difference between groups ($p > 0.05$). Values represent means + standard errors (N = 24). SMPH: semi-minimal pruned hedge, VSP: vertical shoot positioning.

3.3. Fungal Disease

We found no infection by *Botrytis cinerea* (botrytis bunch rot) or *Erysiphe necator* (powdery mildew) on grapevine leaves in any of the treatments. There was also no incidence of *Plasmopara viticola (*downy mildew*)* in 2015 (Table 1). In 2016, we found leaves showing symptoms of *P. viticola* infection all over the vineyard. As expected, higher plant protection intensity had a significant negative effect on *P. viticola* incidence rate and infection levels (incidence rate: t_{18} = −5.03, *p* = 0.0001, infection level: t_{18} = −3.05, *p* = 0.007). There was also a significantly higher percentage of symptomatic leaves (incidence rate, t_{18} = 2.07, *p* = 0.05), although the average affected leaf area (infection level, t_{18} = 0.67, *p* = 0.51) was not significantly larger in the SMPH grapevines than in the VSP-trained vines (Table 1).

Table 1. Infection level and incidence rate of *Plasmopara viticola* compared between grapevine leaves under three plant protection intensities and between the pruning methods VSP (vertical shoot positioning) and SMPH (semi-minimal pruned hedge). Significant differences between groups are indicated by different letters.

Year	**Measure for Fungal Disease**	**Number of Plant Protection Treatments**			**Pruning Method**	
		2	4	12/13	VSP	SMPH
2015	infection level ± SE [%]	0	0	0	0	0
	incidence rate ± SE [%]	0	0	0	0	0
2016	infection level ± SE [%]	11.8 ± 2.9 ^{a}	5.0 ± 1.2 ^{b}	2.5 ± 1.4 ^{b}	5.7 ± 1.4	7.1 ± 2.4
	incidence rate ± SE [%]	65.0 ± 7.2 ^{a}	49.1 ± 6.6 ^{b}	31.8 ± 7.0 ^{c}	43.4 ± 8.0 ^{a}	53.9 ± 5.1 ^{b}

4. Discussion

Minimal pruning and reduced frequency of fungicide sprays enhanced predatory mite abundance in our grape vineyard during both years of the trial. In contrast, densities of pest mites in vineyards under innovative management were generally very low and did not differ significantly between the three fungicide treatment intensities or the two pruning systems. In Europe, both *Ca. vitis* and *Co. vitis* infestations rarely exceed the economic threshold [7]. This is in a large part due to the presence and the active protection of predatory mites such as phytoseiids. Phytoseiid numbers decrease significantly with increasing frequencies and amounts of plant protection applications and there were significantly more mites per leaf area in SMPH-pruned than in VSP-pruned grapevines. The susceptibility of phytoseiid mites to pesticides and fungicides is well described in the literature. For example, Schruft, Wohlfarth and Wegner [15] describe a negative effect on population numbers of *T. pyri* by fungicides containing copper, especially when applied late in the season. They also found a negative influence of sulphur sprayings, but argue that they are not harmful for the population since the effects persisted less than four weeks after the last application. Hanna, et al. [29] confirm reduced numbers of *Metaseiulus occidentalis* (Nesbitt) in a vineyard near Madera that was treated with sulfur compared to triadimefon. Hoffmann [17] found that copper and sulfur, in combination, damage up to 80% of the *T. pyri* population when applied frequently in low concentrations as is common practice in organic viticulture.

The negative impact of a combination of sulfur and copper treatments on predatory mite populations in the current study agrees with these earlier studies. Pest mite population numbers were very low in general and there was no significant effect of either of the two management practices. They also benefit from reduced spraying based on the acaricidal properties of sulfur [30,31]. A possible explanation for the lack of a pattern could be that the higher predation pressure from predatory mites, which are more abundant in the reduced fungicide treatments, negates the positive effect of reduced fungicide input on the pest mite population.

Our second hypothesis can be confirmed as well. There were higher densities of Phytoseiids on SMPH-pruned than on VSP-pruned grapevines. One difference between SMPH-pruned and VSP-pruned grapevines are smaller but more abundant leaves in SMPH, resulting in an overall larger leaf surface [19]. This means that there are not only more predatory mites per leaf, but more predatory mites per SMPH vineyard area, increasing the effect even further.

SMPH grapevines probably offer more favorable living conditions for predatory mites. The larger leaf area and the larger amount of wood and especially old wood surface area may offer a higher potential for population growth, better shelter and more oviposition sites. In cracks and crevices of the old wood, mites can find more and better overwintering opportunities, which allow a fast repopulation of the fresh growth in spring and prevents pest mite outbreaks early in the season. Pozzebon, et al. [32] describe habitat complexity as a major factor in promoting predatory mite populations.

Although fungal material is an important food source for phytoseiids [7,10], these patterns seem to be independent of the infection of leaves with fungal diseases. Both years showed similar patterns for predatory mite and pest mite densities, even though we detected very different levels of fungal infection between the two years. As expected, in the year 2016, the infection level and the incidence level of *P. viticola* were lower in grapevine that received a more intense plant protection regime. This might amplify the direct negative effect of sulfur and copper on phytoseiids by additionally reducing food availability [18]. The slightly higher infection level with *P. viticola* in the SMPH grapevines can probably be explained by the different microclimate within the canopy. It is more humid and less airy, which promotes fungal growth [19]. Since the population densities of mites were similar in 2015 when there was no incidence of *P. viticola* at all, we assume that this factor is likely contributing to overall predatory mite densities, but is probably overruled by mortality through fungicide application.

It is likely that the more structurally complex SMPH grapevines offer a favorable habitat not only to phytoseiids but also to a number of other species, that are also good alternative food sources for predatory mites in the absence or scarcity of pest mites. It will be interesting to see if the reduced number of sprayings and the minimal pruning method can favor additional beneficials and how the balance of other predator–prey relationships is influenced.

5. Conclusions

Reduced fungicide sprayings as well as the more structurally diverse habitat created by minimal pruning both favor beneficial mites, but not pest mites. Thus, minimal pruning of fungus-resistant cultivars improves the conditions for natural pest regulation within the leaf mesofauna of grapevine. Using fungus-resistant cultivars of grapevine and other crops on a larger scale could drastically reduce the frequency of application and thus the impact of plant protection chemicals, not only directly in the crops but also in the surrounding landscape and therefore contribute to the sustainability of agriculture.

Supplementary Materials: The following are available online at www.mdpi.com/link: Table S1: Spraying table.

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Chapter III

Reduced fungicide applications improve insect pest control in grapevine

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Abstract

Agricultural intensification is a major driver of biodiversity decline in many species including arthropods. This may also affect important ecosystem services such as natural pest regulation. Traditional grapevine varieties rely on a high number of fungicide applications, which can be greatly reduced in novel fungus resistant cultivars. Additionally, in contrast to the traditional trellis system, the semi-minimal pruned hedge offers a structurally more diverse habitat for arthropods. We investigated natural control of the grapevine pest *Lobesia botrana* ([Denis & Schiffermüller], 1775) (Lepidoptera: Tortricidae) with artificial inoculation of grape bunches, egg baits, camera surveillance and beatsheet sampling of predators. Reduced fungicide applications enhanced densities of *L. botrana* predators along with increased egg predation and reduced damage of inoculated grape bunches. Minimal pruning did not have a clear effect. Improved pest control potential adds to other advantages of reduced fungicide applications. Planting fungus-resistant varieties should be augmented, potentially also in other crop systems that rely heavily on fungicides.

Keywords

Natural pest control, fungicide, *Lobesia botrana*, minimal pruning, predation, viticulture

1. Introduction

Pesticide use and habitat simplification are main causes for declining biodiversity in agricultural landscapes (Benton et al. 2002; Hallmann et al. 2017). Low levels of biodiversity can compromise ecosystem services such as natural pest control and pollination (Altieri 1999; Benton et al. 2002; Wilby and Thomas 2002; Gurr et al. 2003; Bianchi et al. 2006). In addition to biodiversity loss, heavy use of pesticides can lead to problems such as the development of resistance and environmental pollution including aquatic ecosystems (Geiger et al. 2010; McMahon et al. 2012; Miles et al. 2012; Bundschuh et al. 2016). Viticulture in particular relies on frequent applications of fungicides to protect the commonly grown, highly susceptible grapevine cultivars. These fungicides not only control their target organisms but can also affect the arthropod fauna in the vineyards including pests and their natural enemies (Geiger et al. 2010; Duso et al. 2012; Miles et al. 2012). A reduction in fungicide use requires the cultivation of fungus resistant grapevine varieties, which are considered the most innovative development in viticulture in the last century (Töpfer et al. 2011). They can produce grapes for high quality wine production, while being less susceptible to the two most damaging fungal diseases of grapevine: downy (*Plasmopara viticola (Berk. & Curt. ex de Bary) Berl. & de Toni),* and powdery mildew (*Erysiphe necator* [Schw.] Burr.). Cultivation of these varieties reduces the necessary fungicide usage drastically by up to 85% in some multiresistant varieties (Fischer et al. 2004).

Growing these new varieties as a semi-minimal pruned hedge (SMPH) can further affect health, quantity and quality of the crops compared to vertical shoot positioned (VSP) grapevines (Intrieri et al. 2001; Kraus et al. 2018). Grapevine architecture changes drastically in this cultivation method; the canopy becomes more voluminous and contains more woody branches. Thereby SMPH offers a larger and more heterogeneous habitat for arthropods, including naturally existing predators. Increased amounts of older wood provide additional shelter to the grape mesofauna, resulting in higher predatory mite abundance (Pennington et al. 2017). It is still unclear if this affects the abundance and diversity of pests and other beneficial organisms, although a more complex structure has been shown to enable higher densities of predatory arthropods in both natural and agricultural systems (Langellotto and Denno 2004). Both the pruning system and the reduction of fungicides associated with the cultivation of fungus-resistant varieties might lead to an increased arthropod abundance and biodiversity, and thereby promote dependent ecosystem services. In this study, we focused on the potential for natural pest control.

One of the major grapevine pests in Europe and beyond is *Lobesia botrana* (Denis & Shiffermüller) (Lepidoptera: Tortricidae). The main damage is a quality loss due to *Botrytis cinerea* infections which are often initiated by the feeding of the grapevine moth larvae. In Europe, there are two to four generations of *L. botrana* per year, with two to three generations being common in Germany. The first generation is anthophagous and usually not of economic importance, whereas the following generations are carpophagous and often need to be controlled. Known predators of *L. botrana* are Dermaptera, Hemiptera, Neuroptera, Diptera, and Coleoptera, as well as several families of spiders and mites (Marchesini and Monta 1994). *Lobesia botrana* are also attacked by numerous parasitic hymenoptera, most noticeably by *Trichogramma* species which have been used as a control agent in viticulture with mixed success (Castaneda-Samayoa et al. 1993; Barnay et al. 2001; Hommay et al. 2002; Bagnoli and Lucchi 2006). Several of these natural enemies are susceptible to sulfur and copper, which are commonly applied in organic viticulture against grapevine fungal diseases (Bartlett 1964; Hassan et al. 1998; Thomson et al. 2000; Gent et al. 2009; Moura et al. 2012), which is why we suspect that reducing fungicide applications increase the potential for natural pest control.

The objective of this study was to investigate the separate and combined effects of reduced fungicide input and minimal pruning on natural pest control of *L. botrana* in an experimental vineyard. We tested if the predation pressure on *L. botrana* eggs changes with the number of fungicide applications and with the more heterogeneous canopy structure in SMPH trained grapevines. We also assessed the damage from larval feeding on artificially inoculated grape bunches and checked if it is more or less severe in plots with less fungicide treatments and in plots with SMPH trained grapevines. In both experiments, we expected that (1) less frequent fungicide applications and (2) reduced pruning intensity would enhance predator abundance and therefore the natural suppression of *L. botrana.* This might be caused by direct and indirect effects of fungicides on predatory arthropods, the decreased disturbance by sprayings, or because the more heterogeneous habitat of SMPH vineyards can support a higher diversity of potential predators.

2. Material and methods

2.1 Study site

The experiments took place in an experimental vineyard in Siebeldingen, Germany (N 49°13'13.7", E 8°02'43.0"). The vineyard is planted with four different *Vitis vinifera* cultivars; 'Reberger', 'Villaris', 'Felicia' and 'Gf 84-58-988' resistant against Powdery- (*Erysiphe necator*) and Downy Mildew (*Plasmopara viticola*). Each variety is cultivated in six to ten rows, half of which are VSP and half are SMPH trained. Each of these rows is again divided into three parts which receive a different plant protection regime by using a plot sprayer with tunnel to avoid spray drift to adjacent rows (Tunnel plot sprayer ABS 6/25-TU, Christian Schachtner Fahrzeug- und Gerätebau, Ludwigsburg). Thus, each combination of plant protection intensity and pruning system is replicated four times in the different varieties, resulting in 24 treatment plots. We chose an organic spraying regime consisting of two ('low'), four ('reduced') or twelve/thirteen ('standard') sprayings of Funguran progress® (350g copper per kg (copper hydroxid)), Netzschwefel Stulln (796g sulfur per kg) and VitiSan® (994,9 g potassium bicarbonate per kg) per season (see supplemental material). This allowed us to standardize the amounts of active ingredients in the plant protection products as far as possible. Twelve or thirteen plant protection treatments per year equal an amount and frequency of plant protection which is commonly applied to vineyards planted with traditional, fungus susceptible grape varieties. Reducing the plant protection by as much as 85% while still working with healthy plants is only possible in vineyards that are planted with fungus resistant grapevines.

2.2 Rearing of Lobesia botrana

European grapevine moths were reared in a climate chamber using the method described in Markheiser et al. (2018). The rearing-containers were modified in a way that allows the moths to oviposit on polyethylene strips which can then be used for field experiments (Hoffmann, 2008). Twist-ties are left in the container for 24 hours. The moths distribute their eggs relatively even, resulting in an average number of 47±21 eggs on each twist tie. The egg-laden twist ties were used for experiments on the same day they were removed from the containers (Hoffmann 2008).

2.3 Field experiments

To determine predation pressure, the egg-laden twist-ties were attached to randomly selected inflorescences or bunches and were exposed there for 72 hours. The number of eggs was counted before and after exposition using stereomicroscopes (Zeiss, Jena, Germany). *L. botrana* eggs hatch after three to eleven days (Varela et al. 2010), so that missing eggs are most likely due to predation and not due to hatching. Besides, predated and hatched eggs can be distinguished by the leftover egg shell, since hatching larvae emerge from the side of the eggs, leaving pieces of the shell behind. In case of predation, the egg is usually removed in its entirety. We stored the eggs that remained on the twist-ties in a climate chamber at 70% rh and 21 °C for two weeks to check for *Trichogramma* parasitism, but could not find any parsitised eggs. This part of the experiment was performed in July and August 2015 and June, July, August and September 2016.

In addition to the egg predation experiment, we performed an infestation experiment of grapes in the susceptible development stage. We inoculated four bunches in each of the 24 blocks with an egg-laden zip tie as described in Hoffmann (2008). The infested bunches were harvested after 21 days of exposure and grape berry moth damage was assessed by counting the percentage of damaged berries using stereomicroscopes (Zeiss, Jena, Germany). *L. botrana* feeding sites are easily recognized. Infested clusters contain feces, shriveled or sometimes completely excavated berries, as well as webbing. Dark spots surround the feeding position in a berry, and *L. botrana* larvae can often be found in the bunches. This part of the experiment was repeated three times between July and August 2015.

To determine the most important egg predators of *L. botrana* in our experimental vineyard, we used camera surveillance of egg baits. We built a camera system using a raspberry pi (Raspberry Pi Foundation, UK) and a camera module without IR filter combined with two IR-LEDs (Kuman, Glendora, CA, USA). The camera system was run with an Anker Powercore with 20100 mAH (Anker Technology Co., Seattle, WA, USA) which powered the camera for 24 hrs. The cameras were placed in plastic containers (Lock & Lock, Seocho-Dong Seocho-Gu, Seoul, South Korea) to protect them from moisture and mounted on a tripod (Manfrotto, Cassola, Italy). We used the same egg bait as described for the egg predation experiment and observed them using cameras to see which predators were feeding on the eggs. The cameras were programmed to take a picture every 10 seconds for 24 hours. We observed twelve bait strips with twelve cameras in different locations of the vineyard at the same time. This was repeated 6 times between June and August of 2017. The pictures were analyzed using the open source program VirtualDubMod 1.5.10.3. Since we could only observe a total of eleven egg predation events (three times by Formicidae, 6 times by Dermaptera and twice by Chrysopidae) we refrained from analyzing this data any further due to the small sample size. We plan to continue using this method and present a more comprehensive dataset in the future. Arthropod fauna in the vineyard was sampled using a beat sheet technique. Arthropods were dislodged from the plants by shaking them onto a circular cloth with 1.0 m diameter, from where they were collected in 70% EtOH. Ten vines in each treatment were shaken for 5 seconds and the collected arthropods from those ten vines were combined into one sample. We only counted those predators that we could confirm to be preying on *L. botrana* eggs in the vineyard from our camera surveillance; ants (Formicidae), earwigs (Dermaptera) and green lacewings (Chrysopidae).

2.4 Data analysis

As the results were largely consistent across years, data from each experimental plot were combined across sampling times and years to increase sample size. Data were analysed using R version 3.0.2 (R Development Core Team, 2014). We fitted linear mixed models with 'grapevine variety' as random and 'pruning method' and 'plant protection intensity' as fixed factors and tested for main and interactive effects using the nlme package (Pinheiro et al. 2016). Tukey’s all-pairwise differences among effects of the three plant protection intensities were calculated using the function glht in the multcomp package (Hothorn et al. 2008). Predator abundance was analysed using the same models.

3. Results

3.1 Effects of reduced fungicide applications

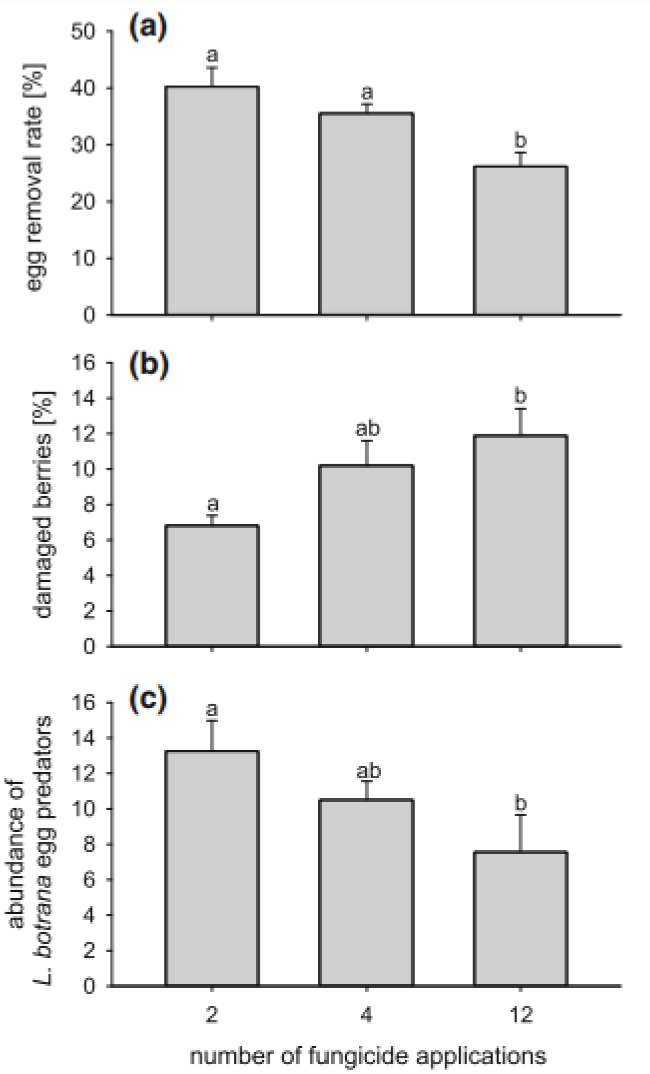

Fig. 1. Effects of the number of fungicide applications on (a) removal rates [%] of *L. botrana* eggs, (b) feeding damage on grape bunches that were artificially inoculated with *L. botrana* eggs, and (c) abundance of *L. botrana* egg predators per 10 vines. Different letters indicate significant differences between groups ($p<0.05$). Values represent means ± standard error (N=24).

No significant interactive effects on egg removal rates were found between minimal pruning and plant protection intensity. Natural pest control potential as estimated by the percentage of removed *L. botrana* eggs was highest in plots with the least fungicide applications. With increasing numbers of applications, the number of removed eggs decreased significantly ($t_{17}=-4.99$ $p=0.0001$; Fig. 1a). The number of berries damaged by *L. botrana* feeding in artificially inoculated bunches also decreased with increasing amounts of applied fungicides ($t_{18}=2.6$, $p=0.02$; Fig. 1b). The number of damaged berries was about 40% higher in artificially inoculated bunches in plots with 12 treatments compared to only two plant protection treatments (Fig. 1b).

The number of *L. botrana* predators decreased significantly in plots with more frequent fungicide applications (t_{18}=-2.44, p=0.025; Fig. 1c). We could not find a significant effect of fungicide applications on the abundance of Dermaptera (t_{18}=-0.34, p=0.74, Tab.1) and Chrysopidae (t_{18}=-0.93, p=0.36, Tab 1). The number of Formicidae was significantly reduced by more fungicide applications (t_{18}=-2.68, p=0.02). A post hoc test revealed that there is a significant difference in ant numbers only between plots with 12 fungicide treatments and those with 2 treatments (z_{18}=-2.9, p=0.01; Tab. 1).

3.2 Effects of minimal pruning

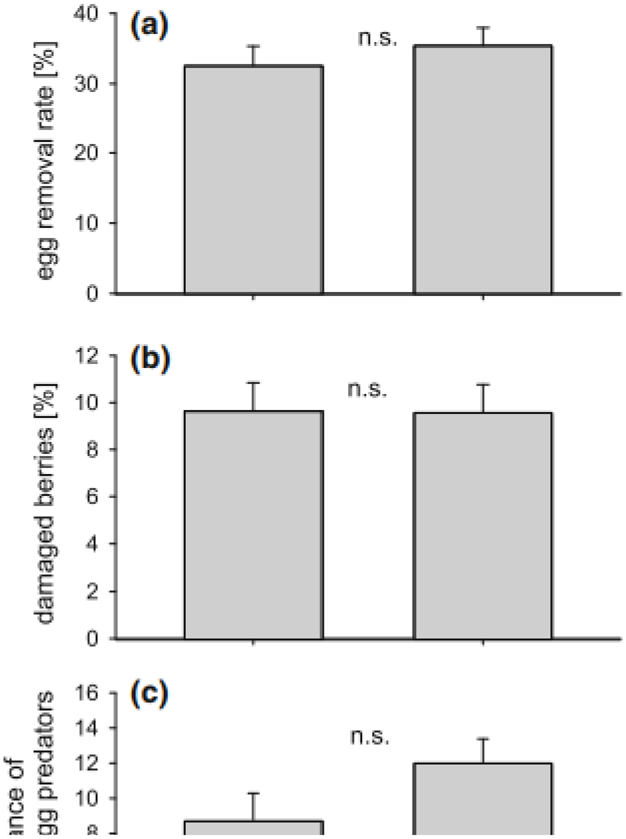

Fig. 2 Differences between a semi-minimal pruned hedge (SMPH) and vertical shoot positioning (VSP) on (a) removal rates [%] of L. botrana eggs, (b) feeding damage on grape bunches that were artificially inoculated with L. botrana eggs, and (c) abundance of L. botrana egg predators per 10 vines. n.s. indicates a non-significant difference between groups (p>0.05). Values represent means ± standard error (N=24)

Minimal pruning had no significant effects on the removal rate of *L. botrana* eggs (t_{18}=1.25, p=0.23; Fig. 2a). Feeding damage on inoculated bunches was not significantly different between the two pruning methods (t_{18}=-0.05, p=0.96; Fig. 2b). There was no interactive effect between plant protection intensity and pruning effects. Pruning did not cause a significant change in predator abundance, although there was a trend towards a higher predator abundance in the VSP system (t_{18}= 1.83, p=0.08; Fig. 2c). This can be explained by the significantly higher abundance of earwigs in the VSP compared to the SMPH system (z_{18}=4.78, p=0.001). Ant and green lacewing abundances show no significant reactions to the different pruning systems (Formicidae: t_{18}= 0.45, p=0.66, Chrysopidae: t_{18}= 1.46, p=0.16, Tab. 1).

Tab.1. Abundance of *L. botrana* egg predator groups in grapevine at three plant protection intensities; 2, 4 and 12 treatments over the vegetation period and grown as a semi-minimal pruned hedge (SMPH) or using vertical shoot positioning (VSP). Values represent the mean abundance of predators on 10 vines ± SE. Different letters indicate significant ($p<0.05$) differences in the abundance of one predator group between different plant protection intensities or pruning methods.

predator group	plant protection intensity			pruning method	
	2	4	12	SMPH	VSP
Formicidae	9.6 ± 1.9 ^{a}	5.7 ± 1.2 ^{ab}	3.7 ± 1.1 ^{b}	6.5 ± 1.6 ^{a}	6.2 ± 1.1 ^{a}
Dermaptera	3.0 ± 0.9 ^{a}	3.9 ± 0.7 ^{a}	3.4 ± 1.0 ^{a}	1.8 ± 0.3 ^{a}	5.1 ± 0.6 ^{b}
Chrysopidae	0.6 ± 0.2 ^{a}	0.9 ± 0.2 ^{a}	0.5 ± 0.2 ^{a}	0.5 ± 0.2 ^{a}	0.8 ± 0.2 ^{a}

4. **Discussion**

4.1 Effects of reduced plant protection

Reduced numbers of fungicide sprayings increased the removal of *L. botrana* eggs and reduced feeding damage to grape berries, indicating improved natural pest control. These effects are likely due to a higher abundance and efficiency of predators feeding on *L. botrana* eggs and larvae. Intensive pesticide applications can be detrimental to abundance as well as biodiversity of arthropod communities in agroecosystems. This negatively impacts the potential for natural pest control (Geiger et al. 2010; Duso et al. 2012). Our study shows that not only insecticides, but also fungicides used in organic viticulture can reduce the natural control of an important pest insect. In addition to direct lethal effects of sulfur and copper (Mansour 1987; Thomson et al. 2000; Childers et al. 2001; Prischmann et al. 2006), they can also have sublethal effects on predators which influence their physiology, fecundity and behavior, making them overall less efficient in their ability to control pests (Beers et al. 2009). Increased mechanical disturbance through the sprayings can also play a role in the decrease of functional biodiversity (Bruggisser et al. 2010). The spraying regime we used in this trial may have affected the behavior, activity, diversity or abundance of relevant predator species. Ant abundance was significantly reduced in plots with more frequent fungicide applications. Ants are highly effective predators in many ecosystems worldwide, including agricultural systems (Way and Khoo 1992). Our camera surveillance showed them to be very efficient predators of *L. botrana* eggs as well. While sulphur is known to have negative effects on beneficial arthropods such as predatory mites and *Trichogramma spp*, (Pennington et al. 2017, Thomson et al. 2000), there is little information about the effects of fungicides on ants. Olotu et al. (2013) found no detrimental effects of Sulphur sprayings on African weaver ant, *Oecophylla longinoda* (Hymenoptera: Formicidae). The susceptibility of leaf-cutting ant colonies to fungicides is based on their mutualistic relationship with a fungus and there is no evidence that fungicides are harmful to the ants themselves (Herz et al. 2008). Migula and Głowacka (1996) and Diehl et al. (2004) suggest that copper may have negative effects on ant populations. Increased disturbance and compression of the soil by the heavy machinery used for spraying might also explain decreased numbers of ants in plots with more frequent fungicide applications. In our experiment this was probably not the cause for the different ant abundances, since all rows were trafficked equally.

Our results are specific for the active ingredients Copper and Sulphur and cannot necessarily be transferred to plant protection regimes based on synthetic fungicides, which can have a variety of active ingredients and modes of action. However, synthetic fungicides often affect non-target organisms (Desneux et al. 2007; McArt et al. 2017), thus benefits for biodiversity and natural pest control can also be expected for reduced application of conventional fungicides.

4.2 Effects of minimal pruning

In contrast to plant protection intensity, no significant effect of pruning system on natural pest control or the abundance of predators was found. This contrasts with the positive effect of minimal pruning on predatory mites in the same system (Pennington et al. 2017). For the predators studied here, there was even a trend in the reverse direction of higher densities in the VSP plots than in the minimally pruned grapevines. While ant abundance was not impacted by minimal pruning, earwigs likely benefit from the more compact grape clusters in VSP grapevine since they use them for breeding and as shelter (Huth et al. 2009). This probably makes them particularly effective as predators of grape berry moth since their primary habitat and the egg-laying site of *L. botrana* overlap. Although earwigs are often seen as pests in viticulture, it was shown that they only cause quality loss in numbers that are far higher than what we have found (Kehrli et al. 2012). The fact that we could not find significant differences between the two pruning systems regarding predation on *L. botrana* might have several causes. Although Langellotto and Denno (2004) suggest that increasing habitat complexity will promote predators and increase their effectiveness as pest control agents, it might become more difficult for predators to find the egg bait due to the more complex structure of the canopy. Lukianchuk and Smith (1997) showed that *Trichogramma minutus* for example is not very efficient in finding host egg clusters in complex foliage. Andow and Prokrym (1990) showed with a simple paper model that structural complexity is negatively correlated with host finding by *Trichogramma nubilale*. The higher volume of the canopy could lead to a dilution effect so that predators have to search for similar numbers of prey animals in a larger ar(Andow and Prokrym 1990)ea. It is also possible that the number of predators caught in SMPH grapevine is lowered for methodological reasons. The SMPH canopy is more dense and dislodged arthropods may not fall all the way onto the sampling sheet but hold on to a plant structure instead. The lack of significant effects of minimal pruning on natural pest control of *Lobesia botrana* means that neither synergies nor interference between these two innovations in viticulture were observed. Thus, our findings suggest that minimal pruning is compatible with the cultivation of fungus-resistant cultivars and that the benefits of reduced pesticide applications are present in both pruning systems.

4.3 Other potential benefits of reduced plant protection

In addition to the improved biocontrol potential against *L. botrana*, reduced fungicide spraying likely has further benefits. Besides the economic advantages of reduced pesticide applications itself (cost of products and labor for application), biodiversity in general and functional soil biodiversity in particular may benefit from reduced fungicide applications.

Accumulation of copper in vineyard soils is a peculiar issue that questions the sustainability of viticulture (Eijsackers et al. 2005; Komárek et al. 2010; Ruyters et al. 2013). High copper concentrations can damage earthworms and ground vegetation and thus have a negative impact on wine production. Earthworms and other soil fauna are crucial for nutrient cycling and bioturbation in vineyards and other agricultural systems(Eijsackers et al. 2005; Lemtiri et al. 2014). Ground vegetation binds nutrients outside the growth period of the vines and protects the soil from compaction, especially under the increased use of harvesting machines. The benefits of reduced chemical inputs on the soil flora and fauna may be further amplified by a reduction in machine traffic under a reduced fungicide application regime.

Biodiversity in agricultural systems is of particular importance, not just for the ecosystem functions it provides but also for conservation. In Europe, vineyards host many rare species because of their location in especially warm and dry climate (Isaia et al. 2006), notwithstanding that viticulture is the most fungicide consuming culture in Europe (Wightwick et al. 2010). Minimization of fungicide use can be achieved by using decision support systems, low impact fungicides and by planting mildew resistant grape varieties (Pertot et al. 2017). Overall, our results show that in addition to other known benefits, the cultivation of fungus-resistant grape varieties promotes predator abundance and improves conditions for natural control of the grape berry moth. Further investigations are necessary to assess to which other vineyard pests this applies. A wider cultivation of fungus-resistant cultivars is currently limited by consumer demand (Gary et al. 2010). Communicating the biocontrol benefits alongside other advantages of fungus-resistant cultivars of grapevine and potentially also of other fungicide-intensive crops such as potato and apple to the public may help to improve consumer acceptance, and to provide incentives to growers to meet the demand for sustainable intensification of agriculture

(Bommarco et al. 2013). Reducing fungicide use should be paramount in establishing environmentally friendly, healthy and highly productive agroecosystems.

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Chapter IV

Effects of canopy architecture and microclimate on grapevine health in two training systems

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Abstract

Semi minimal pruned hedge (SMPH) is a time and cost saving grapevine training system, which is becoming more and more popular in German viticulture. In this study we compared the canopy architecture and its effect on the microclimate of SMPH trained grapevines with those of plants trained in the vertical shoot positioning (VSP). We detected a 3% points higher humidity and a 0.9°C lower mean temperature within the complex canopy architecture of SMPH trained vines compared to VSP. Moreover, we investigated the influence of the differing microclimate, canopy and bunch architecture, as well as berry skin characteristics of the two training systems on the incidence of the major fungal grapevine diseases Downy Mildew, Powdery Mildew and Botrytis Bunch Rot, as well as on the occurrence and damage of the invasive insect pest *Drosophila suzukii*. We demonstrate that SMPH trained vines can be more susceptible to Downy Mildew and Powdery Mildew than VSP trained vines. The incidence of Botrytis Bunch Rot can be higher in the latter system, even if berry skin characteristics are the same in both training systems. We trapped a higher number of *D. suzukii* in SMPH canopies, however no increased berry damage was observed. Based on our results we recommend a more adapted plant protection regime for SMPH trained vines due to their higher susceptibility to the major fungal diseases. Furthermore, we propose a combination of SMPH and fungal resistant grapevine cultivars, e.g. 'Reberger', to achieve a more competitive, environmentally friendly and high quality grapevine production.

Keywords

Training system, plant architecture, Powdery Mildew, Downy Mildew, Drosophila suzukii, Vitis vinifera ssp. vinifera, PIWI, viticulture

1. Introduction

Traditionally, grapevine in Germany is cultivated in the vertical shoot positioning (VSP) system, which is typical for cool climates. This type of grapevine training enables farmers to manage grape yield and quality by controlling the number of buds and their optimal distribution in the trellis (Jackson, 1996). However, the farmer has to undertake a time consuming winter pruning and wire positioning during the season, which causes high labor costs (Clingeleffer, 1993). In order to reduce the costs of manual labor, a novel training method called semi minimal pruned hedge (SMPH) was introduced. The mechanization of pruning, which is the basis of SMPH, in combination with the omission of wire positioning, reduces labor costs to a minimum (Clingeleffer, 1993). This makes SMPH a highly efficient and competitive grapevine production system, which is easily applicable by grapevine growers.

Cultivation of grapevines in SMPH affects plant physiology and as a consequence plant morphology. Compared to VSP, bunches of SMPH trained plants weigh less and have a more loose architecture, due to the fact that they consist of fewer and smaller berries (Intrieri *et al.* 2011). Despite smaller bunches, the number of inflorescences and bunches per plant is elevated in minimal pruned grapevines and thus the yield per plant is enhanced in contrast to the traditional training system (Clingeleffer and Possingham, 1987; Wolf *et al.*, 2003). The average leaf size in SMPH is smaller than in VSP vines (Sommer *et al.*, 1993), but the total leaf number per vine and hence the total leaf area (m^2/m of row) is higher if the grapevines are minimally pruned (Clingeleffer and Posingham, 1987; Schmid and Schultz, 2000; Intrieri *et al.* 2001). We expect that these vast differences in canopy architecture between SMPH and VSP affect the grapevine microclimate. Because of the increased leaf volume SMPH canopies should show poor air movement and less light penetration. We therefore expect a lower temperature and a higher humidity in SMPH canopies than in the less voluminous VSP canopies.

European grapevine, *Vitis vinifera*, is threatened by several pests. Fungal diseases such as Downy Mildew (DM, caused by *Plasmopara viticola*), Powdery Mildew (PM, caused by *Erysiphe necator*) and Botrytis Bunch Rot (BR, caused by *Botrytis cinerea*) are the most destructive. Their development and spreading in the vineyard can be influenced by canopy management. Since disease progress of DM and BR is facilitated by a warm and moist climate, training systems which increase air movement and light penetration are beneficial for controlling these pathogens in the vineyard (Coombe and Dry, 1992). Canopy management during the season such as leaf removal in the bunch zone can additionally reduce wetness and improve light penetration, creating an environment which is less favorable for PM and BR (Gubler *et al.* 1987; Austin and Wilcox, 2011). In addition, characteristics of the berry skin, e.g. thickness of the berry skin and of the cuticle, are described as further important traits influencing susceptibility against BR (Commenil *et al.* 1997; Gabler *et al.* 2003; Becker and Knoche 2012a,b; Herzog *et al.*, 2015).

We expect a higher incidence of DM in SMPH because of the elevated humidity and reduced light penetration in the canopy compared to VSP. Concerning *Botrytis* we assume a decreased rate of BR in SMPH panels as result of the loose bunch architecture. Furthermore we expect the incidence of PM to be elevated in SMPH panels, due to the more favorable microclimate and reduced light penetration (Gadoury *et al.*, 2012).

Drosophila suzukii (Matsumura, Diptera: Drosophilidae), also known as spotted wing drosophila (SWD) is a pest insect native to Asia which has recently spread to the Americas and Europe (Cini *et al*. 2012). In contrast to the common fruit fly *Drosophila melanogaster* which is attracted by overripe or rotten fruit, SWD prefers ripening or ripe red fruit. It may penetrate intact fruit skin with its serrated ovipositor and deposit eggs inside the fruit (Lee *et al.*, 2011). However, laboratory experiments revealed that artificially damaged berries are more attractive for the fly than intact ones (Ioriatti *et al.* 2015; Jarausch *et al.* 2017). SWD damage can be both direct through larval feeding and indirect, since oviposition leaves the fruit skin damaged and susceptible to secondary pathogens such as bacteria and fungi (Ioriatti *et al.*, 2015). SWD has a wide range of host plants including blueberries, strawberries, cherries, and plums, as well as grapevine (Rouzes *et al.*, 2012; Bellamy *et al.*, 2013). Since SWD prefers humid conditions on a large as well as on a smaller scale (Hauser *et al.*, 2009; Tochen *et al.*, 2016) we hypothesize that more flies can be trapped in SMPH panels with their more voluminous canopy than in VSP. As a result of the higher density of SWD we expect a higher infestation rate of grapes in SMPH than in VSP trained grapevines. In addition to the microclimate, the characteristics of the grape skin might further influence the damage by SWD on berries. Thicker and more resilient skin might reduce the egg laying success of SWD females in SMPH trained grapevines (Ioratti *et al.*, 2015).

2. Material and Methods

2.1. Plant material and cultivation practices

For this study the *Vitis vinifera ssp. vinifera* cultivars 'Chardonnay' (planted 2008) and 'Reberger' (planted 2001) were used. Vines were planted at the experimental vineyards of Geilweilerhof located at Siebeldingen, Germany (N 49°21.747, E 8°04.678). Since 2013, half of the rows of each cultivar were pruned mechanically and thereby converted to the SMPH system. Inter-row distance is 2 m and grapevine spacing is 1 m. For pest control plants were treated with an organic plant protection regime consisting of wettable sulphur (AgroStulln, Stulln, Germany), Funguran progress (Spiess-Urania, Hamburg, Germany) and Vitisan (Biofa, Münsingen, Germany). Pesticides were applied fortnightly, 12 times during the season. In 2017 conventional pesticides (Polyram WG, Enervin, Vivando; BASF SE, Ludwigshafen, Germany) were used in the first three plant protection applications, because of the severe plant damage caused by *P. viticola* und *E. necator* in the previous year. After flowering both panels, SMPH and VSP, were pruned mechanically.

All experiments and measurements were performed during the growing season 2016 and 2017. Phenological development of grapevines was determined using the BBCH scale according to Lorenz *et al.* (1995).

2.2. Canopy architecture

To compare the canopy architecture six main characters were chosen and analyzed for each cultivar and training system: (1) the number and distribution of shoots at bud burst (BBCH 10) was evaluated in four random 50 cm wide canopy sections, divided into five horizontal zones (Fig. S1); (2) based on this scheme the number and distribution of inflorescences/bunches at the phenological stages flowering (BBCH 65), pea size (BBCH 75) and veraison (BBCH 81) was determined; (3) for calculation of the leaf area index (LAI) all leaves from four random 50 cm wide canopy sections were removed and measured with a leaf area meter (Modell 3100 area meter, Li-COR, Lincoln, Nebrasca, USA); (4) fifty randomly selected leaves were measured to calculate the average leaf size; (5) the canopy volume was calculated as the product of canopy height [m] x canopy width [m] x 10.000 m^2, divided by the inter-row distance [m] (Siegfried and Sacchelli, 2005). LAI, average leaf size, and canopy volume were also determined at flowering (BBCH 65), pea size (BBCH 75) and veraison (BBCH 81); (6) weight [g], length [cm] and width [cm] of ten randomly selected bunches were recorded as indicator for bunch architecture. Additionally, average berry number and size [mm] of 10 berries per bunch was measured. Bunch architecture was evaluated at ripening (BBCH 89). Data were analyzed using t tests in R (R Core Team, 2013).

2.3. Berry skin characteristics

Physical and morphological berry skin characteristics were determined of cv. 'Reberger' (VSP and SMPH) at ripening stage (BBCH 89) and before harvest.

First, impedance of the berry cuticle was measured at room temperature by using the I-Sensor from 30 berries per training system, 17% Brix and relative impedance Z_{rel} was calculated according to Herzog *et al*. (2015).

The TA.XT Texture analyzer (Stable Micro System, Godalming, Surrey, UK) was used to evaluate the penetration resistance of berries by mean of maximum break force [N] and skin break energy [mJ]. Settings were used according to Letaief *et al.* (2008). For each training system 50 berries were randomly harvested. Results were recorded with software Exponent Lite Express (Stable Micro System, Godalming, Surrey, UK) results were recorded.

The thickness of berry skin was measured using light microscopy in order to detect morphological differences between SMPH and VSP berries. Skin sections of 20 frozen berries were cut from the side and sliced into 6 – 8 µm thick discs with a cryomicrotome (Micro HM 525, Thermo Scientific, Waltham, Massachusetts, USA). 15 skin slices per berry were then fixed on a protein glycerol coated object plate and stained in an Astra Blue solution. Using Leica Application Suite 4.3 and a Leica DM 4000 B light microscope (Leica Microsystems GmbH, Wetzlar, Germany) under 100-fold magnification, the thickness of the berry skins was determined. All means were compared using t tests in R (R Core Team, 2013).

2.4. Microclimate

Temperature and relative humidity in the grapevine canopy were recorded with Tinytag Plus 2 data loggers (Gemini Data Logger Ltd, Chichester, UK). Three loggers per training system and variety were positioned 150 cm above ground in the canopy at random locations in the vineyard. Microclimate measurements were started when three leaves were visible (BBCH 13) and ended by the time of ripening (BBCH 89) with a recording interval of 1 h. For adjustment and reading of the loggers as well as for data evaluation the Tinytag Explorer Software (Gemini Data Logger Ltd) was used. Local climate data including mean temperature, total rainfall and leaf wetness were obtained from the institute DLR Rhineland-Palatinate (www.am.rlp.de). For statistical evaluation of the mean values a permutation test with the program R was performed (R Core Team, 2013).

2.5. Assessment of fungal diseases

Monitoring of fungal grapevine diseases was done according to the European and Mediterranean Plant Protection Organization (EPPO) guidelines: *Plasmopara viticola* (PP 1/31(3)), *Erysiphe necator* (PP 1/4(4)), *Botrytis cinerea* (PP 1/17(3)). For each training system and variety 100 grape bunches were screened and rated for disease symptoms of the particular fungal pathogen. The score ranged from 0% (no symptoms) to 100% (symptoms on the whole bunch) with a scaling interval of 10%. Additionally, a scoring of 5% was added to the ranking for the assessment of minimal symptoms. With this method we determined both incidence rate and level. For statistical evaluation Fisher´s exact test for the incidence rate and Kruskal-Wallis test for the incidence level was performed with the program R (R Core team 2013).

2.6. Spotted wing drosophila (SWD)

2.6.1. Trap design and evaluation

During the season the occurrence of SWD in the two training systems was evaluated from BBCH 83 to BBCH 89 in the variety 'Reberger'. SWD appears almost exclusively on red varieties, which is why the white 'Chardonnay' variety was not sampled for this experiment (Saguez *et al.* 2013). Three traps per training system were randomly distributed in the canopy and analyzed weekly for four weeks. A trap consisted of a 500 mL clear plastic drinking vessel with lid. The vessel was manipulated in the upper third on one side by affixing a red tape and drilling 15 holes with a diameter of 1 mm into it. As trapping liquid we used 100 mL of a 1:1 mixture of water and unfiltered cider vinegar plus a drop of wetting agent (Tween® 20, Sigma-Aldrich, Munich, Germany). *Drosophila suzukii* flies were counted through a stereomicroscope (Zeiss, Jena, Germany). Statistical analysis was done using t test in R (Core team, 2013).

2.6.2. Berry infestation rate

Between BBCH 83 to 89, 50 healthy berries per training system were harvested weekly from random vines and different bunches within the 'Reberger' variety to evaluate the infestation rate. Oviposition of SWD was observed under a stereomicroscope (Zeiss) and the number of eggs per 50 berries was counted. Data was analyzed using t tests in R (R Core team, 2013).

3. Results

3.1. Canopy architecture

In Table 1 all investigated characteristics of the canopy architecture for 2016 (a) and 2017 (b) are listed (the complete Table including a comparison of the different trellis zones can be seen in Table S1). The number of shoots per 0.5 m was significantly higher in SMPH panels than in VSP panels in both years: Eight to 15 times for 'Chardonnay' and six to eleven times for 'Reberger'. We also noticed differences in shoot distribution between the two training systems. While the majority of the shoots were found in the upper zones (Tab. S1; 3-5) in the SMPH trellis, shoots in the VSP trellis were almost completely restricted to the lower zones (Tab. S1; 1-2) and virtually equally distributed.

A similar result was observed for the number of inflorescences/bunches per 0.5m. In 2016 and 2017 at BBCH 65 the total amount of inflorescences in the 'Chardonnay' field was two to four times higher for SMPH compared to VSP and three to six times higher in the 'Reberger' field. The majority of SMPH inflorescences/bunches were located in the higher zones (Tab. S1; 4 and 5). In the VSP panel the inflorescences/bunches were most frequently found in zone two and three.

For 'Chardonnay' the LAI and the canopy volume were at least 1.5 times higher in the SMPH than in the VSP panel during the complete seasons of both experimental years, except for BBCH 75 in 2017 (Tab. 1). In the 'Reberger' variety both parameters showed significant differences between the training systems the entire season of 2017. In 'Chardonnay', leaves of VSP plants were 1.5 times bigger than leaves from SMPH plants at all phenological stages. For 'Reberger' this was only the case at BBCH 75 in 2016 and at BBCH 65 in 2017.

Regarding bunch architecture, all investigated characteristics, weight, length, width, number of berries and mean berry size, were significantly higher for VSP bunches compared to SMPH bunches in both years and cultivars, except for ‘Reberger’ in 2016. For ‘Chardonnay’ the differences in bunch architecture between the two training systems are clearer in 2017 than in the previous year.

Tab. 1: Canopy architecture characteristics of the two grapevine varieties ‘Chardonnay’ and ‘Reberger’ as a function of training system, 2016 (a) and 2017 (b). T test; **P<0.05*, ***P<0.001*.

a)

2016		Chardonnay			Reberger		
	BBCH	SMPH	VSP		SMPH	VSP	
Number of shoots [per 0.5m]	10	68.3 ± 9.6	8.3 ± 1.3	**	44.4 ± 6.9	6.5 ± 1.8	**
Number of inflorescences/bunches [per 0.5m]	65	40.0 ± 5.9	9.0 ± 0.8	**	30.0 ± 7.9	9.3 ± 3.1	*
	75	19.0 ± 7.7	9.5 ± 3.7	n.s.	25.0 ± 11.5	7.8 ± 3.1	n.s.
	81	11.5 ± 2.4	7.5 ± 3.7	n.s.	10.3 ± 3.8	9.5 ± 5.8	n.s.
LAI	65	4.7 ± 0.7	1.0 ± 0.1	**	2.6 ± 1.0	1.2 ± 0.1	n.s.
	75	2.8 ± 0.2	1.7 ± 0.3	*	2.6 ± 0.5	1.9 ± 0.4	n.s.
	81	3.1 ± 0.3	1.8 ± 0.4	*	3.2 ± 0.5	1.9 ± 0.6	*
Average leaf size [cm^2]	65	65.9 ± 6.6	106.7 ± 11.7	*	87.3 ± 4.2	107.4 ± 36.6	n.s.
	75	59.3 ± 3.1	85.0 ± 10.2	*	70.9 ± 4.6	119.5 ± 13.7	**
	81	51.2 ± 3.9	74.4 ± 5.8	**	76.0 ± 13.6	99.5 ± 2.6	n.s.
Canopy volume [m^3]	65	47046.0 ± 6770.8	9945.5 ± 1122.6	**	25884.4 ± 10246.0	12094.6 ± 728.5	n.s.
	75	28041.6 ± 2350.2	17234.5 ± 3139.9	*	26322.2 ± 4797.8	18604.0 ± 3796.0	n.s.
	81	31120.9 ± 2787.9	17986.3 ± 3139.9	**	31812.4 ± 4608.5	19209.8 ± 5902.6	*
Bunch weight [g]	89	96.5 ± 37.3	152.3 ± 40.0	*	126.7 ± 27.1	182.6 ± 69.0	*
Bunch length [cm]	89	11.2 ± 2.0	12.9 ± 1.1	*	11.3 ± 2.5	13.3 ± 3.3	n.s.
Bunch width [cm]	89	6.9 ± 1.3	8.7 ± 1.8	*	8.7 ± 1.9	9.6 ± 1.1	n.s.
Berry number per bunch	89	78.1 ± 26.9	110.1 ± 27.5	*	71.3 ± 17.1	85.6 ± 24.1	n.s.
Ø berry size [mm]	89	12.0 ± 1.0	12.4 ± 1.0	*	14.2 ± 1.2	14.2 ± 1.3	n.s.

b)

2017		Chardonnay			Reberger		
	BBCH	SMPH	VSP		SMPH	VSP	
Number of shoots [per 0.5m]	10	150.0 ± 14.4	10.0 ± 1.2	**	101.0 ± 11.3	9 ± 0.8	**
Number of inflorescences/bunches [per 0.5m]	65	18.5 ± 6.5	9.8 ± 2.2	n.s.	52.0 ± 15.4	8.0 ± 2.1	*
	75	19.0 ± 7.5	9.0 ± 0.8	*	18.0 ± 8.0	7.8 ± 3.0	n.s.
	81	12.3 ± 4.5	9.8 ± 2.2	n.s.	27.5 ± 6.4	7.0 ± 1.6	n.s.
LAI	65	3.5 ± 0.4	1.1 ± 0.3	**	3.7 ± 0.3	0.8 ± 0.1	**
	75	3.0 ± 0.3	2.6 ± 0.5	n.s.	2.6 ± 0.5	1.5 ± 0.5	*
	81	3.5 ± 0.7	2.1 ± 0.4	*	4.0 ± 0.6	2.1 ± 0.2	*
Average leaf size [cm^2]	65	60.4 ± 5.7	105.3 ± 6.7	**	68.1 ± 10.6	98.8 ± 8.4	*
	75	60.7 ± 6.9	76.2 ± 8.7	*	79.7 ± 16.2	95.0 ± 9.8	n.s.
	81	63.4 ± 3.6	96.8 ± 5.6	**	84.0 ± 10.2	96.9 ± 7.7	n.s.
Canopy volume [m^3]	65	35039.0 ± 4414.3	10726.4 ± 2860.0	**	36713.1 ± 3465.7	8288.9 ± 1196.5	**
	75	30205.5 ± 3148.3	25861.5 ± 4969.6	n.s.	26167.3 ± 5174.9	15326.1 ± 5108.7	*
	81	34957.6 ± 6964.7	20611.1 ± 4041.3	*	40488.8 ± 6456.3	20934.6 ± 1851.5	*
Bunch weight [g]	89	76.2 ± 34.8	163.7 ± 33.0	**	101.4 ± 35.7	257.7 ± 83.5	**
Bunch length [cm]	89	8.7 ± 2.6	12.0 ± 1.4	*	10.9 ± 2.3	14.7 ± 2.2	*
Bunch width [cm]	89	6.9 ± 1.7	8.9 ± 1.6	*	6.9 ± 1.7	8.9 ± 1.6	*
Berry number per bunch	89	57.8 ± 23.7	125.1 ± 26.8	**	56.0 ± 20.5	134.1 ± 45.5	**
Ø berry size [mm]	89	12.2 ± 1.0	13.2 ± 1.3	**	12.8 ± 2.0	15.3 ± 1.7	**

3.2. Berry skin characteristics

No significant differences were detected between the two training systems in terms of investigated berry skin characteristics, i.e. impedance of the cuticle, maximum break force, skin break energy and berry skin thickness (Tab. S2).

3.3. Microclimate

*Tab. 2: Local and micro climate during different phenological stages in the trial fields 'Chardonnay' and 'Reberger' as a function of grapevine training system, 2016 (a) and 2017 (b). Except for 'total rainfall' all parameters are mean values. Permutation test; *P<0.05, **P<0.001.*

a)

2016

	local climate				Chardonnay			Reberger		
BBCH	Temperature [°C]	total rainfall [mm]	leaf wetness [%]	Microclimate	SMPH	VSP		SMPH	VSP	
13 - 71	17.4	131.4	49.1	Relative humidity [%]	83.4 ± 17.5	80.5 ± 19.7	**	83.3 ± 16.7	80.0 ± 20.4	**
				Temperature [°C]	18.2 ± 5.3	18.7 ± 5.7	*	17.9 ± 4.8	18.8 ± 6.0	**
71 - 83	19.8	63.1	28.8	Relative humidity [%]	75.4 ± 19.6	72.7 ± 20.2	*	74.7 ± 74.7	74.3 ± 19.9	n.s.
				Temperature [°C]	20.7 ± 5.8	20.9 ± 5.0	n.s.	20.4 ± 5.5	20.5 ± 5.6	n.s.
83 - 89	18.3	32.6	44.6	Relative humidity [%]	77.9 ± 18.8	74.8 ± 20.5	**	75.2 ± 21.0	75.4 ± 21.0	n.s.
				Temperature [°C]	18.8 ± 6.1	19.3 ± 5.5	*	19.1 ± 7.0	18.9 ± 6.7	n.s.

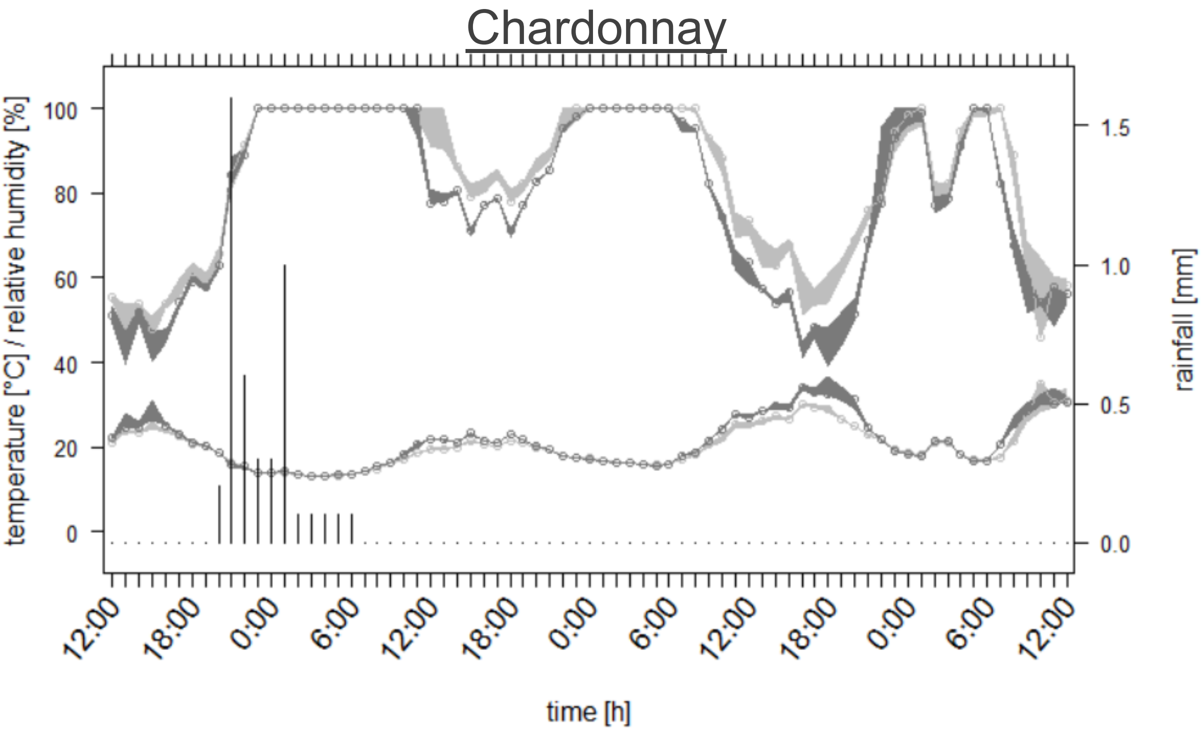
b)

2017

	local climate				Chardonnay			Reberger		
BBCH	Temperature [°C]	total rainfall [mm]	leaf wetness [%]	Microclimate	SMPH	VSP		SMPH	VSP	
13 - 71	18.8	74.8	19.7	Relative humidity [%]	69.6 ± 21.1	67.7 ± 22.4	n.s.	68.9 ± 21.4	68.1 ± 20.5	n.s.
				Temperature [°C]	19.6 ± 6.8	20.2 ± 7.2	n.s.	19.5 ± 6.6	20.4 ± 7.3	*
71 - 83	20.7	65.6	23.0	Relative humidity [%]	73.1 ± 20.4	71.7 ± 20.4	n.s.	72.9 ± 20.1	73.6 ± 19.7	n.s.
				Temperature [°C]	21.3 ± 5.9	21.5 ± 5.9	n.s.	21.1 ± 5.6	21.5 ± 5.9	n.s.
83 - 89	17.1	145.2	29.0	Relative humidity [%]	78.7 ± 18.1	76.8 ± 18.8	*	77.3 ± 18.3	78.1 ± 17.9	n.s.
				Temperature [°C]	19.0 ± 4.9	19.3 ± 5.2	n.s.	19.1 ± 4.8	19.5 ± 5.1	n.s.

The evaluation of microclimate as a function of the training system showed significant differences between SMPH and VSP, predominantly in the ‘Chardonnay’ field in 2016 (Tab. 2, a). In the first year of the study, increased leaf wetness was measured in BBCH 13 – 71 and 83 – 89 due to rainfall and morning dew, respectively. At this time the relative humidity in the SMPH canopy was significantly higher and the temperature lower compared to VSP canopies. This was also the case for ‘Reberger’, but only at BBCH 13 – 71. However, in 2017 (Tab. 2, b), when the leaf wetness only reached a maximum of 29.0%

in spite of intense rainfall, minor differences in the canopy microclimate between the two training systems were noted.


Chardonnay

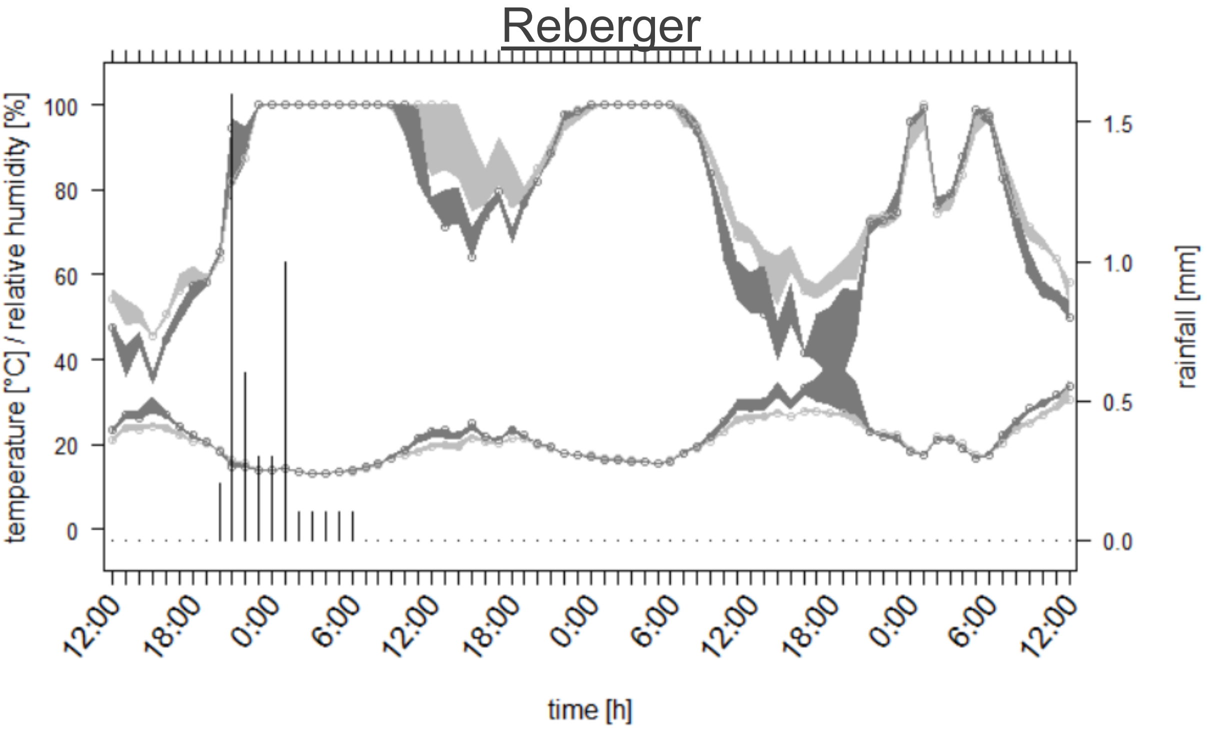

Reberger

Fig. 1: 72 h recording section of the microclimate data from 20th to 23th of June 2016 in the two trial fields 'Chardonnay' (a) and 'Reberger' (b) as a function of grapevine training system SMPH (bright) and VSP (dark). Relative humidity is shown in the upper lines, temperature in the lower lines. Black columns represent rainfall [mm].

A more detailed look on the canopy microclimate during the course of the day revealed that the relative humidity in the SMPH canopy is up to 20% points higher for several hours compared to VSP after a rain event, while the temperature in the two training systems may differ by up to 3°C (Fig. 1 b, 2nd day, 12:00 o´clock). Similar results could also be observed during morning dew (Fig. 1 a and b, 3rd day, 7:00 o´clock). These observations were made in both trial vineyards, 'Chardonnay' and 'Reberger'.

3.4. Fungal diseases

*Tab. 3: Assessment results for the fungal grapevine diseases Downy Mildew, Powdery Mildew and Botrytis Bunch Rot as a function of grapevine training system, 2016 (a) and 2017 (b). Statistical analysis was done with Fisher´s exact test for the incidence rate and Kruskal-Wallis test for the incidence level; *P<0.05, **P<0.001.*

			Chardonnay			Reberger		
a) 2016	BBCH	Incidence	SMPH	VSP		SMPH	VSP	
Downy Mildew	71	rate [%]	48	25	**	48	28	**
	71	level [%]	19,3 ± 26,7	2,9 ± 6,4	**	13,3 ± 22,4	3,8 ± 8,2	**
	83	rate [%]	92	99	n.s.	96	100	n.s.
	83	level [%]	43,3 ± 37,6	37,7 ± 27,2	n.s.	46,5 ± 35,8	45,2 ± 33,3	n.s.
Powdery Mildew	83	rate [%]	78	76	n.s.	0	0	n.s.
	83	level [%]	22,2 ± 23,0	25,8 ± 27,7	n.s.	0 ± 0	0 ± 0	n.s.
	85	rate [%]	97	100	n.s.	0	0	n.s.
	85	level [%]	63,3 ± 29,0	62,1 ± 31,4	n.s.	0 ± 0	0 ± 0	n.s.
Botrytis Bunch Rot	89	rate [%]	3	5	n.s.	0	0	n.s.
	89	level [%]	0,2 ± 1,2	0,6 ± 3,0	n.s.	0 ± 0	0 ± 0	n.s.

			Chardonnay			Reberger		
b) 2017	BBCH	Incidence	SMPH	VSP		SMPH	VSP	
Downy Mildew	71	rate [%]	0	0	n.s.	0	0	n.s.
	71	level [%]	0 ± 0	0 ± 0	n.s.	0 ± 0	0 ± 0	n.s.
	83	rate [%]	0	0	n.s.	0	0	n.s.
	83	level [%]	0 ± 0	0 ± 0	n.s.	0 ± 0	0 ± 0	n.s.
Powdery Mildew	83	rate [%]	77	48	**	3	2	n.s.

	83	level [%]	18,2 ± 23,0	6,4 ± 12,0	**	0,3 ± 2,1	0,3 ± 2,1	n.s.
	85	rate [%]	98	94	n.s.	0	0	n.s.
	85	level [%]	47,4 ± 27,9	36,5 ± 29,3	*	0 ± 0	0 ± 0	n.s.
Botrytis Bunch Rot	89	rate [%]	11	42	**	6	11	n.s.
	89	level [%]	1,8 ± 8,0	8,7 ± 14,4	**	0,4 ± 1,5	2,6 ± 10,5	n.s.

At the first DM assessment in 2016, when young fruits begin to swell (BBCH 71), 25% of the VSP and 48% of the SMPH bunches in the ‘Chardonnay’ trial field were infected with *P. viticola* (Tab. 3). The mean incidence level for SMPH reached 19.3% and was significantly higher compared to VSP with 2.9%. At beginning of veraison, 92% of the SMPH bunches and almost all examined VSP bunches showed DM symptoms. The incidence level was 43.3% for SMPH and 37.7% for VSP. No significant differences between the two training system could be observed at the second DM assessment.

Similar results were observed in the ‘Reberger’ field. In the beginning of the DM infection process, at BBCH 71, 48% of the SMPH and 28% of the VSP bunches showed symptoms. With incidence levels of 13.3% for SMPH and 3.8% for VSP, a significant difference between the training systems could be noticed at this point of plant development. At BBCH 83 an increase of infection pressure was evident in both experimental panels, which led to disease symptom in 96% of the sampled SMPH and 100% of the VSP bunches. In the SMPH panel the mean infection level was 46.5% and for VSP 45.2%, resulting in no significant differences between the training systems. The assessment results for DM in 2017 revealed no infection in either of the both experimental fields during the whole season.

In the ‘Chardonnay’ trial field in 2016 78% SMPH and 76% VSP bunches were infected with PM at the beginning of ripening (BBCH 83). The mean incidence level was 22.2% for SMPH and 25.8% for VSP. At BBCH stage 85 when the berries started to soften, 97% of the SMPH bunches and all tested VSP bunches showed symptoms of *E. necator*. Additionally, an increase in the infection level occurred. Both training systems had a similar mean value of about 62.5%. In the subsequent year 29% more infected bunches were found in the SMPH panel than in the VSP at BBCH 83. The incidence level was three times higher for infected SMPH bunches compared to the VSP bunches. At the second PM assessment the number of infected bunches was almost equal in both training systems. However, the incidence level was still significantly higher in the SMPH panel.

During the whole season 2016 and 2017 no or only minimal PM symptoms could be observed in the trial field of the grapevine cultivar 'Reberger'.

For 2016 no severe Botrytis infection could be noticed in either of the both grapevine cultivars until ripening (BBCH 89). Only 3% of the SMPH and 5% of the VSP bunches in the 'Chardonnay' field showed slight symptoms of BR and all examined 'Reberger' bunches were free of BR. In 2017 significant differences between the two training systems regarding BR infection could be noticed, at least in the 'Chardonnay' field. Here, 42% of the monitored VSP and 11% of the SMPH bunches showed BR symptoms. The incidence level was almost five times higher in the VSP panel compared to the SMPH. Also in the 'Reberger' field BR symptoms could be observed, but the incidence rate as well as the incidence level was quite low with no significant differences between both training systems.

3.5. Drosophila suzukii

*Tab. 4: Number of trapped SWD flies in SMPH and VSP trained 'Reberger' vineyards (mean value, n=12 traps). Counted D. suzukii eggs on 50 randomly selected SMPH and VSP berries with intact skin (mean value, n=4 runs). Results from 2016 (a) and 2017 (b) are shown. T test; *P<0.05, **P<0.001.*

a)

2016	SMPH	VSP	
mean no. of trapped *D. suzukii* flies	31,3 ± 18,7	20,5 ± 20,2	n.s.
mean number of eggs per 50 berries	2,3 ± 3,3	0,5 ± 1,0	n.s.

b)

2017	SMPH	VSP	
mean no. of trapped *D. suzukii* flies	10,3 ± 6,6	4,8 ± 2,6	*
mean number of eggs per 50 berries	0,8 ± 1,0	1,0 ± 1,2	n.s.

In both seasons the mean number of *D. suzukii* flies was up to two times higher in SMPH compared to VSP trained grapevine (Tab. 4). However, only in 2017 this difference is significant. Despite this striking difference we did not observe a higher number of eggs on intact SMPH grape berries. In both panels the number of detected eggs on grapevine berries was marginal over the two seasons and no differences between the training systems were observed.

4. Discussion

The aim of this study was to compare two training systems, SMPH and VSP, with regard to canopy architecture, berry skin characteristics, microclimate and the influence of those factors on incidence of common fungal grapevine pathogens as well as the damage caused by the invasive insect pest *Drosophila suzukii* in German viticulture. We found that the large amount of leaves produced by SMPH trained plants create a bigger and denser canopy structure than VSP trained plants, even if the leaves of the latter are larger in size. SMPH bunches showed a looser structure than VSP bunches, due to a smaller architecture, a reduced number of berries and smaller sized fruits. These findings are in line with other studies, which compared the morphology of minimal and intensely pruned grapevines (Clingeleffer and Posingham, 1987; Schmid and Schultz, 2000; Sommer *et al.* 1993; Wolf *et al.* 2003; Intrieri *et al.* 2011). The analyses of canopy architecture in the different trellis zones demonstrate that the plant vigor in the SMPH system is mainly located in the upper zones (3-5), while in the VSP system it is restricted to the lower zones (1-2), perhaps because of the apical dominance (Jackson, 1996).

These differences in plant morphology between the two training system have a clear effect on the microclimate in the canopy. SMPH canopies dry much slower and need several hours longer after rain or morning dew to achieve a similar humidity level as VSP canopies. Local weather increased leaf wetness and lead to a higher relative humidity, but lower temperature in SMPH canopies compared to VSP canopies. This is clearer for 'Chardonnay' than for 'Reberger', probably because of the hillside location of the latter. The dense leaf structure of the SMPH plants prevents sunlight from reaching the inside of the canopy, thus the moisture takes longer to evaporate. Additionally, air movement is reduced, which also impedes the canopy from drying.

Under certain climate conditions with high leaf wetness, as observed in 2016, we found SMPH trained grapevines more susceptible to DM than VSP trained, for 'Chardonnay' and Reberger. However, in the second assessment no significant differences between the two training systems were found. This is probably caused by a considerable decline of SMPH inflorescences/bunches caused by DM, which are not included in the assessment made at BBCH 83. *Plasmopara viticola*, the causal agent of DM, needs an environment rich in moisture for successful infection and spreading (Blaeser and Weltzien, 1978; 1979). It is possible that the slower drying of the SMPH canopies provides an extended time frame for *P. viticola* to successfully infect grapevine tissue after rain or morning dew.

SMPH bunches were more sensitive to PM infection than VSP bunches in 'Chardonnay' in 2017. The results of Austin *et al.* (2011) demonstrate that training systems showing a high light penetration in the fruit zone are less susceptible to PM, due to sunlight exposure of the pathogen and improved pesticide deposition. According to this assumption, VSP should be the more robust training system, since the SMPH bunches are more often located within the dense leaf canopy. This was only the case in 2017. In the previous year no differences in PM incidence between the two training systems could be observed. Since the infection pressure of DM and PM reached an extraordinary high level in 2016, the first three plant protection applications in 2017 were performed with conventional pesticides to achieve a profound cleaning effect of the plants against the pathogens. It is possible that the use of these pesticides maintained a better protection shield for VSP trained vines than minimal pruned vines, due to the enhanced accessibility of the VSP bunches.

Because of the *E. necator* resistance gene *Ren3* located in the genome of the grapevine variety 'Reberger' no or very few PM symptoms could be observed during the study of this work (Zendler *et al.* 2017).

In this study, differences between SMPH and VSP regarding their susceptibility against BR could only be noticed in the 'Chardonnay' field in 2017. Bunches from minimal pruned vines with their loose bunch structure were less susceptible to BR compared to the densely packed bunches from cane pruned vines, which tend to burst and open the gates for BR infection, which is in consensus with Ashley *et al.*, 2006. Also Emmett *et al.* (1995) reported that the bunch architecture of minimally pruned vines is usually characterized by a smaller and less compact structure, which promotes robustness against BR. However, in

2016 no differences between the training systems could be noted. An explanation for these inconsistent results could be the influence of DM on bunch architecture in 2016. In this year the heavy DM epidemic demolished many berries in the trial fields, creating loose bunch structures in both training systems, SMPH and VSP.

As expected we found significantly more SWD in the SMPH trained than in the VSP trained panels, but the difference was only significant in 2017. SWD prefers shady and humid microhabitats, even within a single plant species (Diepenbrock and Burrack 2017). In this experiment, a high number of captured SWD did not correspond with a high incidence of SWD damage to the grapes. It appears that *D. suzukii* uses the grapevine as a habitat, but does not necessarily use grapes as a substrate for oviposition. Despite their wide host range, grapevine does not seem to be a preferred host for SWD. In laboratory studies only very few eggs were laid on grape berries and those eggs had very slow developmental rates as well as a low survivorship to the adult stage (Bellamy *et al.*, 2013; Jarausch *et al.* 2017; Maiguashca *et al.*, 2010; Lee *et al.*, 2011; Poyet *et al.*, 2015). The small numbers of eggs that we could find in both trial years on grape berries confirms that grapevine appears to be a low quality host for SWD. The resistance of fruit skin to penetration has been previously discussed as a factor driving oviposition in SWD (Lee *at al.*, 2011; Burrack *et al.*, 2013). Ioratti *et al.* (2015) reported that oviposition by *D. suzukii* increases with decreasing penetration force. Since the two training systems did not influence the grape skin characteristics significantly, we cannot directly confirm their results. The fact that we also did not find any differences in oviposition between SMPH and VSP grapevines, despite the difference in number of SDW in the habitat, indicates an important role of skin characteristics in host selection by SWD.

In conclusion, SMPH trained grapevines were more susceptible to DM and PM compared to VSP trained vines, possibly due to differences in canopy microclimate. The incidence of BR in contrast was higher for VSP vines showing a more compact bunch architecture. Regarding SWD, a higher activity was noticed in SMPH canopies. However, the number of damaged berries was the same in both training system. Because of the higher susceptibility of SMPH against the two major fungal grapevine diseases a plant protection regime specifically adapted to this new training system should be established. The benefit of fungus resistant cultivars such as 'Reberger' will be particularly high in SMPH vines, enabling winegrowers to combine advantages of SMPH with the economic and environmental benefits of reduced fungicide applications.

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Chapter V

Do minimal pruning and reduced plant protection enhance spiders on grapevine?

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Abstract. Agricultural management should aim at high densities of beneficial organisms such as spiders. Here, we asked whether spiders in vineyards can be promoted by using novel disease resistant grape varieties that allow to reduce fungicide applications, or by minimal pruning which enhances the structural complexity of the grapevine canopy. We compared spider communities in vineyards planted with fungus-resistant varieties (PIWIs) to vineyards with traditional grapevine varieties, and minimally pruned vineyards to vertical shoot positioned vineyards. Densities of Theridiidae were more than doubled in fungus-resistant grape varieties, but the remaining families were not significantly affected. Minimal pruning enhanced Philodromidae and Dictynidae but reduced Salticidae. However, neither pruning method, nor the reduced fungicide use in PIWI vineyards had significant effects on overall spider abundance and species richness. Hence, effects of vineyard management were family-specific and possible consequences for pest regulation will thus depend on the pest control potential of the involved families.

Keywords: Natural pest control, minimal pruning, fungicide, predation, fungus resistant grapevine

Spiders play an important role in the control of arthropod pests in many crops including grapevine (Young & Edwards 1990; Sunderland & Samu 2000; Pfannenstiel 2008; Pennington et al. 2018). Spiders are often the most abundant group of predatory arthropods in vineyards (Costello & Daane 1999). As generalist predators, they can act against a broad spectrum of potential vineyard pests, such as leafhoppers (Hogg & Daane 2011) or the grape berry moth *Lobesia botrana* ([Denis & Schiffermüller], 1775) (Lepidoptera: Tortricidae) (Pennington et al. 2018). Despite the important role of spiders as biocontrol agents, there are few studies focusing on the effects of different viticultural practices on spider abundance and diversity in the grapevine canopy. Here, we investigate the spider community in the canopy, where the presence of spiders is most relevant for natural pest control in grapevine. We compare spider communities between vineyards managed with: 1) different pruning methods and 2) classical versus fungus-resistant grapevine varieties, receiving different levels of plant protection. The canopy in minimally pruned vineyards (SMPH) is more voluminous and contains more old, woody branches than in vertical shoot positioning, where old wood is removed annually except for one or two short branches from the previous growing season (Intrieri et al. 2011). Therefore SMPH vineyards offer a structurally different habitat with more space and potentially more resources such as prey and shelter (Langellotto & Denno 2004; Kraus et al. 2018), which is why we expect a higher spider abundance than in VSP vineyards. The different microclimate induced by the denser canopy in SMPH than in VSP is expected to affect spider species differently according to their shading and moisture preference (Entling et al. 2007). Our second comparison is between vineyards planted with traditional grapevine varieties and vineyards planted with fungus resistant cultivars (PIWI). Traditional varieties need to be sprayed over ten times per season to prevent fungal disease, whereas spraying can be reduced by 50 - 90% in vineyards planted with fungus resistant cultivars (Töpfer et al. 2011). In the organic vineyards studied here, fungal diseases are mostly controlled with combined sprayings of Copper and Sulphur containing products. Both direct and indirect (via prey organisms) effects of these substances on spiders are likely, but poorly known (Bruggisser et al. 2010; Pekár 2012, 2013). For example, Sulphur and Copper are toxic to other arachnids such as mites (Hanna et al. 1997; Duso et al. 2012; Pennington et al. 2017), they can affect insects that are important spider prey (Thomson et al. 2000; Pennington et al. 2018; Vogelweith & Thiéry 2018), and spraying might physically destroy spider webs. We expected that (i) minimal pruning leads to different, more diverse and abundant spider communities than VSP and (ii), that vineyards planted with PIWI varieties have different, more diverse and abundant spider communities than vineyards with traditional varieties.

Sampling took place in 16 pairs of adjacent vineyards around Nußdorf, Germany (49°13'26.1"N 8°06'33.6"E), which were all managed organically and thus received neither insecticides nor herbicides. We used a paired design with eight pairs consisting of one SMPH and one VSP pruned vineyard to investigate the effects of pruning system, and eight pairs consisting of one vineyard planted with a fungus resistant grapevine variety, paired with a vineyard planted with a traditional, fungus-susceptible variety to investigate the effects of fungicide application frequency. All SMPH vs. VSP vineyards were planted with traditional grapevine varieties and most of them received the same plant protection treatments, consisting of ten Sulphur and 13 Copper sprayings. One of the plots received eight Sulphur and ten Copper sprayings. PIWI vineyards were all VSP trained and received three Sulphur and either no or four Copper sprayings. The adjacent vineyards with traditional grapevine varieties received on average nine Sulphur and eleven Copper sprayings and were also VSP trained. Vineyards were between two and 52 years old and at least two rows wide. Minimally pruned and vertical shoot positioned vineyards were on average 27 and 21 years old. Due to the novelty of fungus resistant cultivars, PIWI vineyards were on average 12.5 years old, whereas the vineyards planted with traditional varieties were on average 27 years old. Where possible, the outer rows were excluded from sampling to prevent edge effects. Spiders were collected using a drop cloth method. They were dislodged from the plants by shaking them onto a beat sheet with a 72 cm diameter, where they were collected and later conserved in 70% EtOH. Fifteen randomly selected vines in each vineyard were shaken for five seconds and the collected spiders were combined into one sample. This process was repeated four times between June and September 2017. Adult spiders were identified to species and immatures to the family level using identification keys of Roberts (1987, 1995) and Nentwig et al. (2018). Nomenclature follows World Spider Catalog Version 19.5 (World Spider Catalog 2018). Since the majority of individuals were immature, our analysis uses pooled family data from all four sampling dates. We used paired t-tests in Sigma Plot (Systat Software, San Jose, CA) to detect effects of pruning and fungicide application frequency on total spider abundance and family richness, as well as the abundance of single spider families. To compare spider communities between treatments we used a permutational MANOVA in R (version 3.0.2) (function 'adonis' in the vegan package (version 2.2-1), 999 permutations) using the number of individuals per family, with vineyard pairs as 'strata' to account for the paired design (R Development Core Team 2010; Oksanen et al. 2015). Since PIWI vineyards were on average 14.5 years younger than traditional vineyards, we also tested for an influence of vineyard age on the spider community, using the same procedure as described above.

Overall we collected 1481 spider individuals, 175 of which were adults. They belonged to at least 25 species in 16 families (Table 1). Most spiders belonged to the family of Salticidae (22.2 % of individuals) closely followed by Theridiidae (20.2 %), Araneidae (19.9 %) and Philodromidae (17.4 %). *Philodromus cespitum* (Walckenaer, 1802) (Philodromidae) (19.4 % of adult spiders), *Marpissa muscosa* (Clerck, 1757) (10.9 %) and *Heliophanus auratus* C. L. Koch, 1835 (10.3 %) (both Salticidae) were the most common species.

While neither the overall abundance nor the species richness of spiders was significantly affected by the applied pruning method, spider communities differed significantly between minimally pruned vineyards and VSP vineyards ($R^2 = 0.21$, $P = 0.002$, Fig. 1A). Dictynidae and Philodromidae were significantly more abundant in SMPH than in VSP vineyards (Dictynidae: $t_7 = 2.56$, $P = 0.04$, Philodromidae: $t_7 = 3.17$, $P = 0.02$). Salticidae on the other hand were more abundant in VSP than in minimally pruned vineyards ($t_7 = -4.47$, $P = 0.003$) (Fig.1A).

Table 1. Adult spiders caught in minimally pruned (SMPH) and vertical shoot positioned (VSP) grapevine, as well as in vineyards planted with PIWI (fungus resistant) and traditional varieties.

Family	Species	Pruning		Grapevine type	
		SMPH	VSP	PIWI	traditional
Araneidae	*Araneus diadematus*	2			
	Araniella cucurbitina		1		
	Araniella opisthographa	3		1	
	Mangora acalypha	1		4	1
	Zygiella x-notata			1	
Dictynidae	*Dictyna uncinata*	4	1	5	2
Linyphiidae	*Agyneta rurestris*		1	1	
	Araeoncus humilis	1	1		
	Erigone dentipalpis			1	

	Tenuiphantes tenuis	5	3	2	3
Philodromidae	*Philodromus cespitum*	6	3	11	12
Salticidae	*Ballus chalybeius*			1	
	Heliophanus auratus	2	6	5	5
	Marpissa muscosa	3	2	6	8
	Pseudicius encarpatus				1
	Salticus scenicus	4	8	3	2
	Salticus zebraneus				4
	Synageles venator	3	2	1	1
Theridiidae	*Dipoena melanogaster*	2	1		
	Enoplognatha latimana			2	2
	Neottiura bimaculata	1			1
	Phylloneta impressa	2	6	4	1
	Theridion asopi		1	3	
Thomisidae	*Synema globosum*			2	
Uloboridae	*Hyptiotes paradoxus*	1	1		

Overall spider abundance and species richness was not significantly higher in PIWI grapevines compared to traditional varieties, which received about three times as many plant protection treatments. More intense plant protection measures in traditional grapevines also didn't cause a significant change in the spider community (R^2 = 0.09, P = 0.11, Fig. 1B). , We found no significant effects of vineyard age on spider communities (R^2 = 0.22, P = 0.82). Theridiidae were the only family in which abundance was significantly affected by plant protection intensity. Their abundance was more than two times higher in PIWI vineyards than in vineyards with traditional grapevine varieties (t_7 = -2.715, P = 0.03, Fig. 1B).

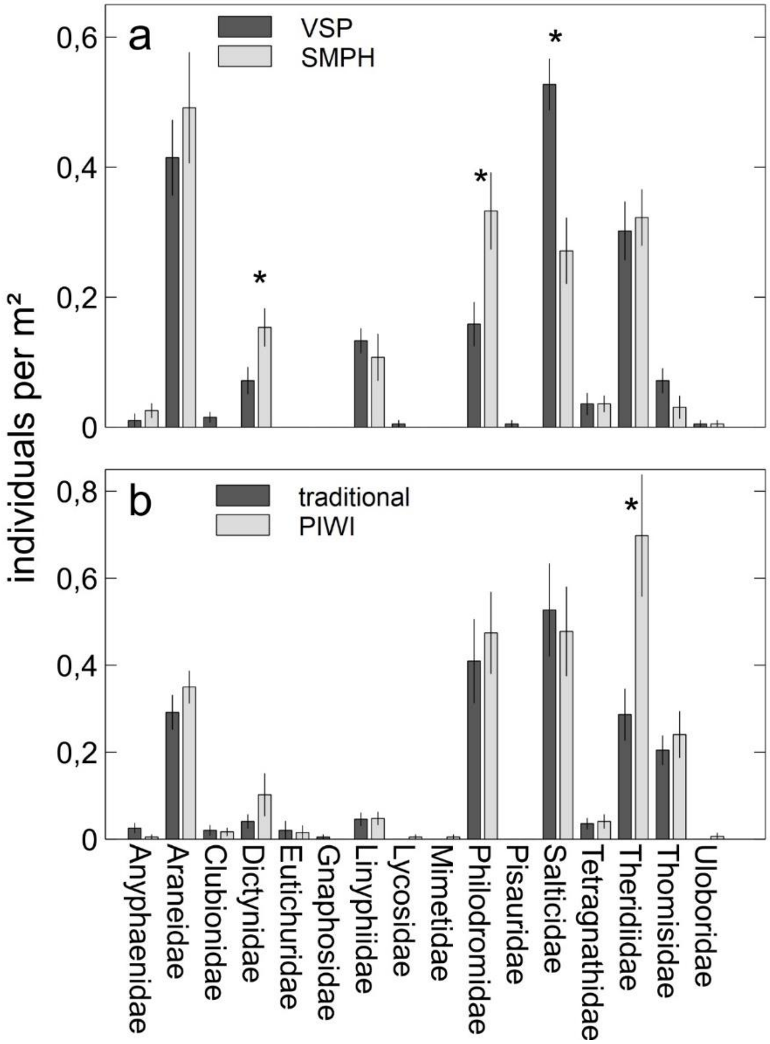

Figure 1a-b. Number (± SE) of spider individuals per m²: **a.** In vertical shoot positioned (VSP) or in minimally pruned (SMPH) vineyards (*n* = 32) **b.** In vineyards planted with traditional grapevine versus fungus resistant (PIWI) grapevine varieties (n = 32) * *P < 0.05.*

The significantly different spider communities between SMPH and VSP vineyards can probably be explained by the drastic differences in the structure of the canopy, which also affects the microclimate, making the SMPH canopy slightly cooler and more humid than in VSP (Kraus et al. 2018). There are examples in the literature describing a positive relation between the structural diversity of vegetation and spider diversity (Greenstone (1984), Scheidler (1990), Whitmore et al. (2002)). We can confirm this positive relation for Dictynidae and Philodromidae, which benefitted from SMPH versus VSP grapevine. The one member of the Dictynidae we could identify to the species level is *Dictyna uncinata* Thorell, 1856. We assume that most immature Dictynidae can be grouped in the same genus or even the same species. *D. uncinata* is often found high in vegetation, especially in brush and hedges, where it builds webs on tips of branches and on the underside of leaves (Harvey et al. 2002). There are significantly more branches and leaves per plant in SMPH than in VSP vineyards, which provides more habitat for these small spiders (Kraus et al. 2018). Philodromidae are cursorial spiders, which have a lower recolonization rate than most web spiders (Öberg & Ekbom 2006). They likely benefit from better and more abundant overwintering opportunities provided by old wood in SMPH vineyards. In VSP vineyards all old wood other than the stem is removed, severely limiting shelter during winter. *Philodromus cespitum* is abundant in orchards across Europe (Bogya et al. 1999; Pekár 1999) and is useful for biocontrol of a wide range of pest insects, but also preys on other spiders (Michalko & Pekár 2015). In contrast to the aforementioned spider families, Salticidae were significantly less abundant in SMPH than in VSP pruned vineyards. They are visually oriented hunters (Harland et al. 2012) and may benefit from more light and higher temperatures in VSP pruned grapevine.

The only family that was significantly affected by PIWI vs traditional varieties were Theridiidae. This pattern might be explained by their web-building lifestyle and the higher frequency of disturbances in traditional varieties, which needed three times as many plant protection treatments during the growing period in our study. Usually Theridiidae build webs that stay in place for an extended period of time (Benjamin and Zschokke 2003). Frequent disturbances by pesticide treatments might destroy those webs and thereby be a fitness disadvantage. Spider webs have also been shown to be efficient at collecting agricultural spray (Samu et al. 1992), so web-building spiders might be even more exposed to the fungicides than cursorial spiders. Such negative effects of fungicides on non-target arthropods can reduce natural biological control of grapevine pests (Pennington et al. 2018), even if reduced activity of ants rather than spiders appeared responsible for the reduced predation of grape berry moth (*Lobesia botrana*) eggs under intensive fungicide regimes in that study.

As the effects of fungicide application frequency and minimal pruning on spiders were family-specific, possible consequences for natural pest control by spiders requires specific knowledge about the roles of the affected spider families in the vineyard canopy. Conclusions from our study are limited by the moderate number of sampled spiders. However, we demonstrate that spiders in grapevine canopies are sensitive to management, and should be more widely considered in ecological studies of vineyards, especially given the known sensitivity of ground-dwelling spiders to different management practices in vineyards and other agricultural systems (Schmidt et al. 2005; Bruggisser et al. 2010).

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Chapter VI

Conclusions & Outlook

Theresa Pennington

The two management methods studied here had an either positive or neutral effect on natural pest suppression in the examined vineyards. Reduced fungicide use in PIWI vineyards either increased or did not significantly affect the numbers of all studied organisms. SMPH vineyards displayed either similar or higher abundance of studied organisms, except for Dermaptera and Salticidae, which seemed to benefit from the structure of VSP grapevines and grape clusters (Chapters III and V) (Table 1).

Table 1: Effects of PIWI and SMPH on studied arthropods at a glance. + stands for an increase in numbers, o represents no effect and - depicts a decrease. *Drosophila suzukii* was only studied in SMPH vs. VSP and there is no data about effects of PIWI on their numbers.

	PIWI	**SMPH**
Predatory mites	+	+
Pest mites	o	o
***Lobesia botrana* predators**	+	o
Formicidae	+	+
Dermaptera	o	-
Chrysopidae	o	o
***Drosophila suzukii* adults**	N/A	+
***Drosophila suzukii* eggs**	N/A	o
Araneae	o	o
Theridiidae	+	o
Dictynidae	o	+
Philodromidae	o	+
Salticidae	o	-

Beneficial mites clearly profited from both reduced fungicide sprayings and minimal pruning, whereas pest mites were not affected by either management system. Since high numbers of predatory mites are desirable for their efficiency as pest control organisms, the conditions for natural pest regulation within the leaf mesofauna of grapevine are improved by both innovative management methods. Predators of *Lobesia botrana* found improved conditions in PIWI grapevines but appeared unaffected by the pruning system. This suggests that reducing fungicide sprayings is beneficial for the natural suppression of *L. botrana* independent of the pruning system. *Drosophila suzukii* was more abundant in SMPH, but this did not lead to a higher incidence of damage to the grapes. It appears that *D. suzukii* prefers the shadier and more humid SMPH canopy as a habitat, but grapevine does not seem to be a preferred host for oviposition. According to the insurance hypothesis (Naeem and Li 1997; Yachi and Loreau 1999), a more biodiverse ecosystem is better at buffering changes. As a result, promoting biodiversity in vineyards, particularly through a reduction of fungicide use and offering a more structurally diverse habitat, should offer a better protection against invasive pests like spotted wing drosophila. Spider abundance was not affected by reduced fungicide use or different pruning systems. Still, some spider families were sensitive to changes in management and their influence on natural pest control warrants further investigation. My results indicate that there are neither synergies nor conflicts when cultivating PIWIs in the SMPH pruning system and that the benefits of reducing fungicides for natural pest suppression should be present in both studied pruning systems. Given the climate change induced earlier ripening of grapes over the last decades (Stoll et al. 2010), cultivating SMPH pruned grapevines can also be a useful short-term solution to mitigate the effects of global warming on viticulture. The reason for their delayed ripening is yet unknown, but it is likely an effect of mechanical thinning of the grapes in the early season.

Overall, the effects of PIWI and especially of SMPH on the vineyard arthropod fauna were less pronounced than expected. Since SMPH is a rather recent innovation, the studied vineyards had not been cultivated in this pruning system for very long. The experimental vineyard at Geilweilerhof had only been mechanically pruned since 2013. The structural complexity of these newly SMPH pruned vineyards is still developing and might offer more habitat and overwintering opportunities for arthropods over time. Especially spiders that rely on plant structures might benefit from a more mature SMPH vineyard (Benton & Rypstra 1998). An extremely important factor influencing vineyard biodiversity in general and the diversity of pest and beneficial arthropods in particular is landscape context (Benton et al. 2002; Schmidt et al. 2005; Isaia et al. 2006; Miles et al. 2012; Rusch et al. 2016). Landscape composition effects on biodiversity are prevalent and can even override the effects of agricultural management (Aavik & Liira 2010, Weibull et al. 2003). The landscape context of the studied vineyards has not been assessed in detail here, but the surrounding landscape mostly consists of other vineyards under unknown management regimes. This homogenous landscape and the use of agricultural chemicals in the surrounding areas likely affected the arthropods observed in the presented studies. It will be interesting to see the effects of reducing fungicide applications on overall arthropod biodiversity in larger vineyards. As more area is planted with PIWI varieties and resistance is further improved, more opportunities for sampling vineyards with fewer fungicide sprayings will become available. The landscape context of sampled vineyards should definitely be taken into consideration for future studies.

Consumers of non-essential foodstuffs such as wine are very selective in the purchases they make. Many of them not only desire a safe product with a pleasing taste but also insist on products that are sustainable and produced in an environmentally friendly manner (Nidumolu et al. 2009). PIWI varieties as well as the SMPH pruning system promise to be a valuable tool on the path towards a more sustainable viticulture and will hopefully become a more popular choice for consumers and growers alike.

Of course, German viticulture is a rich tradition largely carried out by small family enterprises (Deutsches Weininstitut 2018), and introducing new fungus resistant grape cultivars or innovative cultivation systems such as minimal pruning into practice may be difficult. Nevertheless, this thesis endeavors to mitigate some of the uncertainties and prejudices that farmers may have and inspire some growers to take advantage of the benefits that PIWIs and SMPH offer. In addition to its economic advantages, developing and cultivating fungus-resistant cultivars of grapevine and potentially other crops such as apples, potatoes and wheat on a larger scale may drastically diminish the negative environmental impacts of fungicides in agricultural areas and in the surrounding landscapes. Given the dramatic loss of biodiversity in recent years (Dirzo and Raven 2003; Cardinale et al. 2012; Hallmann et al. 2017; WWF 2018), reducing human impacts on the environment should be paramount in order to conserve the inherent value of biodiversity, as well as the benefits it provides for human wellbeing (Costanza et al. 1997; Mae s et al. 2012; McMahon et al. 2012).

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Wiebke, you were a blessing to have around, thank you for your reassurance. I also want to thank you and your family for inviting me into your home and making me feel so welcome.

I am forever grateful for my parents Roswitha and Matthias Thiele’s love, encouragement and endless generosity, for raising me with an appreciation for nature, and for the privilege of being able to follow my dream of becoming a biologist.

Dr. Carolin Thiele, Dr. Catherina Thiele and Dr. Tim Noll, thank you for being such strong role models for me.

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From the shadows, Batman approvingly nods to Dr. Patrick Hanudel who worked some editorial magic that is deeply appreciated.

Geoffrey J. Pennington, I strive to be the woman you see in me. Thank you for everything you do to support me, to inspire me, and to make me smile. I love you.

Appendix

Appendix A: Status and author contributions of publications

Chapter 2

Pennington, T., Kraus, C., Alakina, E., Entling, M. H., & Hoffmann, C. (2017). Minimal Pruning and Reduced Plant Protection Promote Predatory Mites in Grapevine. Insects, 8(3), 86.

TP, CK, MHE and CH designed the study. TP, CK and EA collected data. TP and CK analyzed the data. MHE provided statistical advice. TP wrote the first draft of the manuscript, and CK, MHE and CH contributed to the published version of the manuscript.

Chapter 3

Pennington, T., Reiff, J. M., Theiss, K., Entling, M. H., & Hoffmann, C. (2018). Reduced fungicide applications improve insect pest control in grapevine. BioControl, 63(5), 687-695.

TP, MHE and CH designed the study. TP, JMR and KT collected data. TP analyzed the data. MHE provided statistical advice. TP wrote the first draft of the manuscript and CK, MHE and CH contributed to the published version of the manuscript.

Chapter 4

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R. T., Hoffmann, C., Töpfer, R. & Kicherer, A. (2018). Effects of canopy architecture and microclimate on grapevine health in two training systems. Vitis, 57(2), 53-60.

CK, TP, KH and AK designed the study. CK, TP, AH and AK collected data. CK and TP analyzed the data. CK wrote the first draft of the manuscript and TP, KH, AH, FM, RTV, CH, RT and KA contributed to the published version of the manuscript.

Chapter 5

Pennington, T., Kolb, S., Kaiser, J., Hoffmann, C., Entling, M.H. Do minimal pruning and reduced plant protection enhance spiders on grapevine? *Submitted to Journal of Arachnology*

TP, MHE and CH designed the study. TP, JK and SK collected data. TP analyzed the data. MHE provided statistical advice. TP wrote the first draft of the manuscript and SK, MHE and CH contributed to the submitted version of the manuscript.

Appendix B: Curriculum Vitae

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Born September 17th 1988 in Pirmasens, Germany

Nationality: German

Education

Master of Science
10/2011 - 01/2014
Biodiversity, Ecology and Evolution
University of Kaiserslautern, Germany

Semester Abroad
02/2012 - 08/2012
University of Auckland, New Zealand

Bachelor of Science
04/2008 – 09/2011
Bio Science
University of Kaiserslautern, Germany

Abitur
2008
Leibniz Gymnasium Pirmasens, Germany

Professional Experience

Education Instructor

05/2018 - present
Virginia Zoological Society, Norfolk, Virginia, USA

Volunteer Management Internship

01/2018 - 04/2018
Virginia Zoological Society, Norfolk, Virginia, USA

Graduate Research Assistant

02/2015 - 11/2017
Institute for Environmental Science, Landau, Germany

Environmental Consultant

04/2014 - 02/2015
iSA Ingenieure, Heltersberg, Germany

Teaching Assistant

04/2010 - 02/2014
Department of Plant Ecology and Systematics, University of Kaiserslautern, Germany

Research Assistant

02/2010 - 02/2012
Research Institute for Forest Ecology and Forestry, Trippstadt, Germany

Academic Publications

Pennington, T., Reiff, J.M. Theiss, K., Entling, M. H., Hoffmann, C. (2018) Reduced fungicide applications improve insect pest control in grapevine. BioControl, 63: 687

Kraus, C.; Pennington, T., Herzog, K., Hecht, A., Hoffmann, C., Töpfer, R., Kicherer, A. (2018) Effects of canopy architecture and microclimate on grapevine health in two training systems. Vitis - Journal for Grapevine Research, 57. 53–60

Pennington, T, Kraus, C.; Alakina, E., Entling, M.H., Hoffmann, C. (2017). Minimal Pruning and Reduced Plant Protection Promote Predatory Mites in Grapevine. Insects, 8(3), 86.

Thiele (Pennington), T., Kost, C., Roces, F., Wirth, R. (2014). Foraging leaf-cutting ants learn to reject Vitis vinifera ssp. vinifera plants that emit herbivore-induced volatiles. Journal of chemical ecology, 40(6), 617-620.

Pennington, T., Kolb, S., Hoffmann, C., Entling, M. H. Do innovative management practices enhance spiders on grapevine? (submitted to Journal of Arachnology)

Pennington, T. Wirth, R., Buchheit, R., Kost, C., Roces, F. Odor-based avoidance learning of leaf-cutting ants (*Acromyrmex ambiguus*) towards herbivore-induced plant volatiles. (in prep for PLOS One)

Conference Contributions

Pennington, T., Theiss, K., Hoffmann, C., Entling M.H. (2017) Natural pest suppression in vineyards under innovative management. Entomology 2017, Denver, Colorado.

Pennington, T., Entling, M.H., Hoffmann, C. (2017) Minimal pruning and reduced plant protection promote predatory mites in grapevine. Poster presentation. Entomology 2017, Denver, Colorado.

Thiele, T., Hoffmann, C. Entling M.H. (2016): Biodiversity and Natural Pest Suppression in Vineyards under Innovative Management. Poster & Elevator Pitch presentation. PLANT 2030 Status Seminar 2016. Potsdam, Germany. *Winner Best Elevator Pitch Award*

Pennington, T., Entling M.H., Hoffmann, C. (2016): Auswirkungen von Erziehungssystem und Pflanzenschutzintensität auf die funktionelle Arthropodenbiodiversität der Rebe. 60th German Plant Protection Meeting, Halle, Germany

Thiele, T., Entling M.H., Hoffmann, C. (2016): Assessing biodiversity in vineyards under innovative management practices. 56th Meeting for Grapevine Research in Germany. Bad Kreuznach, Germany

Pennington, T., Wedel, M.F., Hoffmann, C. Entling MH (2016) Plant protection intensity and pruning method influence the effectiveness of natural pest control in vineyards. 9th Young Scientist Meeting, Quedlinburg, Germany.

Thiele, T., Entling M.H., Hoffmann, C. (2015): Arthropod Biodiversity and Natural Pest Suppression in Vineyards under Innovative Management. Poster & Elevator Pitch Presentation. 8th Young Scientist Meeting, Quedlinburg, Germany. *Winner Best Elevator Pitch and Poster*

Thiele, T., Kost, C., Wirth, R. (2013) Reconciling optimal foraging requirements? Foraging leaf-cutting ants learn to avoid plants that emit herbivore-induced volatiles. Annual meeting of the Ecological Society of Germany, Austria and Switzerland, Postdam, Germany

Thiele, T., Kost, C., Wirth, R. (2013) A not so pleasant bouquet: Leaf-cutting ants learn to reject *Vitis vinifera ssp. vinifera* plants with induced defenses. Poster presentation. Annual meeting of the Society for Tropical Ecology. Vienna, Austria.

Appendix C: Declaration according to §8 of Promotionsordnung des Fachbereichs 7: Natur- und Umweltwissenschaften der Universität Koblenz-Landau vom 14.Juni 2013

Ich erkläre hiermit, dass ich die eingereichte Dissertation selbstständig verfasst habe und alle für die Arbeit benutzten Hilfsmittel und Quellen in der Arbeit angegeben sowie die Anteile etwaig beteiligter Mitarbeiterinnen oder Mitarbeiter sowie anderer Autorinnen oder Autoren klar gekennzeichnet habe.

Ich habe keine entgeltliche Hilfe von Vermittlungs- oder Beratungsdiensten (Promotionsberater oder andere Personen) in Anspruch genommen.

Ich habe die Dissertation nicht in gleicher oder ähnlicher Form als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung im In- oder Ausland eingereicht

Die gleiche oder eine andere Abhandlung wurde in keinem anderen Fachbereich oder einer anderen wissenschaftlichen Hochschule als Dissertation eingereicht.

Mir ist bewusst, dass ein Verstoß gegen einen der vorgenannten Punkte den Entzug des Doktortitels bedeuten und ggf. auch weitere rechtliche Konsequenzen haben kann.

Norfolk, Virginia am 15. November 2018