Cyprus University of Technology

Faculty of Geotechnical Sciences and Environmental Management

Doctoral Dissertation

Implementation of novel spraying techniques in vineyards

Costas Michael

Limassol, December 2020

CYPRUS UNIVERSITY OF TECHNOLOGY
FACULTY OF GEOTECHNICAL SCIENCES AND ENVIROMENTAL MANAGEMENT
DEPARTMENT OF AGRICULTURAL SCIENCES, BIOTECHNOLOGY AND FOOD SCIENCE

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Doctoral Dissertation

Implementation of novel spraying techniques in vineyards

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Declaration

The data presented in this PhD dissertation are the results of an original research work conducted by the author at the Cyprus University of Technology (CUT), unless otherwise indicated. The work contained herein has not been submitted, in whole or in part, to obtain any other degree or professional qualification in this or any other academic institution. Data of this study have been published in two peer-reviewed journals. In addition, part of the data of the current dissertation have been presented orally by Costas Michael in a conference, held under the auspices of the 2nd International Conference ADAPTtoCLIMATE 2019.

<u>Scientific publications in referred journals</u>

1. **Michael, C., Gil, E., Gallart, M., Kanetis, L. and Stavrinides, C.M.,** 2020. Evaluating the effectiveness of low volume spray application using air-assisted knapsack sprayers in wine vineyards. *International Journal of Pest Management*, DOI:10.1080/09670874.2020.1807652
2. **Michael, C., Gil, E., Gallart, M., and Stavrinides, C.M.,** 2020. Influence of spray technology and application rate on leaf deposit and ground losses in mountain viticulture. *Agriculture*, DOI:10.3390/agriculture10120615

<u>Oral presentations</u>

1. **C. Michael**, E. Gil, M. Gallart, L. Kanetis, and M. C. Stavrinides, 2019. Evaluating the effectiveness of low volume spray application using air assisted knapsack sprayers in wine vineyards. 2nd International Conference ADAPTtoCLIMATE, 24 – 25 June 2019, Heraklion, Crete, Greece.

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ΓΕΝΙΚΗ ΠΕΡΙΛΗΨΗ

Η μείωση της χρήσης των φυτοφαρμάκων, η βελτίωση της κάλυψης ψεκασμού των καλλιεργειών και η μείωση των απωλειών εκτός στόχου είναι ζωτικής σημασίας για τη βελτίωση της περιβαλλοντικής απόδοσης και της διαχείρισης επιβλαβών οργανισμών. Στόχος της τρέχουσας διατριβής ήταν να δοκιμάσει και να αξιολογήσει την αποτελεσματικότητα σύγχρονων τεχνολογιών εξοπλισμού εφαρμογής φυτοπροστατευτικών προϊόντων, με έμφαση στη μείωση του όγκου ψεκασμού και της ποσότητας των φυτοπροστατευτικών προϊόντων που χρησιμοποιούνται στους αμπελώνες.

Στο Πρώτο Κεφάλαιο αξιολογήθηκε η κάλυψη με ψεκαστικό υγρό από έναν ψεκαστήρα υψηλού όγκου με πέκκα χειρός (1400 L ha^{-1} - HVS1400), έναν επινώτιο μηχανοκίνητο ψεκαστήρα χαμηλού όγκου με υποβοήθηση αέρα (150 L ha^{-1} - LVS150) και έναν επινώτιο μηχανοκίνητο θειαφιστήρα τροποποιημένο ώστε να μπορεί να ψεκάζει υγρά σκευάσματα (250 L ha^{-1} - CS250). Ο μέσος όρος κάλυψης από το ψεκαστικό υγρό σε φύλλα ή τσαμπιά ήταν κάτω από 21% για το CS250, και πάνω από 40 και 55% για τα LVS150 και HVS1400, αντίστοιχα. Η μόλυνση στα φύλλα από την ασθένεια του περονόσπορου της αμπέλου (*Plasmopara viticola)* ήταν κάτω από 5% καθ' όλη τη διάρκεια της καλλιεργητικής περιόδου, με στατιστικά σημαντική επίδραση του ψεκαστήρα, του χρόνου αλλά και αλληλεπίδραση μεταξύ του ψεκαστήρα και του χρόνου (P < 0,001). Ο μέσος όρος προσβολής από την ευδεμίδα της αμπέλου (*Lobesia botrana*) κυμαινόταν γύρω στο 10% και δεν διέφερε σημαντικά μεταξύ των μεταχειρίσεων και του μάρτυρα.

Στο Δεύτερο Κεφάλαιο μελετήθηκε η εναπόθεση της χρωστικής ουσίας, ταρτραζίνης, στα φύλλα και οι απώλειες στο έδαφος από έναν ψεκαστήρα υψηλού όγκου με πέκκα χειρός (1000 L ha^{-1} - HVS1000), έναν επινώτιο μηχανοκίνητο ψεκαστήρα χαμηλού όγκου (200 L ha^{-1} - LVS200) και έναν ψεκαστήρα δενδρωδών καλλιεργειών (νεφελοψεκαστήρα), βαθμονομημένο στα 500 (OS500) ή 250 L ha^{-1} (OS250). Η διάμεσος της εναπόθεσης της χρωστικής ταρτραζίνης σε φύλλα ήταν ανάλογη του όγκου του ψεκαστικού υγρού που εφαρμόστηκε από κάθε ψεκαστήρα. Όταν η τιμή της εναπόθεσης κανονικοποιήθηκε στο 1 kg χρωστικής ανά εκτάριο, το LVS200 είχε την υψηλότερη εναπόθεση, ακολουθούμενο από τα HVS1000,

OS250 και OS500. Η κανονικοποιημένη εναπόθεση στο έδαφος ήταν διπλάσια για το HVS1000 σε σχέση με το LVS200 και τέσσερις φορές υψηλότερη σε σχέση με τα OS250 και OS500.

Στο Τρίτο Κεφάλαιο διερευνήθηκε η κάλυψη και η αποτελεσματικότητα κατά της ευδεμίδας της αμπέλου ψεκασμών με τα OS250, OS500 και HVS σε τρεις διαφορετικές ποικιλίες αμπέλου. Η διάμεσος της προσβολής από την ευδεμίδα στο μάρτυρα ήταν γύρω στο 2,5% για την ποικιλία Carignan, 8% για την ποικιλία Palomino και 3,2% για την ποικιλία Xynisteri. Η διάμεσος της προσβολής στις μεταχειρίσεις των ψεκαστήρων παρέμεινε κάτω από 1,8% για όλες τις ποικιλίες και για τα δύο έτη μελέτης. Τα ευρήματα της τρέχουσας διατριβής εισηγούνται ότι οι ψεκασμοί χαμηλού όγκου είναι μια βιώσιμη και πιο φιλική προς το περιβάλλον λύση σε σχέση με τις εφαρμογές υψηλού όγκου.

GENERAL ABSTRACT

Decreasing pesticide use, improving spray coverage of crops, and reducing off-target losses are crucial for improving the environmental performance of pest management. The goal of the current Dissertation was to test and evaluate the effectiveness of modern pesticide application technologies, with a focus on lowering the volume rate and amount of pesticides used in vineyards. Chapter 1 evaluated spray coverage by a high-volume sprayer (1400 L ha^{-1}, HVS1400), an air-assisted, low volume knapsack sprayer (150 L ha^{-1}, LVS150), and a motorized knapsack dust applicator modified to spray liquid formulations (250 L ha^{-1}, CS250). Mean spray coverage on leaves or bunches was below 21% for CS250, and above 40 and 55% for LVS150 and HVS1400, respectively. Downy mildew leaf infection was below 5% throughout the season, with a significant effect of sprayer, time and an interaction between sprayer and time ($P < 0.001$). Grape berry moth damage did not differ between sprayers and the untreated control.

Chapter 2 evaluated leaf deposit and ground losses generated by a spray gun (1000 L ha^{-1} - HVS1000), a motorized knapsack sprayer (200 L ha^{-1} – LVS200), and a conventional orchard air-blast sprayer calibrated at 500 (OS500) or 250 L ha^{-1} (OS250). When the amount of tracer deposit was standardized to 1 kg ha^{-1}, LVS200 resulted in the highest standardized deposit, followed by HVS1000, OS250 and OS500. Ground losses standardized to 1 kg of tracer ha^{-1} were twice as high for HVS1000 than for LVS200, and four times as high for HVS1000 than for OS250 and OS500.

Chapter 3 assessed spray coverage and pest control effectiveness by OS250, OS500 and HVS. Infestation by the grape berry moth in control plots varied from ca. 2.5% for Carignan, to 8% for Palomino and 3.2% for Xynisteri. Infestation in sprayed plots remained below 1.8% for all sprayer treatments, varieties and both study years. The findings of the current Dissertation suggest that low volume applications are a viable and more environmentally friendly alternative than high volume treatments.

Keywords: Viticulture, volume rate, spray deposition, *Lobesia botrana*, *Plasmopara viticola*, pesticide application methods, losses to the ground, pests, diseases.

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LIST OF ABBREVIATIONS

A.I	Active ingredient
°C	Celsius
ca.	Approximately
cm	Centimetre
CS	Common Sprayer
cv.	Cultivar
e.g	For example
EC	European Commission
et al	And others
EU	European Union
h	Hour
ha	Hectares
HVS	High Volume Sprayer
i.e	In other words
Kg	Kilograms
km	Kilometres
L	Litres
LVS	Low Volume Sprayer
m	Meters
mg	Miligrams
min	Minutes
nm	Nanometers
OIV	International Organization of Vine and Wine
OS	Orchard Sprayer
PAE	Pesticide Application Equipment
s	Seconds
WSP	Water Sensitive Paper
μg	Micrograms

GENERAL INTRODUCTION

Viticulture represents one of the most intensive cultivations worldwide (Pertot et al., 2017). According to OIV (2020) the global area covered by vineyards covers 7.4 million ha, with 37% located in the EU, 34% in Asia and 19% in America. Substantial amounts of plant protection products are used to protect grapes from several pests and diseases, classifying viticulture as a significant polluter of the environment.

Reducing pesticide use in viticulture requires the combination of different approaches, such as cultural, biological, and chemical control in an integrated pest management framework. When other methods fail to control pests and diseases, interventions with pesticides are needed to safeguard yields. Efficient and effective pesticide application relies on the use of modern application technologies and an accurate calibration of the machinery based on the area to be covered (Doruchowski et al., 2012; Gil et al., 2019). Researchers around the globe develop tools (e.g Dosaviña® 2019) in order to determine and reduce, where possible, the volume rate and amount of pesticides used in vineyard sprays.

Cyprus is an island in the eastern Mediterranean, and one of the oldest wine producing countries in the world according to archaeological findings dating more than 4 millennia ago (Constantinou et al., 2017). Vineyards in Cyprus cover ca. 7,000 ha (Cyprus Statistical Service, 2016) and are located mainly in mountainous areas, usually above 600 m in altitude. Mechanization is limited because the average size of the plots is less than 1 ha. Approximately 85% of vineyards lack irrigation facilities. The characteristics of grape growing in Cyprus are typical for most Mediterranean islands and mountainous regions. The term mountain viticulture is used to describe the common characteristics of viticultural practice occurring at an altitude higher than 500 m, slopes greater than 30%, terraces or on small islands (www.cervim.org).

Until recently, vine growers in Cyprus used dust formulations of pesticides to control insect pests and diseases. Dusting of vines simplified pest control, as farmers were able to carry lighter and ready to use formulated products, an important feature especially for vineyards located on steep and rocky hillsides. However, due to stricter EU regulations, dust formulations of synthetic pesticides were banned from the market, because of environmental and health concerns (Directive 79/117/EEC; Directive 91/414/EEC).

In response to the banning of dusts, many farmers modified their dust applicators to spray liquid formulations. However, spray coverage and control of insect pests or diseases by modified dust applicators has never been evaluated. Another group of farmers shifted to the use of High Volume Sprayers (HVS) with spray guns to apply pesticides, but water limitations and the unsuitable vineyard terrain pose insurmountable problems. Furthermore, HVS result in substantial runoff from treated surfaces, polluting the environment and increasing the cost to farmers because of pesticide waste. Spray application technologies available for mountain viticulture are limited, because of the difficulties inherent in cultivating small parcels of land, especially when fields are nested on steep slopes.

Changes affecting Cyprus are a result of the change in the EU policies on pesticide use in the last years. The European Directive 2009/128/EC (Directive 2009/128/EC) on the Sustainable Use of Pesticides aims at reducing the risks and impacts of pesticide use on human health and the environment. The Directive obliged the European member states to set a goal, among others, about the inspection and calibration of sprayers, as well as training on their proper use. In accordance with this goal, initiatives like the “Better Training for Safer Food” (BTSF, 2019), which among other topics, includes training on the proper use and calibration of Pesticide Application Equipment were launched by the European Commission. Setting a concrete and ambitious target for pesticide use reduction, the Farm to Fork Strategy of the EU calls for a 50% decrease in pesticide use by 2030.

Newer pesticide application technologies use lower volumes of pesticide solutions, and rely on assistive technologies, such as air-jets, to improve spray coverage (Matthews et al., 2014). Pesticide coverage (proportion of target area covered with droplets) and deposition (amount of pesticide remaining on target) are two important factors determining the effectiveness of spray applications. HVS on the other hand rely on the use of high volumes of spray liquid to achieve good coverage of target surfaces. As a result, a substantial portion of the spray liquid runs off from the leaves polluting the terrain of the vineyard (Lefrancq et al., 2014). Pesticide drift, i.e., finer droplets that are created by HVS, due to their poorer control of droplet size spectrum is an additional problem (Pergher et al., 1997, Pivato et al., 2015). In addition to the high environmental impact, HVS raise production costs (Otto et al., 2018). Furthermore, HVS are difficult to use in areas with limited access to water resources, since their use requires up to 1500 L ha^{-1} of spray liquid.

The motorized knapsack sprayer is another spraying technology that can be used in viticulture and is classified as a Low Volume Sprayer (LVS). Motorized knapsack sprayers can be used in vineyards with a volume varying from 150 to 250 L ha^{-1} (Michael et al., 2020; Viret et al., 2003). Based on a Venturi system, the pesticide solution passes through a calibration plate and is taken to a diffuser at low pressure, where it meets a high-pressure air jet that micronizes the solution (Matthews et al., 2014).

Another relatively recent spraying technology for vineyards is the axial fan orchard sprayer (OS) equipped with a vineyard tower. The axial fan is driven by the power take-off of the tractor, which uses side air outlets to direct the air-jet into the canopy on the left and right side of the sprayer. The liquid pressure is produced by means of a volumetric pump and a constant pressure valve regulator controls the liquid output. Orchard sprayers are simple in their operation with low labor costs with the main disadvantage being the excessive drift and potential losses to the ground due to the axial fan design (Cross et al., 2001), especially when used for high volume applications. However, OS are versatile machines and can also be used for low volume applications through manipulation of the tractor speed, type of nozzle, and working pressure.

The current state of the art in pesticide application in vineyards includes the use of sensors with real-time data, and geographic information systems to achieve good leaf and fruit coverage and minimize off target spray losses (Gil et al., 2014). Researchers around the globe investigate methods (Grella et al., 2017), tools (Gil et al., 2011; Balsari et al., 2017; Pertot et al., 2017) and machinery (Gil et al., 2007;

Llorens et al., 2010; Wise et al., 2010; Pascuzzi et al., 2017) to improve spray coverage of crops (Miranda – Fuendes et al., 2016). Furthermore, several studies aim at reducing drift (Landers, 2011; Celen et al., 2008; Ambrogetti et al., 2016) and/or losses to the ground (Pergher et al., 1997; Cross et al., 2001) or more generally to reduce pesticide use through correct calibration of agricultural machinery (Siegfried et al., 2007). The most recent research applies new technologies that rely on lower spray volumes to achieve adequate coverage of crops (e.g., Salcedo et al., 2020; Jeongeun et al., 2019).

The goal of the current dissertation was to test and evaluate the effectiveness of modern pesticide application technologies against vineyard pests and diseases, with a focus on lowering the volume rate and amount of pesticide used. Reducing volume rates minimizes pollution of non-target areas, and the amount of pesticide used. The reduction in volume rates benefits the farmer directly, as it lowers the amount of pesticide and water used, and consequently the need for time- and energy-consuming refills of the sprayers.

The first Chapter of the current Dissertation evaluated spray coverage obtained by three types of sprayers: HVS, LVS and CS, using water sensitive papers (WSPs) placed on vines as a proxy (e.g., Gil et al., 2019; Campos et al., 2019 and references therein). In addition, the study quantified the effectiveness of pesticide applications against the grape berry moth [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)] and downy mildew [*Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni (Order: Peronosporales, Family: Peronosporaceae)], two of the most common pest/disease problems affecting viticulture in Cyprus and worldwide. HVS was tested at the volume of 1400 L ha^{-1} following the common spraying practice of the farmers, while CS and LVS at 250 and 150 L ha^{-1}, respectively.

The aim of Chapter 2 was to define the most effective combination of spray technology and volume rate for the specific case of mountainous viticulture in Cyprus, and generate useful recommendations considering the particularities of vines. The study assessed the deposit on the vine canopy and the losses to the ground via runoff for three different types of sprayers: a) an HVS with a spray gun calibrated at 1000 L ha^{-1}, b) A tractor mounted air-blast OS used for both high and low volume applications, calibrated at 500 (OS500) and 250 L ha^{-1} (OS250), respectively and c) An LVS calibrated at 200 L ha^{-1}.

The third Chapter aimed at evaluating spray coverage and pest control effectiveness against the grape berry moth by two different spray technologies and volume rates: An HVS calibrated at 1000 L ha^{-1}, and an OS calibrated at 500 or 250 L ha^{-1}. The HVS represented the current standard practice, while OS500 and OS250 a reduction of 50 and 75% in both volume and pesticide amount compared to HVS. Experiments were carried out in three different grape varieties over two consecutive years in mountain vineyards in the Mediterranean island of Cyprus.

Although very promising, spray coverage and biological efficacy of spray applications with LVS and OS have never been evaluated in vineyards to the best of our knowledge. The increasing availability and adoption of modern pesticide technologies, such as LVS and OS, presents an opportunity to lower volume rates and reap the associated environmental, human health and financial benefits. Nevertheless, limited research has been carried out to evaluate the effectiveness of spray equipment in mountain viticulture (Viret et al., 2003; Wise et al., 2010). The current Dissertation came to cover the knowledge gap in vineyard spraying by combining different spray technologies and volume rates to identify the most effective practice(s) for mountain viticulture, using the island of Cyprus as a case study.

1. Chapter 1: Evaluating the effectiveness of low volume spray application using air-assisted knapsack sprayers in wine vineyards

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1.1 Abstract

We evaluated spray coverage on grape leaves and bunches by a high-volume sprayer (HVS), an air-assisted, low volume knapsack sprayer (LVS), and a motorized knapsack dust applicator modified to spray liquid formulations (common sprayer – CS). At the full development of the vine canopy 1400, 250 and 150 L ha^{-1} were applied via HVS, CS and LVS, respectively. Sprayer type, leaf or bunch orientation, leaf position and leaf side significantly affected spray coverage ($P < 0.001$). Mean spray coverage on leaves or bunches was below 21% for CS, and above 40 and 55% for LVS and HVS, respectively. Downy mildew leaf infection was below 5% throughout the season, with a significant effect of sprayer, time and an interaction between sprayer and time ($P < 0.001$). Grape berry moth damage did not differ between sprayers and the untreated control. The current work suggests that LVS might be a viable alternative to HVS, but further work is needed to establish whether differences in spray coverage affect biological efficacy.

Keywords: Volume rate, spray deposition, *Lobesia botrana*, *Plasmopara viticola*, pesticide application methods

1.2 Introduction

Worldwide vineyard acreage covers 7.6 million ha, with 3.3 million ha located in the EU (OIV, 2017). Several pests and diseases cause significant crop losses in vineyards, and the frequent pesticide use ranks viticulture as one of the most intensively agrochemical-treated crops. The recognition of viticulture as a significant polluter of the environment led to the development of tools to determine and reduce, where possible, the volume rate and amount of pesticides used in vineyard sprays (e.g. Agrometeo, 2018; Dosaviña®, 2018; Optidose, 2018). Similarly, European projects, such as “LIFE-FITOVID” (LIFEFITOVID, 2018) or the “Ecophyto 2018” plan in France (Ecophyto, 2018) aim at the reduction of the use of pesticides, while maintaining high yields and quality in agricultural production. Horizontal initiatives with a wider scope, such as the European Directive 2009/128/EC on the sustainable use of pesticides put further pressure on reducing the environmental impact of pest management.

Modern pesticide application technologies aim at reducing the environmental impact of pesticide applications in vineyards through achieving a better coverage of target surfaces and minimizing off target losses, such as runoff from the vine to the soil (e.g., TOPPS project). The current state of the art in pesticide application in vineyards includes the use of sensors with real-time data, and geographic information systems to achieve good leaf and fruit coverage and minimize off target spray losses (Gil et al., 2014). However, in vineyards on steep slopes where mechanization is limited, growers rely mainly on high volume sprayers with spray guns (HVS) for pesticide applications.

HVS rely on the use of high volumes of spray liquid to achieve good coverage of target surfaces. As a result, a substantial portion of the spray liquid runs off from the leaves polluting the terrain of the vineyard (Lefrancq et al., 2014). Pesticide drift, i.e., finer droplets that are created by HVS, due to their poorer control of droplet size spectrum is an additional problem (Pergher et al., 1997, Pivato et al., 2015). In addition to the high environmental impact, HVS raise production costs (Otto et al., 2018).

Furthermore, HVS are difficult to use in areas with limited access to water resources, since their use requires up to 1500 L ha^{-1} of spray liquid.

An interesting alternative technology for small-scale grapevine cropping systems located on steep terrains is the use of knapsack low volume sprayers (LVS) (Viret et al., 2003). LVS rely on a Venturi system, whereby the product passes through a calibration plate and is taken to a diffuser at low pressure, where it meets a high-pressure air jet that micronizes the solution (Matthews et al., 2014). The system allows users to position diffusers close to the vegetation to be treated, consequently avoiding drift and waste of the product. Although very promising, spray coverage and biological efficacy of spray applications with LVS and HVS have never been evaluated in vineyards to the best of our knowledge.

The challenges faced by growers in complying with and adapting to new environmental regulations and consumer preferences are very well represented in the Mediterranean island of Cyprus. The island is one of the oldest wine producing countries in the world, with archaeological findings dating wine production more than 4 millennia ago (Constantinou et al., 2017). Today, vineyards cover ca. 7,000 ha, with the majority of the acreage consisting of plots of less than 1 ha in size located above 600 m of altitude (Cyprus Statistical Service, 2016). The lack of water, typical for most Mediterranean regions, is very common in Cyprus with approximately 85% of the vineyards lacking irrigation systems. In the recent past, dust formulations of pesticides simplified plant protection in Cypriot vineyards, as farmers were able to carry lighter, ready to use formulated products, an important feature especially for vineyards located on steep and rocky hillsides. However, due to stricter EU regulations, dust formulations of synthetic pesticides were banned from the market, due to environmental concerns. Many farmers have adapted to the new legislation by modifying dust applicators to spray liquid formulations, but coverage effectiveness obtained by such common sprayers (CS) has never been evaluated. In many cases, local farmers shift to the use of HVS with spray guns to apply pesticides to runoff, but water limitations and the unsuitable vineyard terrain pose insurmountable problems.

Pesticide coverage (proportion of target area covered with droplets) and deposition (amount of pesticide remaining on target) are two important factors determining the effectiveness of spray applications. The purpose of the current study was to evaluate spray coverage obtained by three types of sprayers: HVS, LVS and CS, using water sensitive papers (WSPs) placed on vines as a proxy (e.g., Gil et al., 2019; Campos et al., 2019 and references therein). In addition, we quantified the effectiveness of pesticide applications against the grape berry moth [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)] and downy mildew [*Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni (Order: Peronosporales, Family: Peronosporaceae)], two of the most common pest/disease problems affecting viticulture in Cyprus and worldwide.

1.3 Materials and Methods

1.3.1 Sprayers tested

In the present study the following sprayers were tested (Figure 1): 1) High Volume Sprayer with spray gun (HVS) (Honda GX 120, 4.0 HP), 2) Motorized air assisted Low Volume knapsack Sprayer (LVS) (CIFARELLI, Mist Blower M1200), and 3) Common knapsack Sprayer (CS) (Dinyi, Model 3WF-3, JAPAN). The CS had been originally used for dust applications and was adjusted for liquid sprayings, by equipping it with an electric pump (FLO-2203, Singflo, 12V), which was operating on battery power for constant supply of spraying liquid. This type of modification is very popular among grape farmers in Cyprus, who prefer it rather than buying a new type of sprayer. The velocity of the air jet produced by LVS and CS was 14.3 and 12.7 m/s, respectively (measurement at 50 cm using a Davis #271 electronic wind speed indicator - anemometer). The size of droplets produced by each sprayer is an important factor that may influence spray effectiveness, but it was beyond the scope of the current work to quantify droplet size.

Figure 1: Sprayers tested from left to right: 1) High Volume Sprayer with spray gun (HVS) (Honda GX 120, 4.0 HP), 2) Motorized air assisted Low Volume knapsack Sprayer (LVS) (CIFARELLI, Mist Blower M1200), and 3) Common knapsack Sprayer (CS) (Dinyi, Model 3WF-3, JAPAN)

1.3.2 Experimental design and spraying technique

The study was conducted in 2014 in a 0.6 ha vineyard planted to the indigenous white variety “Xynisteri” in Koilineia village, Paphos, Cyprus (latitude 34.5345^{o}: longitude 32.3813^{o}, altitude: 930 m). The vines were planted in 2010 and trained as a sprawled (goblet) system. Vine spacing was 1.5 m within and 2.7 m between rows.

Vines were assigned into four treatments as follows (Figure 2): 1) Control treatment (C) where vines were left untreated, 2) HVS, 3) CS and 4) LVS. Each of the four treatments was replicated four times in a Latin square design. Each replicate consisted of 5 rows x 8 vines. The study covered the period from May 5^{th} to September 16^{th}, 2014, when the grapes were harvested.

Figure 2: Experimental vineyard and study design (Latin square with four replicates per treatment): Control (C), HVS (High Volume Sprayer), CS (Common Sprayer), and LVS (Low Volume Sprayer)

Applications were made to both sides of each treated row, by the same operator at a constant walking speed of 0.25 m/s. The nozzle of the sprayer formed a ca. 30° angle with the row axis (Figure 3). Because the vine canopy develops within a short period of time (Siegfried et al., 2007; Gil et al., 2014), we adjusted and measured the flow rate (L min^{-1}) and volume applied (L ha^{-1}) by each sprayer at three different stages of vine growth (Raisigl et al., 1991 - Caliset method), a) Early stage (mid-May): BBCH 65, b) Medium stage (mid-June): BBCH 75, c) Final stage (late July): BBCH 81 (Table 1). The concentration of pesticides in the spray solution was kept constant for all three sprayers (Table 2), which resulted in different amounts of pesticide applied per ha because of the variation in the volume rate among sprayers (Table 1).

Table 1: Volume rate (L ha^{-1}), flow rate (L min^{-1}) and the resulting active ingredient (AI) dose ha^{-1} as a percentage of the AI dose reported on the label (Table 2) for the three different sprayers at subsequent vine developmental stages.

Sprayer	Vine stage					
	BBCH 65		**BBCH 75**		**BBCH 81**	
	volume (L ha^{-1}) / flow rate (L min^{-1})	% of AI dose ha^{-1}	volume (L ha^{-1}) /flow rate (L min^{-1})	% of AI dose ha^{-1}	volume (L ha^{-1}) / flow rate (L min^{-1})	% of AI dose ha^{-1}
CS	170 / 0.69	17%	220 / 0.89	22%	250 / 1.01	25%
LVS	100 / 0.40	10%	120 / 0.49	12%	150 / 0.60	15%
HVS	800 / 3.24	80%	1250 / 5.06	125%	1400 / 5.67	140%

1.3.3 Determination of spray coverage on leaves and bunches

Spray coverage was evaluated at the full development stage (BBCH 81), on August 5, 2018. Canopy height was 1 m and canopy width 1.5 m. Prior to spray applications water sensitive papers (WSP - Syngenta, Switzerland, 26 x 76 mm) were placed on leaves and clusters on the 3rd and the 6th vine of the 3rd row of each replicate (Figure 2).

On each vine, WSPs were placed on leaves on two different orientations: Facing perpendicular to the direction of the row axis and facing parallel to the direction of the row axis (Figure 3). At each orientation, WSPs were placed at two positions: 1) Interior leaf (inner area of the canopy), with the WSP folded widthwise so that one half of it (26 x 38 mm) lied on the upper side of the leaf (adaxial), and the other half on the lower leaf side (abaxial), 2) Exterior leaf, with the WSP folded widthwise to cover both the upper and lower leaf side as for the interior leaf (Figure 3).

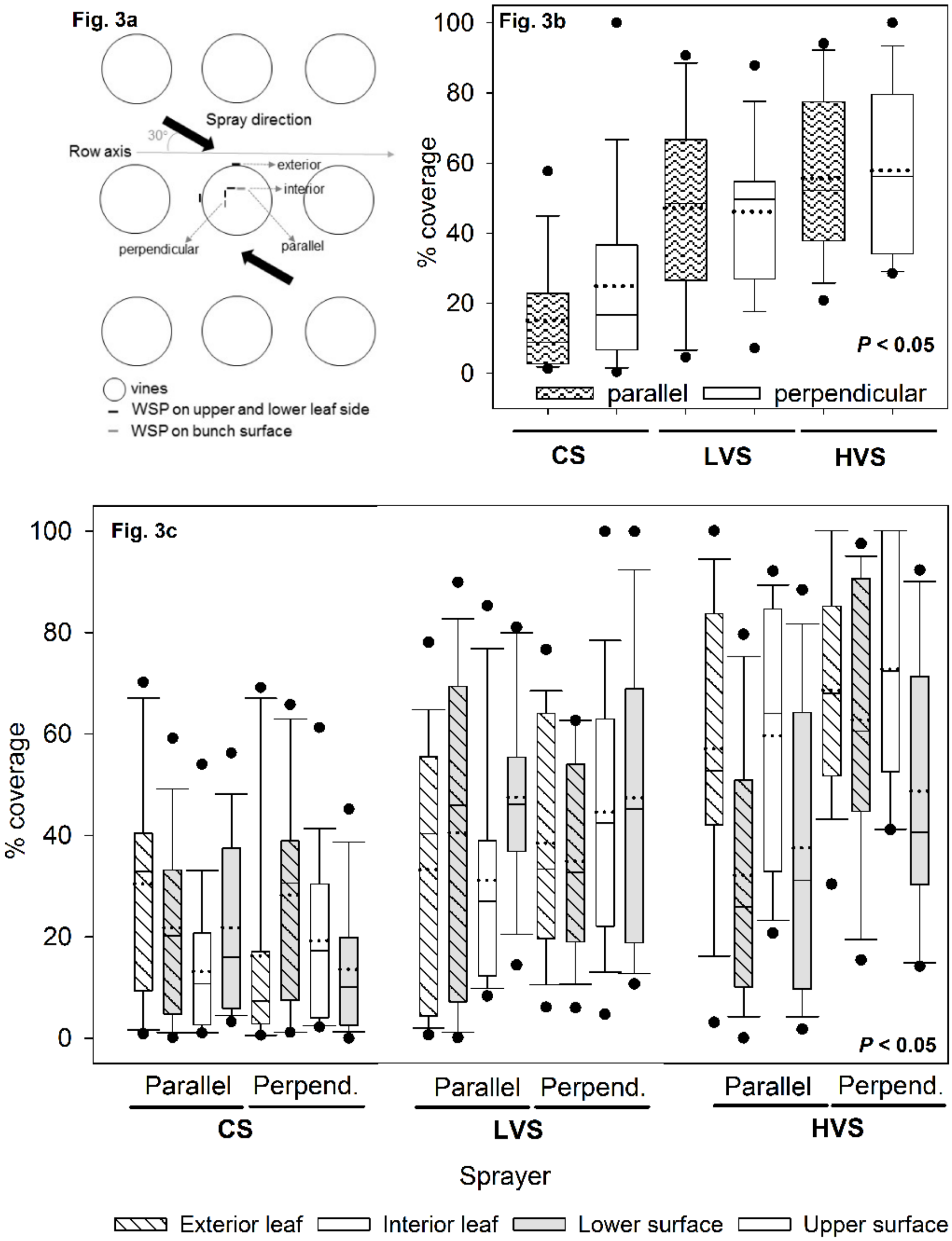

Figure 3: a) Schematic representation of the positioning of water sensitive papers (WSPs) on leaves and bunches of vines. WSPs were placed on two bunces at two orientations, with one facing parallel and the other perpendicular to the row axis. WSPs on leaves were placed at different locations (interior or exterior leaves), orientations (facing parallel or perpendicular to the row axis), and sides (upper or lower leaf side) – see text for details on

number and placement of WSPs, b) Effect of sprayer type (CS: Common Sprayer; LVS: Low Volume Sprayer; HVS: High Volume Sprayer) on coverage of WSPs on bunches, and c) Effect of sprayer type on coverage of WSPs placed on leaves. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers the 10th and 90th percentile. Points outside the 10th and 90th percentile (outliers) are shown by dots. The dashed line shows the mean. See text for details on results of statistical analysis)

In addition, on each vine we attached one WSP (26 x 38 mm) on the surface of two grape bunches at each of the two orientations (one WSP per punch placed either perpendicular or parallel to the row axis –Figure 3a). Both grape bunches were in interior areas of the canopy.

In total, the treated area was monitored with 512 WSPs for leaves (4 treatments x 4 replicates x 4 vines per replicate, x 4 leaves per vine x 2 sides per leaf) and 128 WSPs for bunches (4 treatments x 4 replicates x 4 vines per replicate x 2 bunches per vine). On control vines, WSPs were placed before any spraying and were picked up at the end of all sprays, to evaluate drift potential from other treatment plots. In the three remaining treatments, (CS, LVS, HVS), WSPs were placed just before the spraying and were picked up after the spraying of each specific treatment. WSPs were placed separately in envelopes and transferred to the laboratory to quantify spray coverage.

Water-sensitive papers were processed using the software ImageJ (Rasband 1997-2008, version 1.49b). The images were taken at a resolution of 24 pixels·mm^{-1}. On each image the coverage (percentage of surface covered by all the droplets present in the image) was obtained.

1.3.4 Control measures against the grape berry moth and fungal diseases

The grape berry moth initially attacks grape flowers and at later generations unripe and ripe berries. Infestations by the moth during berry maturation reduce the quantity and quality of harvested grapes, and lead to secondary infections by botrytis bunch rot, caused by *Botrytis cinerea* (Fermaud et al., 1992). Sprays against *L. botrana* were performed following recommendations by the Department of Agriculture of Cyprus, based on captures of male moths in pheromone traps placed in selected vineyards of the region from early March through late September. The traps were checked weekly and agricultural announcements were issued accordingly. The dates of insecticide applications for grape berry moth management and the dose of active ingredient (AI) ha^{-1} are shown in Table 2. In total six insecticide applications were applied for grape berry moth management.

Excessive rainfall and high humidity during the 2014 growing season were considered predisposing factors to downy mildew. In total five fungicide applications were made for downy mildew management (Table 2). In the last two fungicide applications, a botryticide was also included to protect berries from botrytis bunch rot.

Table 2: Dates, active ingredient (AI) / commercial products and the AI dose (L or kg ha^{-1}) used for pesticide applications by the three sprayers.

Each of the three sprayers under evaluation was used to apply the pesticides listed in the table to four replicate groups of vines per sprayer, as outlined in Error! Reference source not found.**.**

Date	AI	Commercial name	AI dose (L or kg ha^{-1})*	Insect / disease
May 20	chlorpyriphos ethyl 48%	Nufos 48 EC	1.5	Grape berry moth
	tebuconazole 25%	Mystic 25 EC	0.4	Powdery mildew
	fosetyl-Al 80%	Alfil 80 WP	3	Downy mildew
June 18	chlorpyriphos ethyl 48%	Nufos 48 EC	1.5	Grape berry moth
	tebuconazole 25%	Mystic 25 EC	0.4	Powdery mildew
	fosetyl-al 80%	Alfil 80 WP	3	Downy mildew
July 2	cypermethrin 50%	Valliant 50 EC	0.2	Grape berry moth
	penconazole 10%	Topas 100 EC	0.4	Powdery mildew
	mancozeb 64% + cymoxanil 8%	Cymoprem	3	Downy mildew
July 15	lambda cyhalothrin 10%	Capoeira 10 CS	0.25	Grape berry moth
	penconazole 10%	Topas 100 EC	0.4	Powdery mildew
	mancozeb 64% + cymoxanil 8%	Cymoprem	3	Downy mildew
	pyrimethanil 30%	Pyrus 300 SC	0.1	Botrytis bunch rot

August 5	cypermethrin 50%	Valliant 50 EC	0.2	Grape berry moth
	trifloxystrobin 50%	Flint 50 WG	0.125	Powdery mildew
	fosetyl-Al 80%	Alfil 80 WP	3	Downy mildew
	pyrimethanil 30%	Pyrus 300 SC	0.1	Botrytis bunch rot
August 28	lambda cyhalothrin 10%	Capoeira 10 CS	0.25	Grape berry moth

* Based on a volume rate of 1000 L ha^{-1}, as reported on the label.

1.3.5 Control efficacy against infestations by grape berry moth and infections by downy mildew

For the evaluation of grape berry moth infestations, three bunches per vine for ten vines per replicate were collected at harvest (September 17, 2014) and the number of infested and non-infested berries per cluster were recorded (following the OEPP/EPPO guidelines – PP1/11(3)). Grape bunches were collected from the 2nd to the 6th vine in rows 2 and 3 of each replicate, leaving at least three vines on the row and one guard row between replicates.

For the assessment of downy mildew infection, three leaves per vine were collected from the 10 plants used for grape berry moth damage assessment (30 leaves per replicate). The percentage of leaf area with downy mildew infection symptoms was recorded at intervals of 5%. Assessments of downy mildew infection took place at biweekly intervals beginning on June 18 and ending on September 16, 2014, one day before harvest. There were no downy mildew infections by June 18, and therefore only data from the second assessment (June 27, 2014) and onwards were included in the analyses.

1.3.6 Statistical analysis

Data on spray coverage of water sensitive paper on either bunches or leaves were analyzed with a generalized mixed effects model in R statistical package (R Core Team 2017) using the package lme4 (Bates et al. 2015). Models were fitted to the data with the function glmer, using the binomial distribution family and logit link function. The glmer function allows weighting of samples by sample size and applies the LaPlace approximation to parameter estimation. Model dispersion was calculated as the square root of the penalized residual sum of squares divided by *n*, the number of observations, with the function dispersion in the package blmeco (Korner-Nievergelt et al. 2015).

The model on coverage on WSPs placed on leaves included as fixed factors sprayer type, leaf orientation (perpendicular or parallel to row axis), leaf position (exterior or interior of the vine canopy), and leaf side (upper - adaxial or lower - abaxial) as well as their interactions as fixed effects. Leaves nested within vines within blocks were included as random effects. We used the bobyqa optimization with the maximum number of function evaluations set to 10^9 to achieve model convergence.

The model evaluating the coverage of WSPs placed on bunches included as fixed effects sprayer type and bunch position (perpendicular or parallel to spray direction), as well as their interaction as fixed effects, and vines nested within blocks as random effects.

Data on infestation of berries by the grape berry moth were also analyzed with a generalized mixed effects model in R statistical package. The model included as fixed effects sprayer type and vines nested within blocks as random effects. The model converged without the need for using the bobyqa optimization.

Data on downy mildew leaf infection were analyzed in a similar framework with treatment, time and their interaction as fixed effects and vines nested within blocks as random effects. For the downy mildew analysis, we used the bobyqa optimization with the maximum number of function evaluations set to 10^9 to achieve model convergence.

Because the models were slightly over-dispersed, indicating the presence of extra-binomial variability, significance of treatment effects was evaluated using conditional *F* tests with corrected degrees of freedom (Bolker et al. 2009, Pinheiro and Bates 2000, p. 90-92) calculated by fitting a linear mixed effects model with the function lme in the package nlme (Pinheiro et al. 2017).

1.4 Results and Discussion

1.4.1 Determination of spray coverage on leaves and bunches

The assessment of spray coverage of WSPs placed on bunches or leaves, was carried out once, at the BBCH 81 stage, when the vines attained their full development. The mean spray coverage of WSPs placed on grape bunches remained below 20% for CS and was ca. 47 and 57% for LVS and HVS, respectively (Figure 3b). There were significant differences in spray coverage of WSPs both between sprayers and orientation with a significant interaction between sprayer and orientation (Table 3). Mean spray coverage was similar for the two orientations for LVS and HVS, but for CS it was higher on WSPs placed perpendicular to the row axis (Figure 3b). The model was slightly over-dispersed (dispersion parameter = 3.2). The standard deviation associated with the random factor block was 0.36 and that for vines nested within blocks 0.92, suggesting that between vine variation is an important component of spray coverage.

Mean spray coverage of WSPs placed on leaves was around 21% for CS, and 40 and 55% for LVS and HVS, respectively (Figure 3c). There were significant differences between sprayers and leaf side (upper or lower), but not between orientation (parallel or perpendicular to the row axis), or leaf position (interior or exterior – Table 3). However, the four-way interaction, as well as three- and two-way interactions were also significant (Table 3). The model was slightly over-dispersed (dispersion parameter = 3.2). The standard deviation associated with the random factor block was 0.24, for vines nested within blocks 0.48, and for leaves nested within vines within blocks 0.91.

Coverage of WSPs placed on the upper side of leaves was consistently higher in HVS for all positions and orientations (Figure 3c). However, for CS and LVS there was not a consistent trend of higher coverage of the upper leaf side compared to the lower side of the leaf (Figure 3c). In addition, HVS was the only sprayer with a consistently higher coverage of leaves oriented perpendicular to the row lines, with no clear trend for CS and LVS (Figure 3c). Coverage of exterior vs interior leaves did not follow a consistent trend for any of the sprayers.

Spray coverage of WSPs in the control was below 1% for both bunches and leaves (data not shown). While drift was not evaluated as part of the study design, the virtually zero coverage of WSPs placed on control plants, indicates that movement of spray droplets between neighboring rows is low. However, further research is required to determine the quantity of spray liquid that is lost to the air and the vineyard soil. Placement of boards with WSPs on the ground would enable the assessment of the runoff, and needs to be carried out in future research.

Table 3: Model results for the analysis of the effect of different factors on coverage of water sensitive papers (WSPs).

Factor	*df*	*F*-value	*P*-value
A) Model for WSP on leaves			
sprayer	2, 44	30.64	< 0.001
leaf.orientation	1, 134	3.95	0.05
leaf.position	1, 134	0.01	0.94
leaf.side	1, 177	85.84	< 0.001
sprayer x leaf.orientation	2, 134	4.78	0.01
sprayer x leaf.side	2, 134	3.30	0.04
leaf.orientation x leaf.position	1, 134	0.10	0.76
sprayer x leaf.side	2, 177	244.76	< 0.001
leaf.orientation x leaf.side	1, 177	1.15	0.29
leaf.position x leaf.side	1, 177	0.11	0.74
sprayer x leaf.orientation x leaf.position	2, 134	0.75	0.48
spraer x leaf.orientation x leaf.side	2, 177	44.05	< 0.001
sprayer x leaf.position x leaf.side	2, 177	23.12	< 0.001
leaf.orientation x leaf.position x leaf.side	1, 177	96.60	< 0.001
sprayer x leaf.orientation x leaf.position x leaf.side	2, 177	38.01	< 0.001

B) Model for WSP on bunches			
sprayer	2, 42	21.17	< 0.001
orientation	1, 43	22.97	< 0.001
sprayer x orientation	2, 43	21.12	< 0.001

The general trend of higher coverage with HVS rather than LVS was expected because of the higher volume of spray liquid applied with each sprayer, at 1400 L ha^{-1} for HVS and 150 L ha^{-1} for LVS (Table 1). However, the difference in coverage between the two sprayers was not proportional to the difference in the amount of spray liquid used. For instance, overall mean coverage by HVS was around 55% for both leaves and bunches, while for LVS it ranged between ca. 40% for leaves and 47% for bunches. A key finding of the present work is that the current practice of HVS spraying until runoff can result in an exceedance of the AI dose ha^{-1}. For example, at the full development stage, the LVS applied 15%, while the HVS 140% of the AI dose ha^{-1} reported on the pesticide label. Subsequently, a large amount of the HVS applied pesticide solution runs off to the soil, increasing the risk for environmental pollution.

In the CS treatment, mean coverage was around 20% for both leaves and bunches (Figure 3b & c), even though the amount of spray liquid used per unit area was higher to that for LVS, at 250 L ha^{-1} (Table 1). CS is a motorized knapsack dust applicator which was equipped with an electric pump to perform liquid sprays. The modification of the CS, as adopted by growers in Cyprus, does not seem to be effective, since despite the fact we used almost twice as much spray liquid, the achieved target coverage was less than half of LVS. It is possible that the lower air velocity of the CS in comparison with the LVS did not result in sufficient leaf movement to allow the spray penetrate into the foliage. Out of the three types of sprayers, only LVS and HVS achieved coverage of WSPs higher than 30%, which is considered effective for pesticide applications (Gil et al., 2014). We note that the 30% limit is an empirical assumption, which may vary per physical and biochemical mode of action of applied AI. While we did not quantify the uniformity of coverage within the WSPs, we did not observe any trends between sprayers, i.e., the distribution of spray droplets within each WSP appeared to be uniform. Further work needs to measure the uniformity of spray coverage, as well as spray coverage and deposition at the early stages of vine growth.

An interesting finding of the current work is the consistently higher coverage of the upper side of the leaves in HVS but not for the other two sprayers (Figure 3c). HVS was also the only sprayer with a consistently higher coverage of leaves oriented perpendicular to the row axis. This is probably a result of the spray angle, which was ca. 30° for leaves oriented in parallel to the vineyard rows, and ca. 60° for leaves oriented perpendicular to the row axis (Figure 3c). The non-consistent differences in coverage of the two leaf sides and orientations in CS and LVS are probably a result of the leaf movement created by the air-jet. Additionally, the air-assisted spraying in CS and LVS achieved a more similar distribution of the spray coverage of leaves, as indicated by shorter whisker extensions and outliers of the boxplots, compared to HVS (Figure 3c). There were no consistent differences in spray coverage of exterior vs interior leaves for any of the three sprayers (Figure 3c). The Xynisteri variety does not develop a dense canopy, and therefore the spray liquid penetrates equally well external and internal leaves.

LVS can achieve dispersion and distribution of the spray material similar to that of HVS (Wise et al., 2010). Furthermore, Viret et al., (2003) showed that knapsack LVS achieve good coverage of the foliage and bunches with a spray volume of 400 L ha^{-1} at the full development of the vine canopy. In dry areas with inaccessible terrain that usually lack irrigation infrastructure, the transportation of large water quantities for spraying applications is extremely difficult (Otto et al., 2018) thus, the use of LVS is a potential reliable alternative that can be adopted by growers.

In addition, LVS are more environmentally friendly compared to HVS, because they reduce runoff (Viret et al., 2003). During spraying it was observed that HVS application resulted in substantial runoff. The excessive runoff of the spraying material from the outer leaves of the vine often misleads farmers who consider that they fully and effectively sprayed vines. However, a relatively small amount of spraying liquid penetrates the foliage and reaches the grape bunches of the sprawl system. A high runoff of spraying liquid is not desirable, since there is a waste of spraying material, resulting in economic loss for the producer and soil and subsequent groundwater pollution through leaching (Lefrancq et al., 2014). No runoff was observed with the LVS, because most of the spraying liquid ended up on target. However, the application of low volume rates with air assisted knapsack sprayers is a harder and a more hazardous task than the implementation of high-volume sprays, because the farmer must carry continuously the knapsack sprayer within the vineyard, resulting in higher operator dermal exposure (Tsakirakis et al., 2014; Thouvenin et al., 2016).

1.4.2 Control efficacy against infestations by grape berry moth and infections by downy mildew

Although spray coverage varied between sprayers (Figure 3b & c), there were no significant differences in berry infestation by the grape berry moth between treatments at harvest (F = 0.74, df = 3, 142, P = 0.50,Figure 4). There was no indication of any serious overdispersion for the model (dispersion parameter = 1.5). The standard deviation associated with the random factor block was 0.45 and that for vines nested within blocks 1.07. Mean infestation remained at around 10% for all treatments (Figure 4).

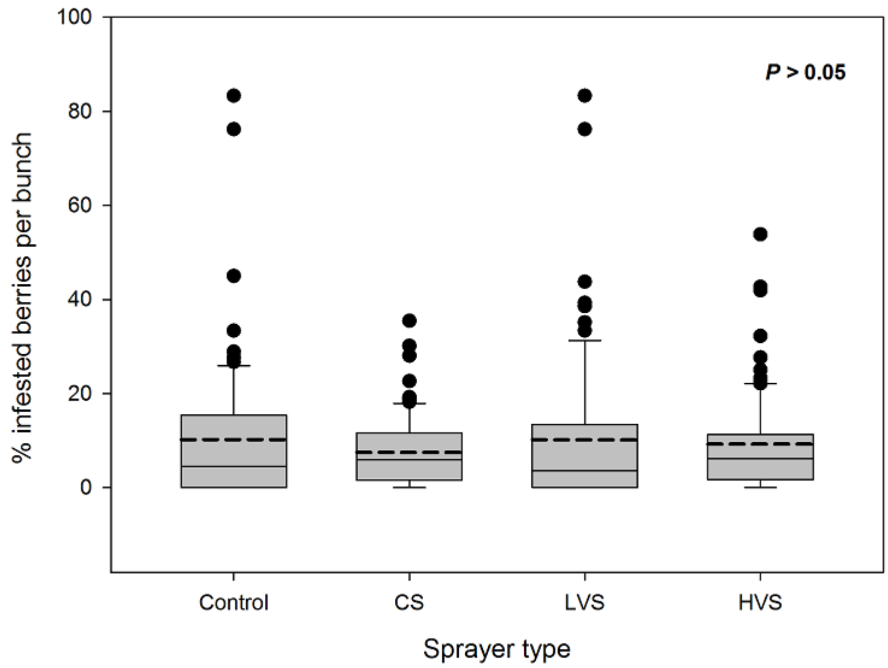

Figure 4: Effect of sprayer type (CS: Common Sprayer; LVS: Low Volume Sprayer; HVS: High Volume Sprayer) on infestation of grape berries by the grape berry moth at harvest.

Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile,while whiskers the 10th and 90th percentile. Points outside the 10th and 90th percentile (outliers) are shown by dots. The dashed line shows the mean. See text for details on results of statistical analysis

The effectiveness of spray applications depends among other factors on spray coverage, the used AI, the levels of pesticide resistance in the target population, and the timing of pesticide applications (Berger-Neto et al., 2017, Buchholz et al., 2016, Van den Berg et al., 2016). In the current work, it seems that factors other than spray coverage may have affected control effectiveness. A potential problem is the lack of a specialized day degree model for grape berry moth development for the Cypriot populations (Baumgärtner et al., 2012). Incorrect timing of pesticide applications can miss the window of effectiveness between larval hatch and boring into grape berries, where the larvae are better protected from pesticide applications.

Downy mildew infection remained at low levels (Figure 5) throughout the season.

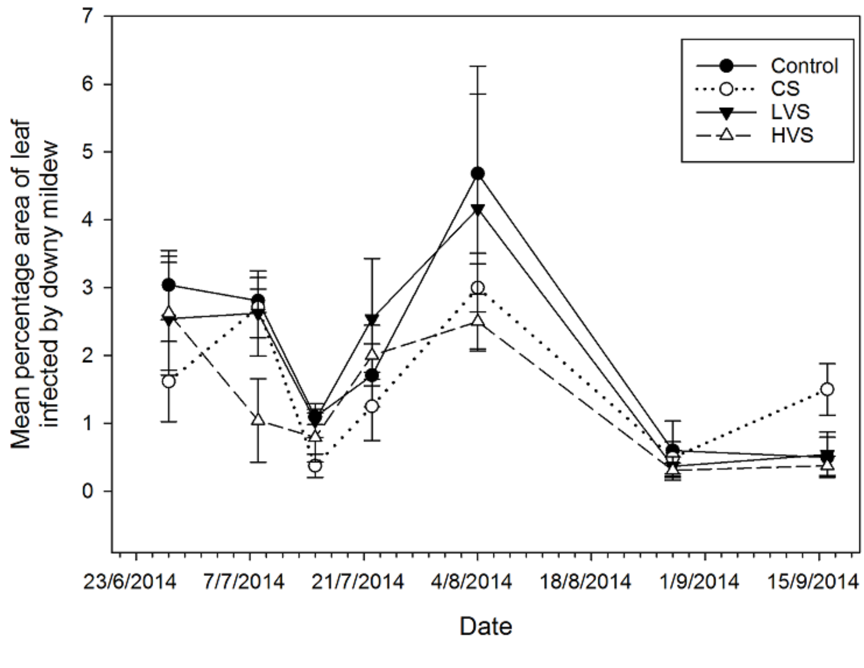

Figure 5: Effect of sprayer type (CS: Common Sprayer; LVS: Low Volume Sprayer; HVS: High Volume Sprayer) on infection of vine leaves (mean ± 1 S.E.) by downy mildew through the growing season.

There were significant differences in leaf infection by downy mildew between treatments (F = 5.6, df = 3, 189, P = 0.001). There was also a significant effect of application time (F = 273.7, df = 6, 3140, P <0.001), and a significant interaction between treatment and time (F = 20.4, df = 18, 3140, P <0.001). The model was slightly overdispersed (dispersion parameter = 2.4). The standard deviation associated with the random factor block was 0 and that for vines nested within blocks 0.85. Downy mildew peaked in early August (Figure 5) and infection rates fluctuated through mid-August, when high temperatures and low humidity led to a decrease in disease incidence. Downy mildew infection was generally consistently lower in HVS and higher in the control treatment. Disease incidence in CS and LVS varied, and was on some occasions higher than control, although differences were small and not biologically meaningful. The highest overall infection by downy mildew in the vineyard was recorded on August 4 at 4.7, 4.2, 3.0 and 2.5% for control, CS, LVS, and HVS, respectively. Although there were significant differences between treatments and an interaction with time, the differences for the most part were not considered important from a disease management perspective, as infection rates were low throughout the season (Figure 5). The current study did not evaluate pesticide deposition, and spray coverage was only assessed at the full development stage only. Therefore, additional work is needed to establish the relationship between biological efficacy and spray deposition/coverage, a challenging task as spray deposition and coverage are only two of several factors influencing control effectiveness.

1.5 Conclusions

At the full development of the vine canopy 1400, 250 and 150 L ha^{-1} were applied via HVS, CS and LVS, respectively. Mean spray coverage on leaves or bunches was below 21% for CS, and above 40 and 55% for LVS and HVS, respectively. Downy mildew leaf infection was below 5% throughout the season, with a significant effect of sprayer and time and an interaction between the two (P < 0.001). Grape berry moth damage did not differ between sprayers and the untreated control. The results of the study show that adequate spray coverage can be achieved with volumes as low as 150 L ha^{-1}, in accordance with previous research (Holowniki et al., 2000; Chen et al., 2013). Spraying with the HVS to runoff can lead to an exceedance of the dose of AI ha^{-1} (Table 1).

The reduction in spray volume with LVS is associated with a proportional reduction of AI applied per unit area (Table 1). This reduction will lead to an equivalent reduction of the amount of pesticide in the cases where concentration of AI is used as dose recommendation. In those cases, results obtained in this research will allow the reduction of pesticide amount used, in accordance with the European Directive 2009/128/EC on the Sustainable Use of Pesticides. This scenario, where a reduced amount of water (and AI) is applied following accurate procedures to determine the optimal volume rate, allows the achievement of a high level of efficacy and efficiency, and guarantees an optimal biological efficacy of the process (Gil et al., 2019).

However, for those pesticide dose recommendations based on kg or L ha^{-1} of AI, lowering the spray volume without reducing the quantity of AI does not decrease pesticide use. Furthermore, since AI doses are expressed as kg or L ha^{-1}, they do not account for changes in canopy structure through the season. Determination of the AI dose based on the stage of plant growth and the surface of the leaf area (Barani et al., 2008; Gil et al., 2007) can lead to a reduction of the quantity of pesticides applied and therefore the production cost without a corresponding compromise in the effectiveness of sprays.

Spray coverage and deposition are only two of several factors affecting control effectiveness, and factors such as timing of pesticide applications and the presence of resistance in the target pest may have affected control effectiveness in the current study.

2 Chapter 2: Influence of spray technology and application rate on leaf deposit and ground losses in mountain viticulture

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2.1 Abstract

Leaf deposit and ground losses generated from spray application in mountain viticulture were evaluated. Four treatments were examined: A spray gun (1000 L ha^{-1}) (High-Volume Sprayer - HVS), a motorized knapsack sprayer (200 L ha^{-1}) (Low Volume Sprayer - LVS), and a conventional orchard mist blower calibrated at 500 L ha^{-1} (OS500) and 250 L ha^{-1} (OS250). The four treatments were assessed using the same tank concentration of tracer in two training systems: a trellis and a goblet. Sprayer treatment, vine side and vine height had a significant effect on leaf deposit ($P < 0.05$). The absolute amount of leaf deposit increased with application volume, but when the amount of deposit was standardized to 1 kg ha^{-1}, LVS resulted in the highest deposit, followed by HVS, OS250, and OS500. Deposition for the goblet system was ca. half that for the trellised vineyard. Ground losses standardized to 1 kg of tracer ha^{-1} were twice as high for HVS than for LVS, and four times as high for HVS than for OS250 and OS500, in both training systems. The current work suggests that low volume applications in vineyards are a viable and more environmentally friendly alternative than high volume treatments.

Keywords: Volume rate, spray deposition, losses to the ground, viticulture.

2.2 Introduction

European member states are obliged to implement the European Directive 2009/128/EC (Directive, 2009/128/EC) on the Sustainable Use of Pesticides, which aims at reducing the risks and impacts of pesticide use on human health and the environment. Among the major goals of the Directive are the inspection and calibration of sprayers, as well as training on their proper use. To achieve the goals of the Directive, the European Commission launched the initiative “Better Training for Safer Food” (BTSF, 2019), which among other topics, includes training on the proper use and calibration of Pesticide Application Equipment (PAE).

Pesticide applications aim at depositing the highest-possible amount of the active ingredient on the target surface (e.g., the leaf), where the target pest resides and/or feeds (Matthews et al., 2014). However, even with state-of-the-art sprayers, a quantity of pesticide can drift through the air or can be lost to the ground. Pesticide drift and losses to the ground result in environmental pollution and tools are being developed to measure and reduce off target losses (Balsari et al., 2017; Lefrancq et al., 2014; Cross et al, 2001). A major cause of ground losses is the runoff of spray liquid from the treated surface, a consequence of not using an appropriate dosing system, or because of performing low uniformity treatments from inadequate use and poor maintenance of application equipment (Miranda-Fuentes et al., 2016). ISO 22866 (2005) defines drift as the quantity of a plant protection product that is carried out of the treated area by the action of air currents during the application process. Many authors have attempted to quantify spray drift and direct ground losses generated by different circumstances, types of equipment, and working parameters (Arvidsson et al., 2011; Gil et al., 2013a; Gregorio et al., 2014; Nuyttens et al., 2010; Landers, 2010)

Substantial amounts of plant protection products are used for protecting grapevines, placing viticulture amongst the most intensive cultivations worldwide (Pertot et al., 2017). Vineyards cover a surface area of 7.5 million ha globally, with 37% of the grape production being in Europe, 34% in Asia and 19% in America (OIV, 2018). Mountain viticulture is an extreme form of vine growing occurring at an altitude higher than 500 m, slopes greater than 30%, terraces or on small islands (www.cervim.org). A common feature of mountain viticulture is the small size of vineyards that precludes intensive mechanization. Mountain viticulture is also characterized by a difficulty of using high amounts of water for pesticide applications, because of scarce water resources and/or the lack of irrigation facilities. The options of spray application technologies available for mountain viticulture are limited, because of the difficulties inherent in cultivating small parcels of land, especially when fields are nested on steep slopes.

Application of plant protection products in mountain viticulture relied traditionally on spray guns, also characterized as High-Volume Sprayers (HVS). HVS can be either on tractor (mounted or trailed) or motorized (mobile units) and require high volumes of water, up to 1500 L ha^{-1} (Michael et al., 2020). Spraying using high volumes results in high drift and runoff (Pergher et al., 1997; Koch, 2007; Pivato et al., 2015). Furthermore, many farmers often apply pesticides to the point of runoff as a guarantee of high biological efficacy (Miranda-Fuentes et al., 2015a). Spray guns are still in use, although today the majority of orchards and vineyards are sprayed with machine operated air blast sprayers. Spray guns are still the most common spraying technique used by farmers in many mountainous vineyards, usually at volumes higher than 1000 L ha^{-1}.

The motorized knapsack sprayer is another type of sprayer used in viticulture. The sprayer relies on a Venturi system, whereby through a calibration plate, the product passes and is taken to a diffuser at low pressure, where it meets a high-pressure air jet that micronizes the solution (Matthews et al., 2014). Motorized knapsack sprayers can be used in vineyards with a volume varying from 150 to 250 L ha^{-1} (Michael et al., 2020; Viret et al., 2003), and are classified as Low Volume Sprayers (LVS).

Another relatively recent spraying technology for vineyards is the axial fan orchard sprayer (OS) equipped with a vineyard tower. The axial fan is driven by the power take-off of the tractor, which uses side air outlets to direct the air-jet into the canopy on the left and right side of the sprayer. The liquid pressure is produced by means of a volumetric pump and a constant pressure valve regulator controls the liquid output. Orchard sprayers are simple in their operation with low labor costs with the main disadvantage being the excessive drift and losses to the ground due to the axial fan design (Cross et al., 2001), especially when used for high volume applications. However, OSs are versatile machines and can also be used for low volume applications through manipulation of the tractor speed, type of nozzle, and working pressure.

Research on pesticide deposition and ground losses in viticulture has included the testing different types of sprayers (Pergher et al., 1997; Baldoin et al., 2008) or more advanced equipment such as ultrasonic sensors for target detection (Gil et al., 2007). Nevertheless, limited research has been carried out to evaluate the effectiveness of spray equipment in mountain viticulture (Viret et al., 2003). The aim of the current study was to define the most effective combination of spray technology and volume rate for the specific case of mountainous viticulture in Cyprus, and generate useful recommendations considering the particularities of vines. Our work assessed the deposit on the vine canopy and the losses to the ground via runoff for three different types of sprayers: a) an HVS with a spray gun, b) A tractor mounted air-blast OS used for both high and low volume applications, and c) An LVS.

2.3 Materials and Methods

2.3.1 Spray application equipment

In the present study the following combinations of sprayers and volume rates were tested (Figure 6):

1. A High-Volume Sprayer (HVS) with spray gun (Honda GX 120, Hamamatsu, Japan) equipped with a 4.0 HP engine, with a hose length of 100 m, calibrated at a nominal volume of 1000 L ha^{-1}
2. A conventional Orchard Sprayer (OS) equipped with a vertical tower (Arcadia Terra, Model Cronos, Greece) calibrated at 500 L ha^{-1} (OS500)
3. The same conventional Orchard Sprayer calibrated at 250 L ha^{-1} (OS250)
4. A Motorized air assisted knapsack sprayer (CIFARELLI Mist Blower M1200, CIFARELLI, Voghera, Italy) adapted for Low Volume Spray (LVS) calibrated at 200 L ha^{-1}.

For both OS treatments, the sprayer was equipped with 12 nozzles arranged on two vertical booms (6 nozzles per side), fixed at the mid-point between the consecutive air outlets. To adapt the sprayer to the height of the vines, only the three lower nozzles on each side were used. Sprayings were made by moving the sprayer along two consecutive rows of crops. In this way the vines were sprayed on both sides. The equivalent performance is one row per pass.

Figure 6: Sprayers tested: a) HVS with a spray gun b) LVS (Motorized knapsack sprayer) c) OS (Axial fan orchard sprayer)

2.3.2 Experimental design and spraying technique

The study was conducted in 2016 in two 0.3 ha^{-1} vineyards, planted with the indigenous white variety Xynisteri. The vineyards were located in Lemona village, Paphos, Cyprus (34° 51′ 47″ N, 32° 33′ 26″ E, altitude: 308 m). Both vineyards were planted in 2004. The first vineyard was trained as a trellis system and the second as a goblet (sprawled) system. Vine spacing was 1.65 m within and 2.25 m between rows in both vineyards.

Spray deposition was evaluated on July 13, 2016 at the BBCH 79 stage (majority of grape berries touching). The sprayers were used to spray 154 plants per treatment (7 rows x 22 plants per row) (Figure 7). Applications were made to both sides of each treated row, by the same person – sprayer, at the same speed and technique. Working parameters and calibration values of the sprayers during the tests are provided in Table 4.

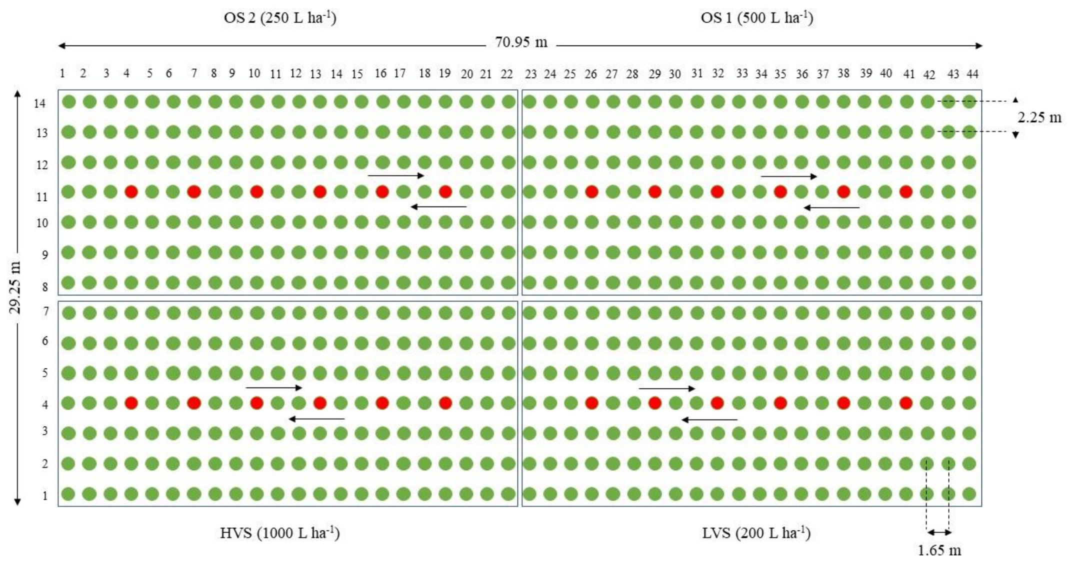


Figure 7: Experimental design. Red circles show sampling vines.

Spraying was carried out with an aqueous solution of a tracer, the food color adjuvant Tartrazine (E 102) 85% at a nominal concentration of 4000 mg L^{-1}. Tartrazine is photostable, non-toxic and has high recovery rates since it remains on the leaves when it dries and can be washed out from the leaves in the lab with distilled water (Naud et al., 2014, Pergher, 2001). Before and after every test by each sprayer, a tank sample was taken to measure the actual tracer concentration, while the sprayer was activated at the set operating pressure in a static position. The samples were collected and stored in a dark recipient for laboratory analysis to obtain the reference absorbance value.

During the spraying, best management practices for a good and safe spray application process were followed (TOPPS). Air temperature, relative humidity and wind speed were measured by a WatchDog 2000 Series Weather Station (Spectrum Technologies, Inc., USA). The weather station was placed at a height of 2.0 m, free from obstacles. For the trellis system, the mean wind velocity during the trial was 0.3 m s^{-1} and the mean values for temperature and RH were 33.7 °C and 26.9%, respectively. For the goblet system, mean wind velocity was 0.2 m s^{-1} and the mean values for temperature and RH were 35 °C and 25.8%, respectively.

Table 4: Forward speed (km h^{-1}), actual volume rate (L ha^{-1}), flow rate (L min^{-1}) and number of nozzles for the four different treatments.

Treatment - nominal volume rate (V_R)	**Forward speed (km h^{-1})**	**Actual volume rate (L ha^{-1})**	**Flow rate (L min^{-1})**	**Number of Nozzles**
HVS (High Volume Sprayer - 1000 L ha^{-1})	1.5	1077	10.00	1
OS500 (Orchard Sprayer – 500 L ha^{-1})	4.0	524	12.96	6
OS250 (Orchard Sprayer 250 L ha^{-1})	4.0	283	7.00	6
LVS (Low Volume Sprayer - 200 L ha^{-1})	1.5	188	1.75	1

2.3.3 Characterization of the canopy

Canopy size characterization parameters for the vines for the two training systems were measured in the vineyard at the BBCH 79 stage (Table 5). The leaf area index (LAI) was determined by the area-weight ratio estimation (Cross et al., 2001; Gil et al., 2007; Llorens et al., 2010). For the purposes of the study, a canopy area of 1.0 m in length for the trellis training system and a single vine for the goblet training system, were randomly selected. All the leaves were collected into plastic bags (one for each training system) and the weight of each leaf was determined in the laboratory.

Table 5: Canopy characterization parameters for the two training systems where the trials took place at BBCH 79.

Vineyard	Row distance (m)	Distance between plants (m)	Canopy height (m)	Canopy width (m)	LAI
Trellis system	2.25	1.65	1.18	0.85	1.34
Goblet system	2.25	1.65	0.98	1.05	1.00

To determine the relationship between leaf weight and leaf area, 18 leaves were collected randomly from each training system (trellis & goblet). Each leaf was weighted and its surface (one side only) was measured with the software ImageJ (Rasband 1997-2008, version 1.49b). The relationship between leaf weight and leaf area was determined using linear regression.

2.3.4 Leaf sampling procedure

Before spray application, 25 leaves from each training system were collected as blank samples. Those leaves were taken so that the pre-spraying amounts of tartrazine (expected to be near zero) could be determined.

Leaf samples to evaluate spray deposit were collected from the central row of each treatment in order to avoid cross contamination from neighboring treatments (Figure 7). Additionally, the first three and last three plants on each row were excluded from the sampling process for the same reason.

Once the spray residues dried out, leaves were collected from six vines per treatment (Figure 7). Nine leaves were collected from each vine, representing nine different zones: three heights (top, middle and bottom of the canopy) x three depths (outer left, center and outer right side) (Figure 8), following the methodology used in previous trials in vineyards (Gil et al., 2007; Llorens et al., 2010; Gil et al., 2011). Subsequently, there were three positions on the left side of the vine, three in the middle and three on the right side, that resulted in a total of nine zones, covering the whole canopy. Collected leaves were placed individually in plastic bags and were stored in a cool box, until transportation to the laboratory, where they were placed in a refrigerator until measurements took place.

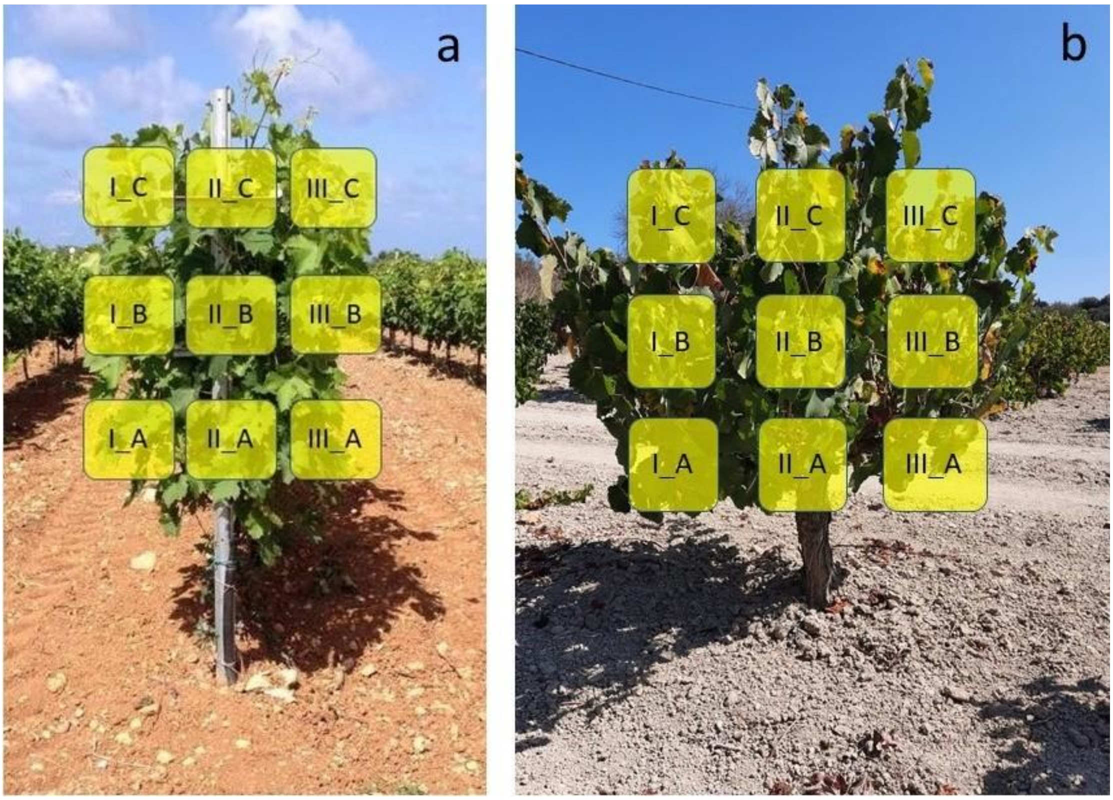


Figure 8: Leaf sampling positions for a) Trellis trained vines and b) Goblet trained vines. Leaves were taken from three heights (A-C) and three sides (I-III), resulting in a total of nine leaf samples per vine.

2.3.5 Quantification of spray deposition on leaves

In the laboratory, each plastic bag containing samples was weighted. The weight of the bag was subtracted from the total to estimate the weight of the leaf. The leaf surface area was estimated based on the relationship between leaf weight and leaf area (see section on Characterization of the canopy).

The total amount of tracer per unit leaf surface (μg cm^{-2}) was measured following Llorens et al. (2010). Briefly, 20 mL of deionised water were added to each plastic bag containing the sample. The bag was shaken for at least one minute to allow tartrazine dissolve in the water. The tracer concentration in the solution was measured using a Tecan Infinite M200 Pro Fluorometer (Tecan Austria GmbH, Austria, Europe) by using absorbance spectrometry at L = 423 nm (Naud et al. 2014).

The amount of tracer deposited on each sample was determined by dividing the amount of tracer deposited on each leaf by the area of the collector (leaf) according to Equation 1, as proposed by Gil et al. (2007) and Llorens et al. (2010):

$$d = (T_{cl} \text{ x } w) / L_a \qquad \text{(Eq. 1)}$$

where d is the actual deposit (μg cm^{-2}) per leaf area, T_{cl} is the tracer concentration in the washing solution of the sample (mg L^{-1}), w is the deionized water volume (ml), and L_a is the surface area of the upper leaf side (cm^2).

Data normalization

The normalized deposition d_N was calculated to account for differences between nominal and actual tracer concentration and volume rate for each sprayer (Table 4) (Gil et al., 2007; Llorens et al., 2010; Salcedo et al., 2020).

$$d_N = d \times f_{Tcs} \times f_{VR}, \qquad \text{(Eq. 2)}$$

where d_N is the normalized tracer deposit (μg cm^{-2} leaf), f_{Tcs} is a factor correcting for differences between the nominal (T_{cs} - 4000 mg L^{-1}) and actual concentration of the tracer in the spray tank, and f_{VR} compensates for the difference between the nominal (V_R) and actual volume rate for each sprayer (Table 4).

The deposit on leaves standardized to one kg of tracer per ha (d_G) was calculated as follows (Codis et al., 2018):

$$d_G = (d_N \times 10^6)/(T_{cs} \times V_R), \qquad \text{(Eq. 3)}$$

where d_G is the amount of deposit per unit of tracer applied per hectare (μg cm^{-2}/kg tracer ha^{-1}), d_N is the normalized tracer deposit (μg cm^{-2}), T_{cs} is the tracer concentration in the tank (mg L^{-1}), and V_R is the nominal application rate (L ha^{-1}) (Table 4).

Following Codis et al. (2018) the amount of tracer deposit (µg cm^{-2}) standardized over a volume of 100 L ha^{-1} was determined as follows:

$$d_{100} = (d_N \times 100)/V_R \qquad \text{(Eq. 4)}$$

where d_{100} is the deposit (µg cm^{-2}/100 L ha^{-1}), d_N is the normalized tracer deposit (µg cm^{-2} leaf), and V_R is the nominal application rate (L ha^{-1}).

2.3.6 Evaluation of spray losses to the ground

To assess spray losses to the ground for each treatment, a wooden board (40 cm x 20 cm) with two round pieces (11 cm Ø) of absorbent filter paper (Whatman, Nº 4 Qualitive) was placed on the ground (Pergher et al., 1997) under each vine from which leaves were sampled to collect spray deposits (total of six boards per treatment). Tartrazine has a high recovery rate from absorbent paper (Pergher et al., 1997). The determination of the spray losses was assessed in the same way as for the leaves. Each filter paper, after the spray, was placed in a plastic bag, stored in a coolbox in the field and afterwards in a refrigerator until extraction in the laboratory.

2.3.7 Statistical analyses

Statistical analyses were carried out using the statistical software R (R Core Team, 2019). The relationship between leaf weight and leaf area was determined using linear regression (function lm) as implemented in the base package of R (R Core Team, 2019).

The data on spray deposition on leaves were analysed in a linear mixed effects model framework in the package lme4 with the function lmer (Bates et al., 2015). Treatment, vine side, vine height and their interactions were included as fixed factors and vine (plant) as a random factor to account for the multiple measurements per plant. A natural logarithm transformation was applied to fulfill the assumption of homoscedasticity. Degrees of freedom for *F*-tests were estimated with Satterthwaite's approximation as implemented in the Anova function of the package lmerTest (Kuznetsova et al., 2017). The difflsmeans function of the lmerTest package was used to compare treatment means for the losses to the ground data. A similar approach was followed for the analysis of losses to the ground, with vine included as a random factor.

2.4 Results

2.4.1 Characterization of the canopy

The canopy characterization parameters are presented in Table 5. There was a significant relationship between leaf area and leaf weight for both varieties (Figure 9). For leaves from trellised vines, the intercept was estimated at 22.07 ± 4.45 (estimate ± 1 SE), while the slope at 34.04 ± 1.86 (leaf area = 22.07 + 34.04 * leaf weight), and the regression was statistically significant (F = 336.2; df = 1, 16; P < 0.001; R^2 = 0.95). For leaves from vines trained in the goblet system, the intercept was estimated at 28.34 ± 4.16, the slope at 29.48 ± 1.69 (leaf area = 28.34 + 29.48 * leaf weight), and the relationship was also statistically significant (F = 304.8; df = 1, 16; P < 0.001; R^2 = 0.95). The LAI for the trellis system was 1.34 and for the goblet 1.02.

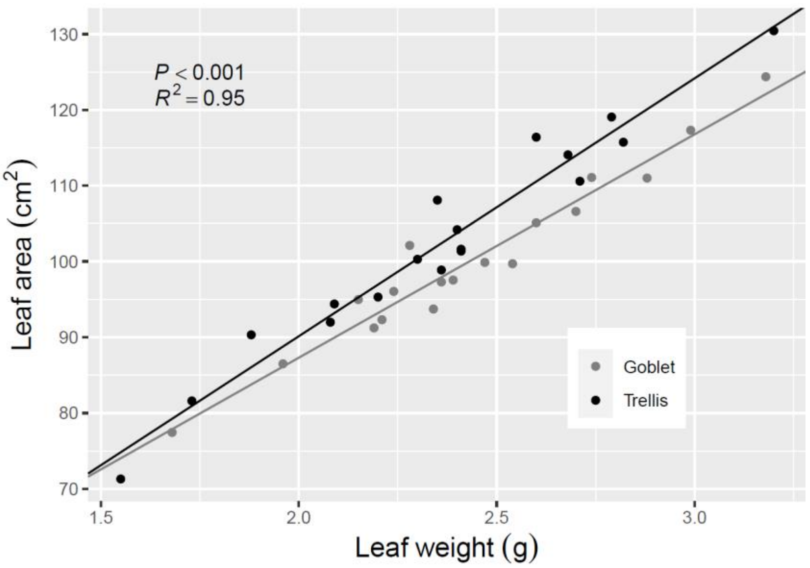
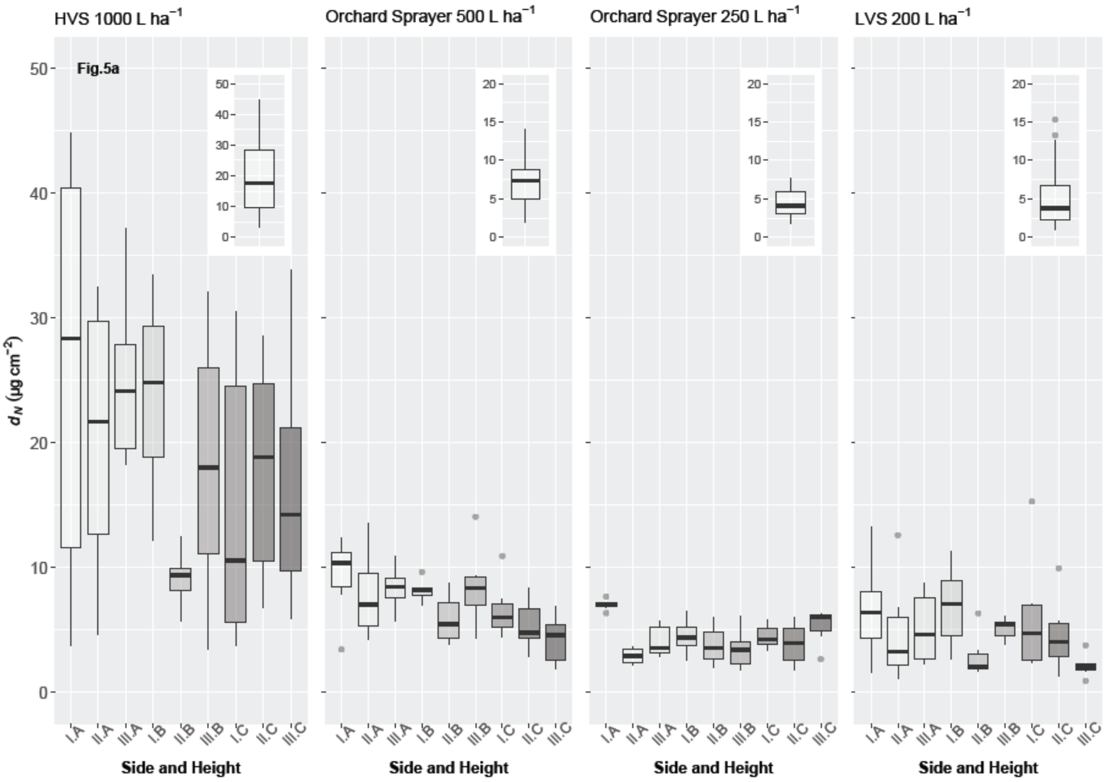


Figure 9: Relationship between leaf area and leaf weight for leaves collected from Xynisteri vines trained as either goblet or trellis. See text for results of statistical analyses.

2.4.2 Quantification of spray deposition on leaves

Tracer concentration in the blank leaf samples was lower than the detection limit of the spectrophotometer (<0.01 ppm) for both training systems.

The d_N for the trellis system was higher for HVS, followed by OS500, OS250 and LVS (Figure 10a). The median d_N was 17.57, 7.33, 4.12 and 3.80 μg cm^{-2} for HVS, OS500, OS250 and LVS, respectively. The main effects for sprayer, side and height sampling position were statistically significant (Table 6). The interactions between side and height, and sprayer, side and height were very close to significance, and were retained in the model (Table 6). The d_N was generally higher on the lower and middle than the top part of the vine (Figure S2.1), and there was a trend of higher d_N on the outer sides of the vine compared to the interior part (Figure S2). Low d_N values were reported from the central middle part of the canopy (sampling area IIB – Figure 8) for all sprayers (Figure 10), and especially HVS. The variability in d_N was higher in HVS, followed by LVS and the two OS treatments. Among vine variation was important source of variability for d_N (Table 6Table 6: Results of the linear mixed effects model for the effect of sprayer, side and height on d_N on leaves for the trellis and goblet training systems.).



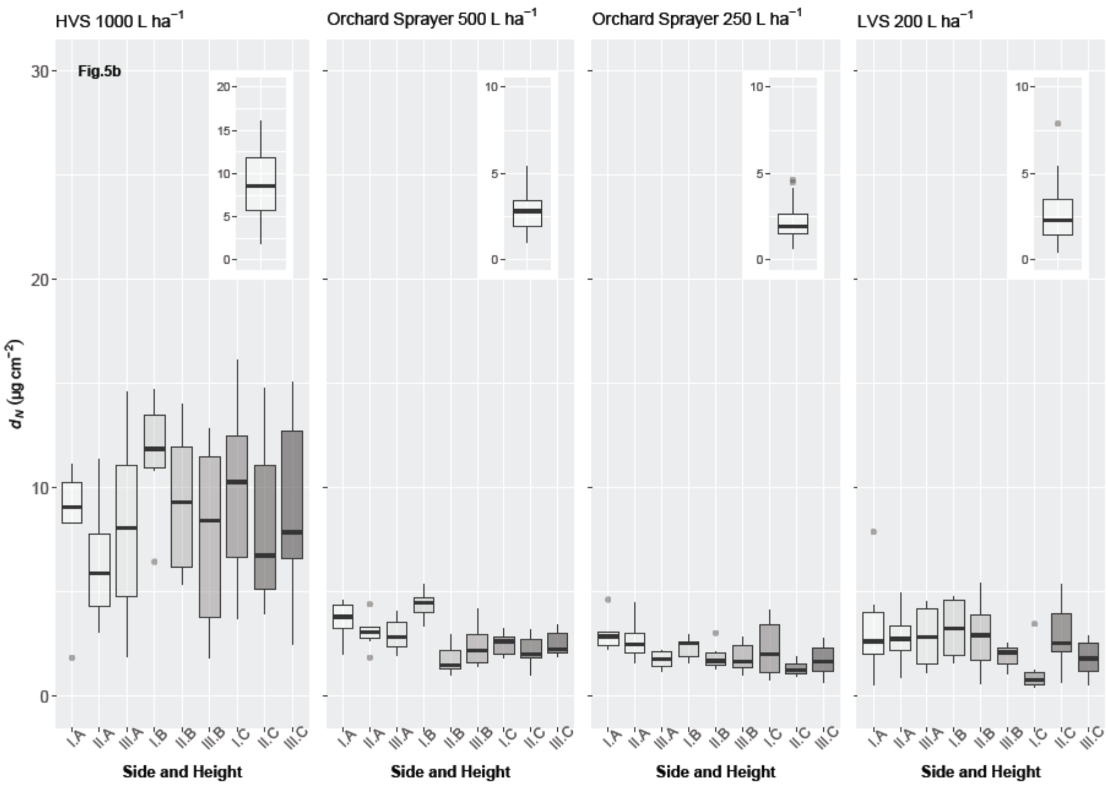


Figure 10: Normalized deposition (d_N) for different sides (II - interior part of the vine) and heights (A - lower – see Fig. 3 for details) of vines for (a) the trellis and (b) the goblet training system. The insets show d_N values for all leaves for each sprayer (note the different scale for the HVS inset). Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually. See text for results of statistical analyses.

Table 6: Results of the linear mixed effects model for the effect of sprayer, side and height on d_N on leaves for the trellis and goblet training systems.

Fixed effects	***df***	***F*-value**	***P*-value**	***F*-value**	***P*-value**
		Trellis		**Goblet**	
Sprayer	3, 20	58.17	0.02	63.32	< 0.001
Side	2, 160	8.96	< 0.001	3.71	0.03
Height	2, 160	5.74	0.004	5.05	0.01
Sprayer: Side	6, 160	0.72	0.63	1.62	0.15
Sprayer: Height	6, 160	1.32	0.25	1.99	0.07
Side:Height	4, 160	2.31	0.06	2.12	0.08
Sprayer: Side:Height	12, 160	1.74	0.06	1.06	0.40
Random Effect	Vine	0.114		0.102	
(standard deviation)	Residual	0.510		0.503	

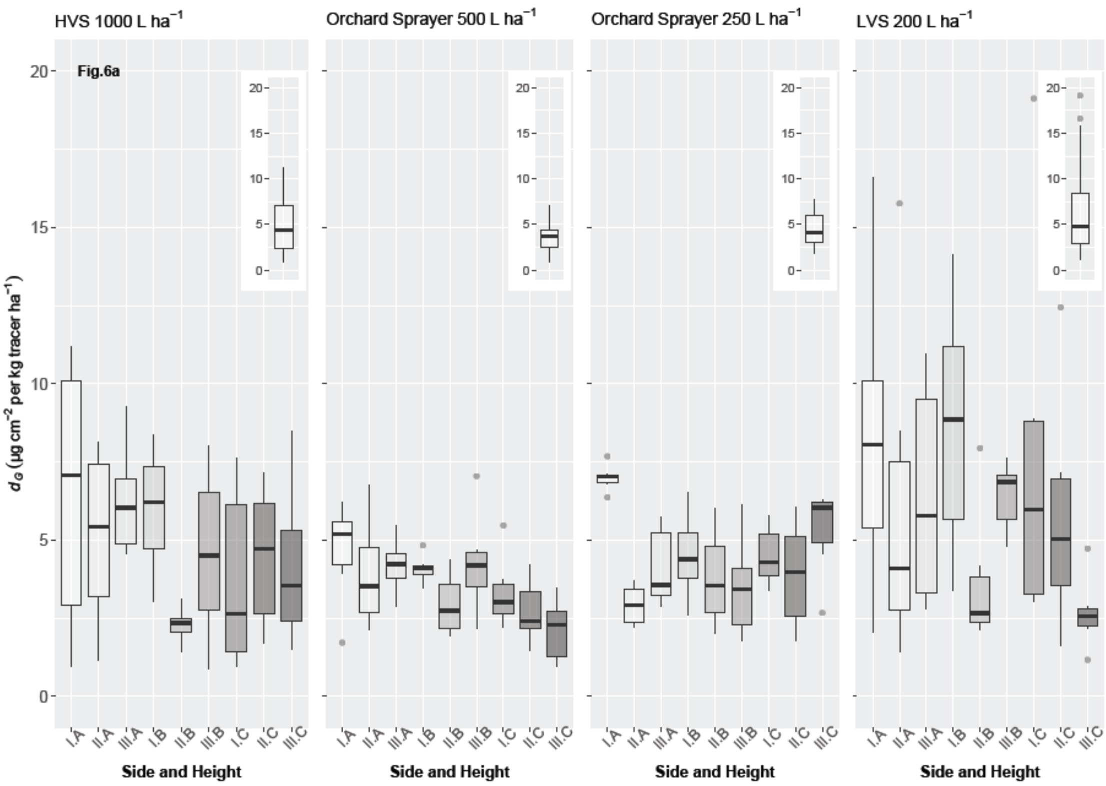
The d_N for the goblet system was higher on leaves sprayed with the HVS, followed by OS500, LVS and OS250 (Figure 10b). The median d_N was 8.59, 2.83, 2.32 and 1.96 µg cm^{-2} for HVS, OS500, LVS and OS250, respectively. The main effects for sprayer, side and height were statistically significant (Table 6). The interactions between sprayer and height, and side and height were very close to significance. With the exception of HVS, d_N was higher on lower parts of the vine (Figure S2.1Figure S2.1: Interactions between sprayer and height). A weak trend of lower d_N in the internal part of the vine was observed only for OS250 and OS500 (Figure S2). The variability in d_N was higher in HVS, followed by LVS and the two OS treatments. Among vine variation was important source of variability for d_N (Table 6).

The median d_G values for trellised vines were 4.75, 4.39, 4.12 and 3.67 µg cm^{-2}/kg tracer ha^{-1} for LVS, HVS, OS250 and OS500 respectively (Figure 11a). The results of the statistical analysis showed that the main effects for sprayer, side and height were statistically significant (Table 7). The interactions between side and height, and sprayer, side and height were very close to significance. The variability in d_G for LVS and HVS was generally greater than for OS500 and OS250. For each sprayer, the trend among sides and height was the same as for d_N. Among vine variation was important source of variability for d_G (Table 7).

For the goblet training system, the median d_G values were 2.90, 2.15, 1.96 and 1.42 µg cm^{-2}/kg tracer ha^{-1} for LVS, HVS, OS250 and OS500, respectively (Figure 11b). The main effect for sprayer, side and height was significant (Table 7). The interactions between sprayer and height, and side and height were not far from significance (Table 7). The variability in d_G was higher for LVS and HVS than for OS500 and OS250. Within each sprayer the trend among sides and height was the same as for d_N. Among vine variation was important source of variability for d_G (Table 7).

Table 7: Results of the linear mixed effects model for the effect of sprayer, side and height on d_G or d_{100} on leaves for the trellis and goblet training systems. The analysis for d_{100} is equivalent to that for d_G as the two parameters differ only by a divisor of 2.5 (see equations 3 and 4).

Fixed effects	***df***	***F*-value**	***P*-value**	***F*-value**	***P*-value**
		Trellis		**Goblet**	
Sprayer	3, 20	4.17	0.02	11.95	< 0.001
Side	2, 160	8.96	< 0.001	3.70	0.03
Height	2, 160	5.74	0.004	5.05	0.01
Sprayer: Side	6, 160	0.72	0.63	1.61	0.15
Sprayer: Height	6, 160	1.32	0.25	1.99	0.07
Side: Height	4, 160	2.31	0.06	2.11	0.08
Sprayer: Side: Height	12, 160	1.74	0.06	1.06	0.40
Random Effect	Vine		0.114		0.102
(standard deviation)	Residual		0.510		0.503


HVS 1000 L ha⁻¹
Orchard Sprayer 500 L ha⁻¹
Orchard Sprayer 250 L ha⁻¹
LVS 200 L ha⁻¹
Fig.6a
d_G (µg cm^{-2} per kg tracer ha^{-1})
I.A
II.A
III.A
I.B
II.B
III.B
I.C
II.C
III.C
Side and Height

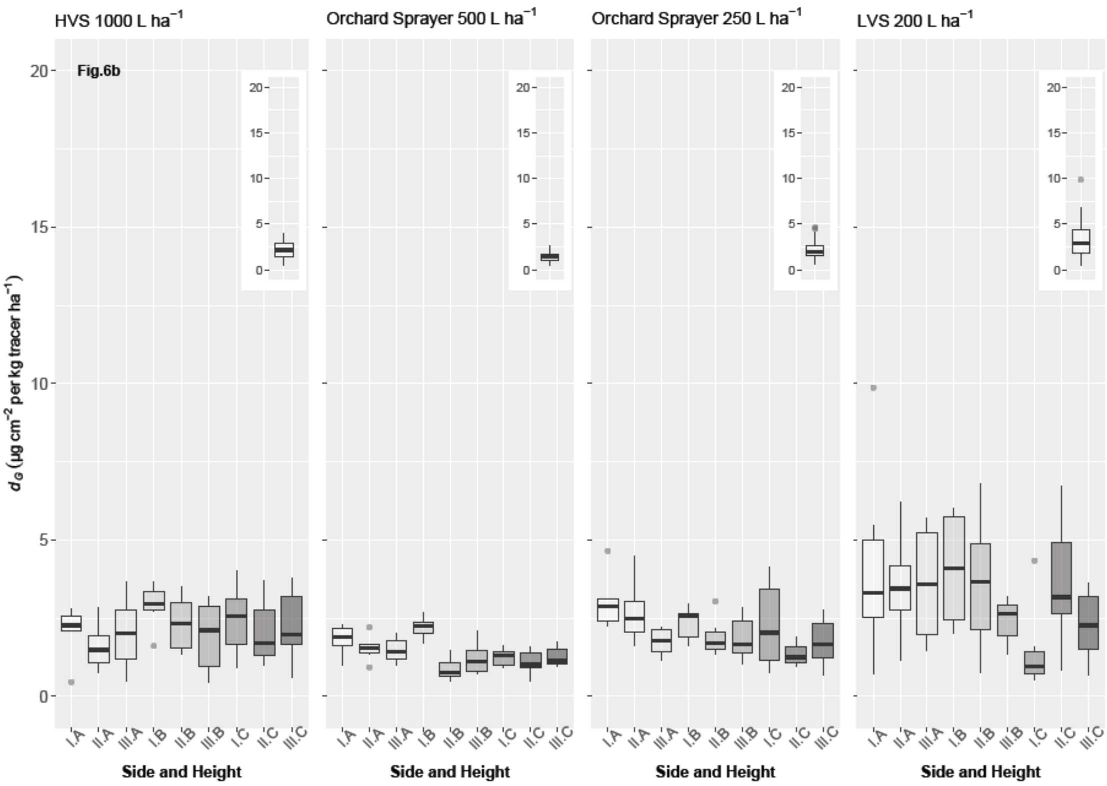

HVS 1000 L ha⁻¹
Orchard Sprayer 500 L ha⁻¹
Orchard Sprayer 250 L ha⁻¹
LVS 200 L ha⁻¹
Fig.6b
d_G (µg cm^{-2} per kg tracer ha^{-1})
I.A
II.A
III.A
I.B
II.B
III.B
I.C
II.C
III.C
Side and Height

Figure 11: Normalized deposition (d_G) per kg of tracer per ha (µg cm^{-2} per kg of tracer per ha) for different sides (II - interior part of the vine) and heights (A - lower – see Figure 8 for details) of the vines for (a) the trellis and (b) the goblet training system. The insets show d_G values for all leaves for each sprayer. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually. See text for results of statistical analyses.

The median d_{100} values for trellised vines were 2.47, 1.91, 1.75 and 1.46 µg cm^{-2} per 100L for LVS, HVS, OS250 and OS500 respectively (Figure 12). Because the nominal tracer concentration was the same for all sprayer treatments, the statistical analysis for d_{100} is equivalent to that for d_G (Table 7) as the two parameters differ only by a divisor of 2.5 (see equations 3 and 4). For the goblet training system, the median d_{100} values were 1.28, 0.87, 0.84 and 0.57 µg cm^{-2} per 100L for LVS, HVS, OS250 and OS500, respectively (Figure 12 and Table 7 for the results of the statistical analysis).

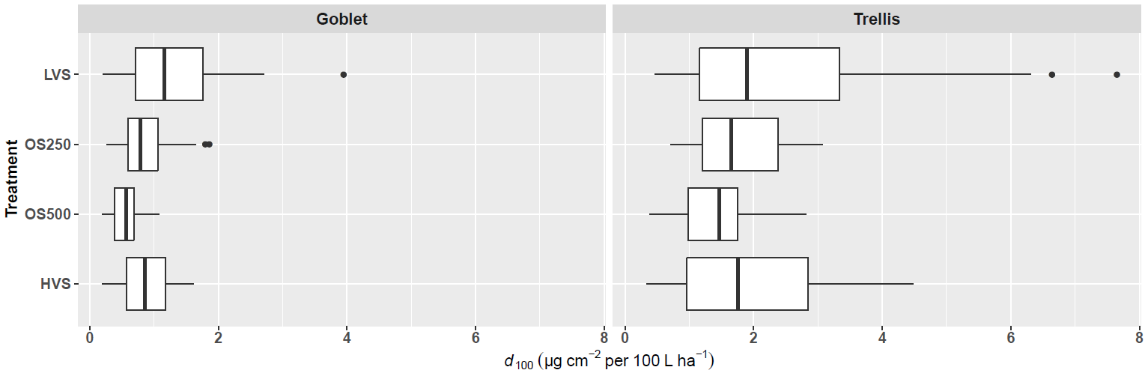


Figure 12: Normalized deposition on leaves (µg cm^{-2}) per 100 L of spray liquid per ha (d_{100}) for the four different sprayers for the goblet and trellis training systems. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile,while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually.

2.4.3 Losses to the ground

The median d_N for the ground losses for the trellis system was 32.26, 3.80, 3.62 and 1.85 µg cm^{-2} for HVS, LVS, OS500 and OS250 respectively (Figure 13 top). The d_N for HVS was significantly higher than that of the other three treatments, while that for OS250 significantly lower than the rest of the treatments (Table 8 and Figure 13 top). The median d_N for the goblet system was 24.54, 3.80, 2.33 and 1.67 µg cm^{-2} for HVS, OS500, LVS and OS250 respectively (Figure 13 top). As for the trellis system, the d_N for HVS was significantly higher than that of the other three treatments, while that for OS250 significantly lower than the rest of the treatments (Table 8 and Figure 13 top).

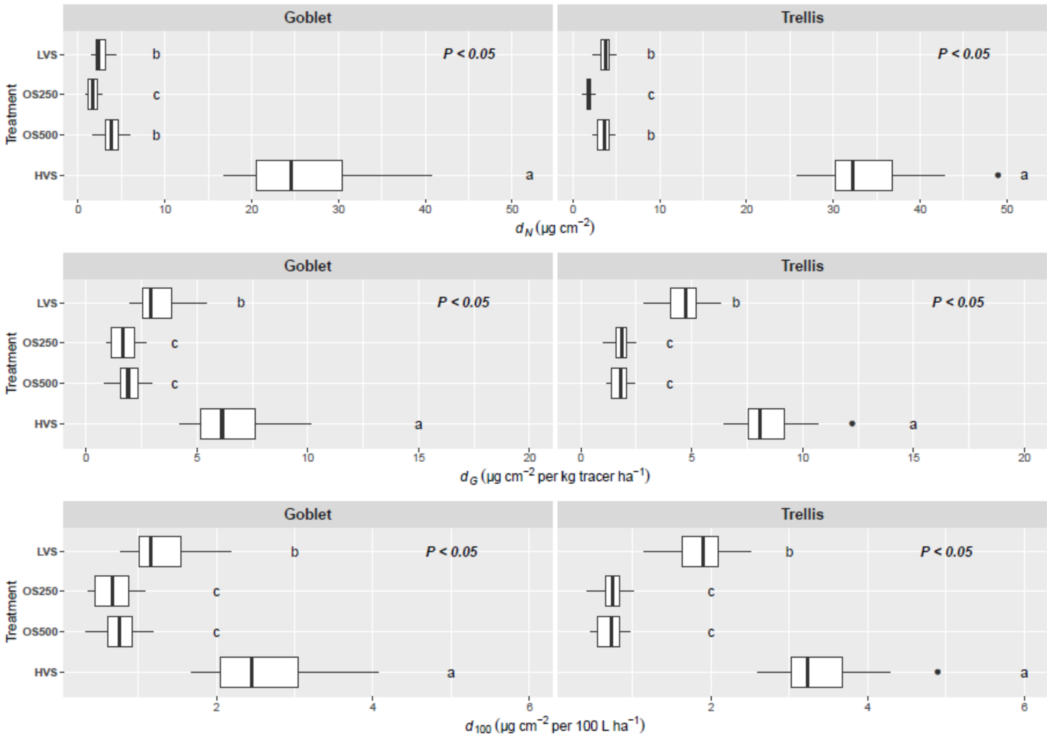


Figure 13: Deposition on the ground for the four sprayer treatments.

Normalized deposition (d_N - µg cm^{-2}) [top], normalized deposition (d_G) per kg of tracer per ha (µg cm^{-2} per kg of tracer per ha) [middle] and normalized deposition per 100 L of spray liquid per ha (d_{100} - µg cm^{-2} per 100 L ha^{-1}) [bottom] for the four different sprayers for the goblet and trellis training systems. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually. See text for results of statistical analyses. Note the different scale for the three graphs.

Table 8: Results of the linear mixed effects model for the effect of sprayer on d_N and d_G or d_{100} on ground losses for the trellis and goblet training systems. The analysis for d_{100} is equivalent to that for d_G as the two parameters differ only by a divisor of 2.5 (see equations 3 and 4).

	df	*F*-value	*P*-value	*F*-value	*P*-value
		Trellis		**Goblet**	
***d*$_N$ (Fixed effect)**					
Sprayer	3, 20	253.79	<0.001	79.641	<0.001
Random effect (standard deviation)	Vine id Residual	0.143 0.192		0.298 0.216	
***d*$_G$ *or d*$_{100}$**					
Sprayer (Fixed effect)	3, 20	92.28	< 0.001	21.41	< 0.001
Random effect (standard deviation)	Vine id Residual	0.143 0.192		0.298 0.216	

Normalized deposition on the ground per kg of tracer per ha (d_G) and d_{100} were almost twice as high for HVS than for LVS for both the goblet and trellis training systems (Figure 13 middle and bottom, respectively). The d_G and d_{100} values for HVS were significantly higher than that for the other three treatments, and d_G and d_{100} for LVS significantly higher than that for OS250 and OS500 (Table 8, Figure 13 middle and bottom).

2.5 Discussion

The current work assessed the deposition on leaves and losses to the ground for four different sprayer treatments in a trellis and a goblet training system. The tank concentration of the tracer was selected using the HVS as the base level, because the sprayer represents the commercial practice currently applied in vineyards in the study region.

2.5.1 Deposition on leaves

In the trellis system, HVS achieved the highest median d_N at 17.57 µg cm^{-2}, followed by OS500 at 7.33, OS250 at 4.12 and LVS at 3.80 (Figure 10a). The d_N for the goblet system was ca. 50% lower than that for the trellis system for all sprayers (Figure 10b). HVS resulted in the highest d_N for the goblet system at 8.59 µg cm^{-2}, which was at least three times higher than that of the other three treatments. As Manktelow et al. (2004) and Michael et al. (2020) found, both the leaf deposit and plant surface coverage tend to increase with increasing application volume.

Comparing, however, just the d_N among treatments provides a misleading picture of spraying efficiency because of the different volume rates used for each sprayer. The HVS applied 4 kg of tracer per ha, while the OS500, OS250, and LVS applied 2, 1 and 0.8 kg ha^{-1} respectively. The d_G which standardizes the leaf deposit at 1 kg of tracer per ha decreased with increasing application volume for the air-assisted sprayers (LVS, OS250, and OS500) in both training systems (Figure 11). The HVS was ranked second in terms of d_G in both the goblet and trellis systems. Previous authors (Manktelow et al., 2004; Gil et al., 2011) found that as the application volume decreases, normalized deposition increases.

The same trend as for d_G was evident when comparing d_{100} (Figure 11), which standardizes deposition for both volume rate (100 L per ha) and tank concentration. Lower volume rates yield savings in time and fuel consumption, as shown by Gil et al. (2011), since they reduce the need for water and pesticide refilling.

Low volumes at 187 and 468 L ha^{-1}, represent the typical range of application used in Michigan (USA) vineyards (Wise et al., 2010). Gil et al. (2011) tested a wide range of sprayers with optimal volume rates estimated by the decision support system (Dosavina), and found that these rates yielded higher leaf deposits than the conventional higher volumes typically applied by farmers. Savings in the applied volume were greater than 50% in accordance with previous research (Gil et al., 2007; Solanelles et al. 2006; Moltó et al., 2000). Manktelow et al. (2004) stated that if chemical application rate is held constant and application volume is adjusted to canopy and sprayer effects on deposits, highest overall deposits will be achieved at low volumes at which runoff losses are minimized. The emerging evidence shows that high volume rates increase losses, with a corresponding reduction in efficiency and a higher risk of environmental contamination. The majority of pesticide labels in Cyprus and elsewhere prescribe application rates still tailored to high volume sprayings, inhibiting the transition to low volume applications.

Variation in spray coverage among different vine areas is a prime factor influencing pest control success (Viret et al. 2003). Control of diseases and pests depends on the amount of active ingredient deposited and its distribution on the target surfaces (Viret et al.2003). Furthermore, Viret et al. (2003) proposed that the incidence of fungal diseases is correlated with the amount of leaf deposit and the uniformity of its distribution on both leaf surfaces. The higher and more evenly distributed the deposit on both leaf sides, the less prevalent the disease incidence. In the current work, the highest variation in d_N was observed for HVS followed by LVS and the two OS treatments (Figure 10). Within vine d_N followed a similar trend for all sprayers (Figure S2.1 & Figure S2), with deposition generally higher on the lower and middle than the top part of the vine. There was also a trend of higher d_N on the outer sides of the vine compared to the interior part. Variation in d_G - which standardizes the amount of tracer used to 1 kg per ha - was highest in LVS, followed by HVS and the two OS treatments (Figure 11). Both the LVS and HVS rely on the operator to move the nozzle to cover the foliage, which inevitably increases deposition variation (Koch, 2007).

OS500, OS250 and LVS rely on air stream to achieve good coverage of the leaves. The air assistance increases the penetration of the spray liquid to the foliage since it creates a small amount of turbulence within the canopy (Landers, 2011; Pergher and Petris, 2008) and also allows better coverage of the plant surface, including the underside of leaves (Michael et al., 2020). The advantages of air support for orchard spraying are unquestioned. Without air assistance, the dispersion of the spray liquid is not adequate, especially in the interior layers of the canopy, in either goblet or trellis training systems (Pergher and Gubiani, 1995).

In addition to perceived effectiveness and cost, farmers select sprayers based on their ease of use. LVS operation is labour demanding since the farmer needs to carry the loaded knapsack sprayer on his back. On the other hand, LVS use requires only one person and uses very low volumes of water compared to HVS. The operation of an HVS requires usually two persons, the operator and a helper to carry the hose, a difficult task in mountainous viticulture. Furthermore, HVS require high volumes of water, which is not always readily available in mountainous areas.

The training system had an important impact on deposition. The d_N was twice as high for the trellis than for the goblet training system (Figure 10 and Figure 11). This might be due to the non-uniform and spherical shape of the vines in the goblet system, in contrast to the trellis system where the foliage is spread as a continuous leaf wall. In addition, the canopy of the goblet vines was smaller (Table 5). The narrower width of the trellised systems facilitates the penetration of the spray liquid. Training a grapevine accomplishes many objectives besides spray distribution, such as the exposure of leaf area to maximize the interception of light, leading to higher yield potential, optimization of the leaf area to fruit ratio, higher quality, and better disease control. Additionally, trellised systems facilitate the movement of equipment through the vineyard and in general facilitate mechanization of vineyard operations (Reynolds and Heuvel, 2009). Different training systems in vineyards exist and the criteria about the choice of the proper one depends on the target ratio of leaf to fruit (Deloire, 2012).

2.5.2 Losses to the ground

The losses to the ground (d_N) were much higher for the HVS followed by OS500, LVS and OS250 which were at a similar level (Figure 13). This indicates that the volume of 1000 L ha^{-1} appears to cause an excessive runoff to the ground. Normalized deposition d_G and d_{100} again point out that HVS resulted to the higher losses to the ground in both training systems, with LVS ranked second (Figure 13). LVS losses were half that of the HVS, showing that the former represents a more environmentally friendly approach regarding the pollution and waste of chemicals especially in areas with mountainous viticulture. Losses to the ground were higher in the trellis training system for the HVS and LVS than the goblet training system (Figure 13). It is possible that the spherical canopy of the goblet system, intercepted less of the spray liquid as shown by the leaf deposit amounts, and therefore resulted in lower losses to the ground. Additionally, the differences might be a consequence of the placement of the spray collectors under the canopy. Adding collectors between rows can give a more representative picture of spray losses. OS250 and OS500 losses to the ground were similar for both training systems (Figure 13).

2.6 Conclusions

The current work assessed the deposition on leaves and losses to the ground for four different spraying treatments in a trellis and a goblet training system located in a mountainous vineyard. Although normalized tracer deposit (d_N) was higher at higher volumes, standardizing the amount of sprayer used per ha^{-1} (d_G) showed that there was a trend of increasing normalized deposition with decreasing volume rate, especially for the three air-assisted treatments. The normalized leaf deposit for the high-volume treatment of 1000 L ha^{-1} was in between that for the 200 and 250 L ha^{-1} treatments, showing the potential of low volume applications to replace high volume pesticide sprayings. The high-volume sprayer resulted in the highest normalized deposit on the ground (Figure 13), suggesting that runoff is excessive compared to the other types of sprayers. Furthermore, volume reduction results in savings of time and fuel consumption, as shown by Gil et al. (2011), as more area is covered with one refill reducing the time needed for water and pesticide refilling.

The training system had an important impact on leaf deposit. We note that d_N was twice as high for the trellis than for the goblet training system (Figure 10), possibly because of the spherical shape of the vines in the goblet system, in contrast to the trellis where the foliage spread as a continuous wall. In addition, the narrower width of the trellised systems facilitates the penetration of the spray liquid.

In conclusion, the current work demonstrates the potential of low volume applications in mountainous viticulture for reducing the environmental and financial costs of pest control. Low volume applications need to be an integral part of EU policies for sustainable pest management. Future work needs to focus on assessing the drift potential of different spray technologies. In addition, follow up studies must assess the effectiveness of low volume sprayings against vine pests and diseases.

3 Chapter 3: Evaluation of the effects of spray technology and volume rate on the control of grape berry moth in mountain viticulture

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3.1 Abstract

The current work evaluated spray coverage and pest control effectiveness against the grape berry moth [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)] by two different spray technologies and three volume rates: A spray gun (High volume sprayer - HVS) calibrated at 1000 L ha^{-1} and a conventional orchard mist blower calibrated at 500 L ha^{-1} (OS500) or 250 L ha^{-1} (OS250). The OS500 and OS250 treatments used 50 and 75% less pesticide than the HVS, respectively. Experiments were carried out in three different grape varieties over two consecutive years in mountain vineyards in the Mediterranean island of Cyprus. The median coverage percentage for HVS remained above 80% for all three varieties, while that for OS500 ranged from 26 to 56%, and that for OS250 from 18 to 37%. Infestation by the grape berry moth varied from ca. 2.5% for Carignan, to 8% for Palomino and 3.2% for Xynisteri. Infestation in sprayed plots remained below 1.8% for all sprayer treatments, varieties and study years. Although infestation levels in OS250 were not different than the control in two varieties, the infestation levels among sprayer treatments did not differ by more than one percentage

point. The results of the current work suggest that lowering application volume and pesticide amount to 50% or more provides adequate levels of control and represents an effective option for reducing pesticide use in vineyards.

Keywords: Vineyards, pest control, pesticide use, Sustainable Use of Pesticides Directive, Farm to Fork Strategy.

3.2 Introduction

Public concerns about the negative effects of pesticide applications to human health and the environment catalyze the development of policies and methods aiming at reducing the environmental impact of spray applications. The recently published Farm to Fork Strategy of the EU calls for a decrease in “the overall use and risk of chemical pesticides by 50%, and the use of more hazardous pesticides by 50% by 2030”. A major policy instrument for reducing the reliance on synthetic pesticides is the European Directive 2009/128/EC on the Sustainable Use of Pesticides. The Directive addresses explicitly pesticide application equipment, requesting from member states to set up inspection procedures for the correct calibration and functioning of sprayers (Barzman and Dachbrodt–Saaydeh, 2011). A parallel initiative demonstrating the commitment of the European Commission on the topic is the “Better Training for Safer Food” program (BTSF, 2019), which includes training on pesticide application equipment.

In response to the zeitgeist, researchers around the globe investigate methods (Grella et al., 2017), tools (Gil et al., 2011; Balsari et al., 2017; Pertot et al., 2017) and machinery (Gil et al., 2007; Llorens et al., 2010; Wise et al., 2010; Pascuzzi et al., 2017) to improve spray coverage of crops (Michael et al., 2020 – Dissertation Chapter 1; Miranda – Fuentes et al., 2016). Furthermore, several studies aim at reducing drift (Landers, 2011; Celen et al., 2008; Ambrogetti et al., 2016) and/or losses to the ground (Pergher et al., 1997; Cross et al., 2001;) or more generally to reduce pesticide use through correct calibration of agricultural machinery (Siegfried et al., 2007). The most recent research applies new technologies that rely on lower spray volumes to achieve adequate coverage of crops (e.g. Salcedo et al., 2019; Jeongeun et al., 2019).

Viticulture represents one of the most intensive cultivations worldwide (Pertot et al., 2017) where substantial amounts of plant protection products are used to protect grapes from pests and diseases. The global area covered by vineyards is estimated at 7.5 m ha, with 37% of the acreage in Europe, 34% in Asia and 19% in America (OIV, 2018). Ongoing efforts involve the development of tools as DOSAVIÑA ® (Gil et al., 2019) to determine and reduce, where possible, the volume rate and amount of pesticides used in vineyards.

A straightforward option for reducing the amount of pesticide used is to reduce the spray volume while maintaining the same concentration of active ingredient in the spray tank (Wise et al., 2010). Volume reduction is also important for practical reasons in mountain viticulture, a special type of vine growing occurring at altitudes higher than 500 m, slopes greater than 30%, terraces or on small islands (www.cervim.org;). Refilling of sprayers with water becomes particularly challenging in mountainous vineyards because of the generally low availability of irrigation facilities, and the difficulties associated with transporting large amounts of water on steep slopes.

The practicing of mountainous viticulture is typical in the Mediterranean island of Cyprus, where small-sized vineyards are nested on terraces on the island's mountains. The most important insect pest attacking grapes in Cyprus and other parts of the world is the grape berry moth [*Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae)], a global pest of grapes. The larvae of the pest attack initially the flowers and at later generations the unripe and ripening berries (Michael et al., 2020 – Dissertation Chapter 1). The moth damages the berries directly through larval feeding, and usually results in secondary infections by botrytis bunch rot, caused by *Botrytis cinerea* (Fermaud et al., 1992).

The current standard for vine spraying in many mountainous vineyards consists in the use of high-volume sprayers connected to spray guns that typically use more than 1000 L ha^{-1} (Koch, 2007; Viret et al., 2003; Michael et al., 2020 – Dissertation Chapter 1). The increasing availability and adoption of modern pesticide technologies, such as orchard sprayers (OS), presents an opportunity to lower volume rates and gain the associated environmental, human health and financial benefits. While a range of coverage values considered adequate for pest control have been reported in the literature (Mangado et al., 2013; Chen et al., 2013), no relationship with insect control effectiveness has been demonstrated yet.

The current work aimed at evaluating the control effectiveness against the grape berry moth by two different spray technologies and volume rates: An HVS calibrated at 1000 L ha^{-1}, and an OS calibrated at 500 or 250 L ha^{-1}. The HVS represented the current standard practice, while OS500 and OS250 a reduction of 50 and 75% in both volume and pesticide amount compared to HVS. Experiments were carried out in three different grape varieties over two consecutive years in mountain vineyards in the Mediterranean island of Cyprus.

3.3 Materials and Methods

Study vineyard and experimental design

The study was conducted in 2017 and 2018 in three different vineyards (Figure 14). The characteristics of each vineyard are shown in Table 9. The three vineyards were located in Lemona village, Paphos, Cyprus (latitude 34.5101°: longitude 32.3300°, altitude: 260 m). Each vineyard was planted with a different wine grape variety: Palomino, Carignan and Xynisteri (Table 9). "Palomino" is a white variety grown mostly in Spain, France and South Africa, but is also found elsewhere, such as Australia and California (USA) (Anderson and Aryal, 2013). "Xynisteri" is a popular indigenous white variety, known for its resistance to drought (Chrysargyris et al., 2020) which takes up 33% of the vineyard area of the island (Cyprus Statistical Service, 2016). "Carignan" is a red variety of Spanish origin that is planted around the globe due to its high yield (Anderson and Aryal, 2013).

Figure 14: The three vineyards a) Palomino var., b) Xynisteri var., c) Carignan var.

Each vineyard was divided in four equal parcels, and each parcel was assigned to each of the three sprayer treatments and a non-sprayed control. Seven rows x 18 plants per treatment were selected for the Palomino and Xynisteri varieties while for the Carignan variety each parcel comprised seven rows x 50 plants (Figure 15).

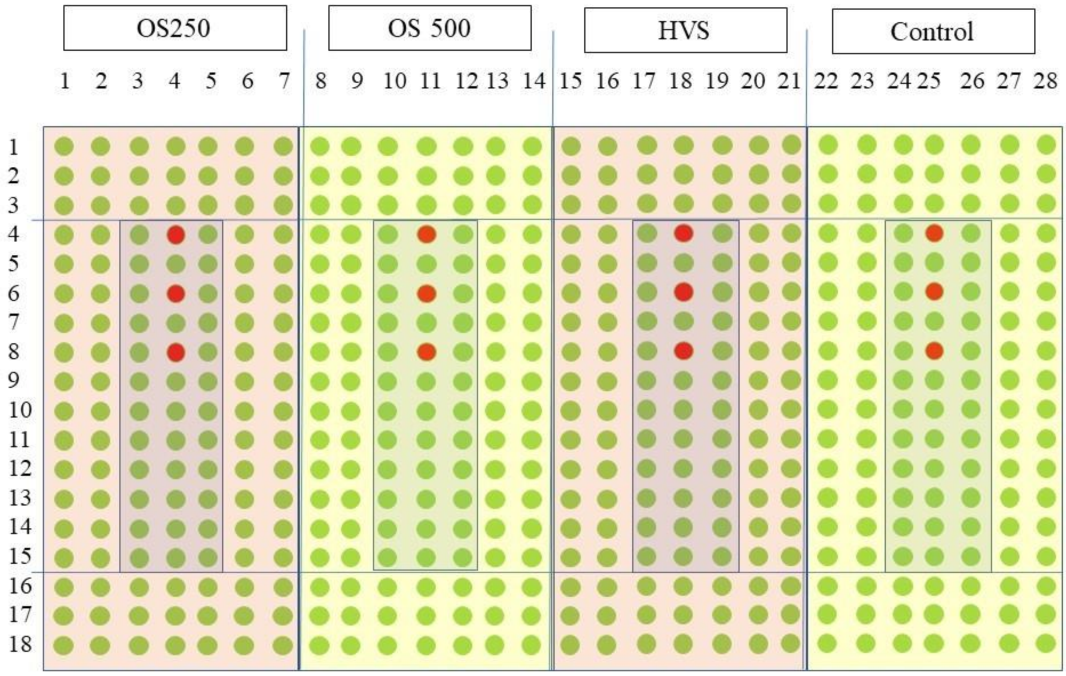

Figure 15: Experimental design. Red dots show the sampling vines for coverage. From the vines within the blue area bunches were collected randomly for grape berry moth assessment. The experimental design for Carignan included 50 vines per row instead of the 18 for Palomino or Xynisteri.

Table 9: Characteristics of the three study vineyards

No	Variety	Training system	Vine spacing (m)	Average canopy height x width (m)		Size (ha^{-1})	Age (years)
				BBCH 65	BBCH 85		
1	Palomino	Trellis	2.4 x 2.4	0.72 x 0.66	1.05 x 1.01	0.3	6
2	Xynisteri	Trellis	2.4 x 2.4	0.76 x 0.81	1.12 x 1.08	0.3	16
3	Carignan	Goblet	2.4 x 2.4	0.68 x 0.86	1.18 x 1.28	1.6	21

3.3.1 Sprayers used

The following combinations of sprayers and volume rates were evaluated (Figure 16):

1. A High-Volume Sprayer (HVS) with spray gun (Honda GX 120, Hamamatsu, Japan) equipped with a 4.0 HP engine, with a hose length of 100 m, calibrated at a nominal volume of 1000 L ha^{-1}. The HVS sprayer represents the current standard practice of grape farmers in Cyprus and in several other parts of the world.

2. A conventional Orchard Sprayer (OS) equipped with a vertical tower (Arcadia Terra, Model Cronos, Greece) calibrated at a nominal volume of 500 L ha^{-1} (OS500), or 50% reduction compared to the HVS treatment.

3. The same conventional Orchard Sprayer calibrated at a nominal volume of 250 L ha^{-1} (OS250), or 75% reduction compare to the HVS treatment.

Figure 16: Sprayers tested. On the left the HVS with the spray gun and on the right the orchard sprayer used for the OS500 and OS250 treatments.

For OS500 the sprayer was calibrated using six nozzles, three per side, type TXR 80028VK (TeeJet Technologies, Springfield, USA) set at a pressure of 5.0 bar and a tractor speed of 4 km h^{-1}, resulting on a flow rate of 1.38 L min^{-1} and a total spray volume of 518 L ha^{-1}. For OS250 the orchard sprayer was calibrated using six nozzles, three per side, type TXR 80015VK (TeeJet Technologies, Springfield, USA) at the same pressure and tractor speed as OS500 and a flow rate of 0.75 l min^{-1}, which resulted in a spray volume of 281 L ha^{-1}.

For the HVS treatment the pressure was set at 20 bar. The walking speed of the operator handling the spray gun was 3.5 km h^{-1}, which resulted in a spray volume of 1086 L ha^{-1}.

For all treatments spray applications were made to both sides of each treated row, by the same person – sprayer, at the same speed and technique. During spraying, the environmental conditions (air temperature and RH, wind speed and direction) were measured by a WatchDog 2000 Series Weather Station (Spectrum Technologies, Aurora) to comply with best management practices recommended for safe spray application (TOPPS Project, 2014). The station was placed at a height of 2.0 m from the ground, at a position free from obstacles.

3.3.2 Determination of spray coverage on leaves

Spray coverage was evaluated on May 24, 2017 at the BBCH 65 stage (full flowering: 50% of flowerhoods fallen) and on August 14, 2017 at the BBCH 85 stage (softening of berries). Prior to spray application, rectangular water sensitive paper strips (26x76 mm) (Syngenta, Basel, Switzerland) were placed (stapled) on the upper side of the leaves of three vine – replicates per treatment located in the middle (fourth) row, on vines 4, 6 and 8. Nine water sensitive papers were positioned and collected from each vine (Figure 17), representing nine different zones: three heights (top, middle and bottom of the canopy) x three depths (outer left, center and outer right side) following ISO 22522. Subsequently, there were three positions on the left side of the vine, three in the middle and three on the right side, that resulted in a total of nine zones, covering the whole canopy. Once the spray residues dried out, the WSPs were collected from the vines and transferred to the laboratory to quantify spray coverage. The spray coverage of water-sensitive papers was analyzed using the software ImageJ (Rasband 1997-2008, version 1.49b). The images were taken at a resolution of 24 pixels·mm^{-1} and the WSP coverage (percentage of surface covered by droplets) was calculated.

Figure 17: Water sensitive paper's positions for a) Trellis trained vines b) Goblet trained vines

3.3.3 Pesticide applications against the grape berry moth

The timing of sprays against the grape berry moth was determined following recommendations by the Department of Agriculture of Cyprus, based on captures of male moths in pheromone traps placed in selected vineyards of the region from early March through late September. The traps were checked weekly and agricultural announcements were issued accordingly.

The dates of insecticide applications for grape berry moth management and the active ingredient dose as indicated on the pesticide label are shown in Table 10. In total four insecticide applications were applied for grape berry moth management each year. For most sprayings, fungicides were also added in the spray tank to protect against powdery mildew (Uncinula necator) following common practice and the recommendations by the Department of Agriculture of Cyprus.

Table 10: Dates, active ingredients / commercial products and the dose (ml or gr / 100L) of the products as indicated on the pesticide labels.

Date	Active ingredient	Commercial name	Recommended Dose (ml or gr / 100 L)	L ha^{-1}	Actual Dose Used/100L	Insect / disease
May 24, 2017	Indoxacarb 30%	Steward 30WG	15	500 - 1500	15	Grape berry moth
	Proquinazid 20%	Talendo EC	20 - 25	300-1000	20	Powdery mildew
June 14, 2017	Spinosad 48%	Tracer 48 SC	10 - 15	400 - 1200	15	Grape berry moth
	Difenoconazole 6% Cyflufenamid 3%	Dynali 60/30 DC	50-65	1000	60	Powdery mildew
July 13, 2017	Esfenvalerate 2.5%	Plinto 2,5 EC	40-120	500 - 1000	40	Grape berry moth
	Boscalid 20% Kresoxim-methyl 10%	ENTOL 20/10SC	40	1000	40	Powdery mildew
August 14, 2017	Alpha cypermethrin 10%	Fastac 10 SC	10-15	500 - 1500	15	Grape berry moth
May 18, 2018	Indoxacarb 30%	Steward 30WG	15	500 - 1500	15	Grape berry moth
	Proquinazid 20%	Talendo EC	20 - 25	300 - 1000	20	Powdery mildew

June 15, 2018	Spinosad 48%	Tracer 48 SC	10 - 15	400 - 1200	15	Grape berry moth
	Difenoconazole 6% Cyflufenamid 3%	Dynali 60/30 DC	50-65	1000	60	Powdery mildew
July 13, 2018	Esfenvalerate 2.5%	Plinto 2,5 EC	40-120	500 - 1000	40	Grape berry moth
	Boscalid 20% Kresoxim-methyl 10%	ENTOL 20/10SC	40	1000	40	Powdery mildew
August 17, 2018	Alpha cypermethrin 10%	Fastac 10 SC	10-15	500 - 1500	15	Grape berry moth

3.3.4 Assessment of infestations by the grape berry month

For the evaluation of grape berry moth infestations twenty bunches per treatment were collected at harvest and the number of infested and uninfested berries per cluster were recorded. Each grape bunch was collected from a separate vine from the middle rows (three to five) of each parcel, leaving at least three vines on the row and two guard rows between parcels. The same vines were sampled on year 1 and 2 of the study.

3.3.5 Statistical analyses

All statistical analyses were carried out in the open-source R language and environment for statistical computing (R Core Team, 2019). Data were curated in dplyr (Wickham et al., 2020) and plotted in ggplot2 (Wickham et al., 2016).

Statistical analyses were carried out separately for each variety. The data on spray coverage of WSPs and berry infestation by *L. botrana* were analysed in a generalized linear mixed effects model framework using the packages glmmTMB (function glmmTMB) (Brooks et al., 2017) for coverage and lme4 (function glmer) (Bates et al., 2015) for berry infestation.

Both packages allow for the analysis of proportion data using a logit link, and a betabinomial (glmmTMB) or binomial (glmer) distribution. Preliminary data analyses showed that the coverage data were over-dispersed, with substantially greater variation than that predicted by the binomial model, and the extra-binomial variation was modelled using the betabinomial distribution. For the glmer models, we used the bobyqa optimization with the maximum number of function evaluations set to 10^9 to achieve model convergence. Dispersion for the glmer models was estimated using the function dispersion_glmer of the blmeco package (Korner-Nievergelt et al., 2015). Model diagnostics were performed in the DHARMa package (Hartig, 2020) using the function simulateResiduals. The function runs tests for correct distribution (KS test), dispersion and residuals.

A likelihood ratio test was used to test for the effects of treatments by comparing the full model to a simpler model with the effect removed using the function drop1 of the stats package of R. For the coverage data, the full model included as main effects sprayer (HVS, OS500, OS250) and vine growth stage (BBCH 65 or BBCH 85), as well as the interaction between sprayer and vine growth stage. For the *L. botrana* infestation data the full model included as main effects sprayer (Control, HVS, OS500, OS250) and year (year 1, year 2) as well as the interaction between sprayer and year. For both coverage and infestation data, vine was included as a random factor in the models, to account for the potential correlation of measurements from the same vine. Comparisons among treatment means were carried out using the emmeans package (Lenth, 2020) with the function emmeans.

3.4 Results

3.4.1 Environmental conditions during the spraying trials

Temperature ranged from 23.3 to 26.8 ºC and wind speed was zero with the exception of Carignan at BBCH 85 (0.1 ms^{-1}). Relative humidity ranged between 44% to 50% for the BBCH 65 sprayings and from 15 to 18% for BBCH 85.

3.4.2 Coverage of Water Sensitive Papers

The assessment of spray coverage of WSPs placed on leaves was carried out twice in the first year (2017), at the BBCH 65 stage (full flowering) and at the BBCH 85 stage (berries start to soften).

The model diagnostics showed that the models for coverage for Carignan and Xynisteri fitted the data well. In the model for Palomino, the *P*-value for the dispersion test was 0.03, indicating a potentially higher dispersion than what modelled through the use of the betabinomial distribution. Visual examination of the quantile-quantile plot of observed vs expected values did not reveal a major overdispersion pattern.

The main effect for sprayer treatment was significant for all three varieties, while the interaction between sprayer treatment and vine stage was not significant for any of the varieties (Table 11). The main effect for vine stage was significant only for Xynisteri with a *P*-value at 0.02. The overdispersion parameter for the betabinomial distribution ranged from 3.09 for Xynisteri to 4.79 for Palomino. The standard deviation for the random effect for vine was 0.28 for Carignan, and practically zero for the other two varieties.

The highest coverage was observed for HVS for all three varieties, with median coverage ranging from 80 to 96 % (Figure 18). Coverage for OS500 varied from 26 to 56%, while that for OS250 from 18 to 37%. Spraying with the HVS resulted in significantly higher coverage than the other two sprayers for all varieties and vine growth stages (Figure 18). OS500 coverage was in general higher than that for OS250, with differences being significant for Palomino and Xynisteri only. Mean coverage was in general higher at BBCH 65 than at BBCH 85, but the difference was significant only for Xynisteri (Table 11).

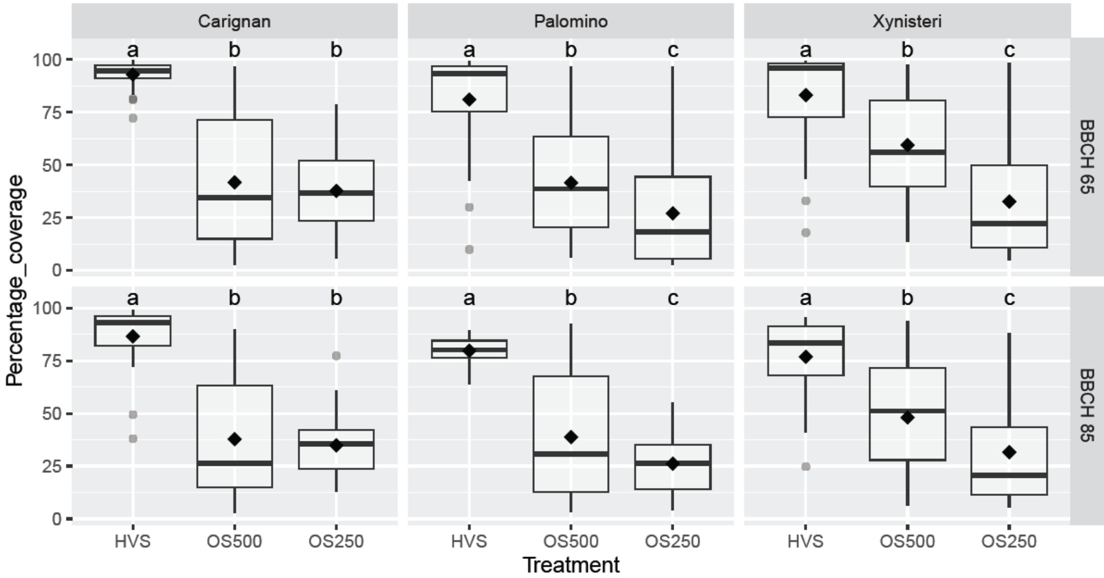

Figure 18: Coverage of Water Sensitive Papers (WSP) at the BBCH 65 and BBCH 85 stages. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually. Diamonds show the mean. See Results for details of the statistical analysis. Different letters within each variety and year represent *P* < 0.05.

HVS spraying resulted in spray coverage higher than 50% for virtually all leaves of the three varieties. Although spraying with OS500 and OS250 resulted in more leaves falling in the 20 – 50 % coverage class (Figure 19), in general coverage for most leaves (with the exception of OS250 in Carignan) fell outside the 20 - 50% class (Figure 19).

Table 11: Results of the statistical analyses for the effect of sprayer/volume rate on coverage for three varieties: Carignan, Palomino, Xynisteri.

Fixed effects*	***df***	***LRT***	***P*-value**	***LRT***	***P*-value**	***LRT***	***P*-value**
		Carignan		**Palomino**		**Xynisteri**	
Sprayer	2	21.53	<0.001	28.44	<0.001	25.00	<0.001
Stage	1	1.59	0.21	1.14	0.29	5.29	0.02
Sprayer: Stage	2	0.56	0.75	2.31	0.32	2.32	0.31
Random effect for vine (st. deviation)		0.28		$4.84 * 10^{-5}$		$6.24 * 10^{-5}$	
Overdispersion parameter for betabinomial family		4.79		3.41		3.09	
Residual degrees of freedom		154		154		154	

*The standard deviation for the random effect (vine) and the residual degrees of freedom are provided for the full model. See Materials and Methods and Results for more information on statistical analyses.

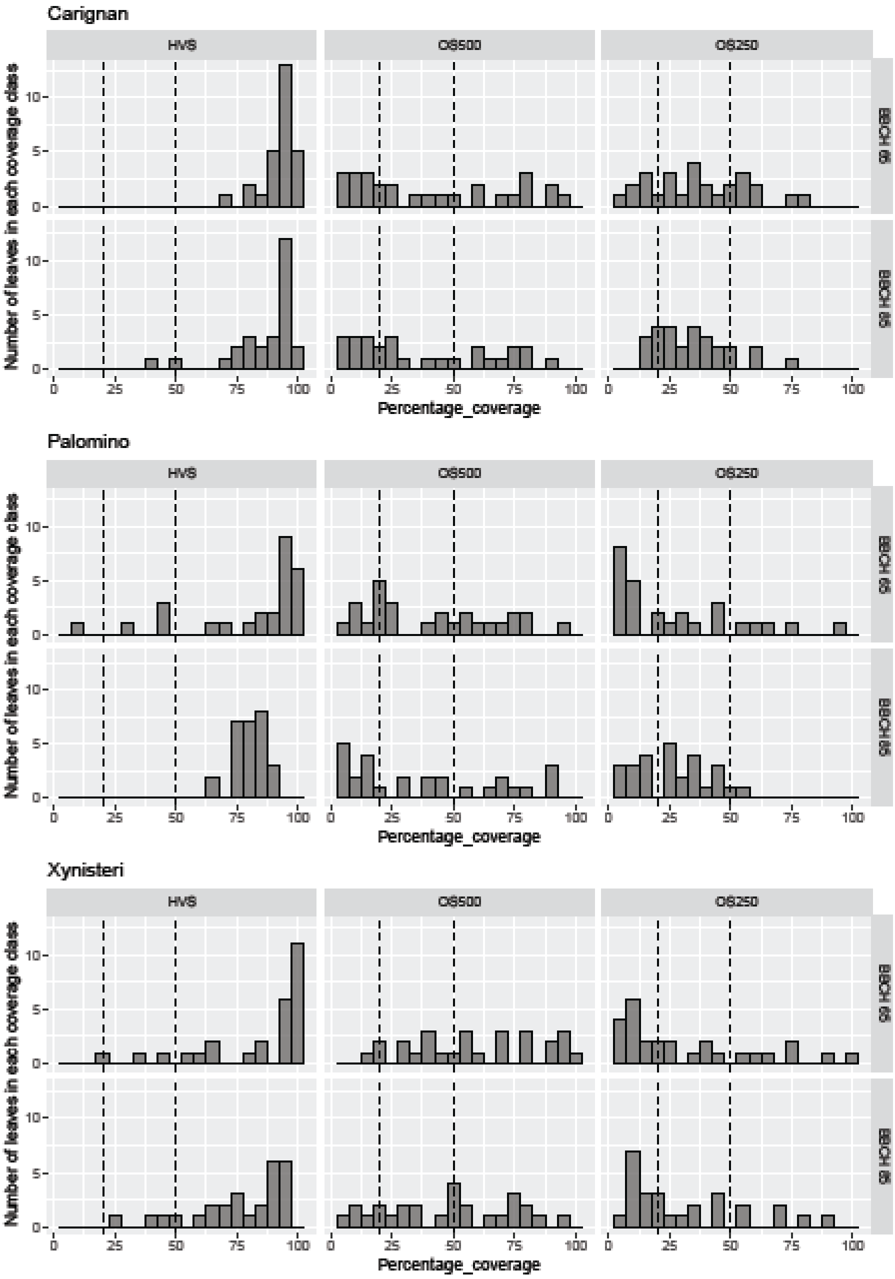

Figure 19: Histograms of the distribution of the number of leaves in different coverage classes for the different treatments and vine stages for the three varieties. The dashed vertical lines correspond to coverage from 20 to 50%, which is considered adequate (Mangado et al., 2013). Values below 20% are classified as underspray, while over 50% as overspray.

3.4.3 Infestation by the grape berry moth

Model diagnostics showed that the models for the three varieties fitted the data well (data not shown). The interaction between sprayer and year was significant for Xynisteri but not for Carignan or Palomino (Table 12). For all three varieties there was a significant effect for sprayer, and a non-significant effect for year. The dispersion parameter for the models was ca. 0.85, and the random effect for vine ranged from 0.71 to 0.86 (Table 12).

In Carignan, the median infestation percentage for the Control remained around 2.50% for both study years (Figure 20). The infestation percentage for bunches from sprayed vines remained below 1.2, 1.5 and 1.8% for HVS, OS500 and OS250, respectively. Differences were statistically significant between Control and HVS, or OS500. The OS250 treatment did not differ significantly from either control, or the other two treatments.

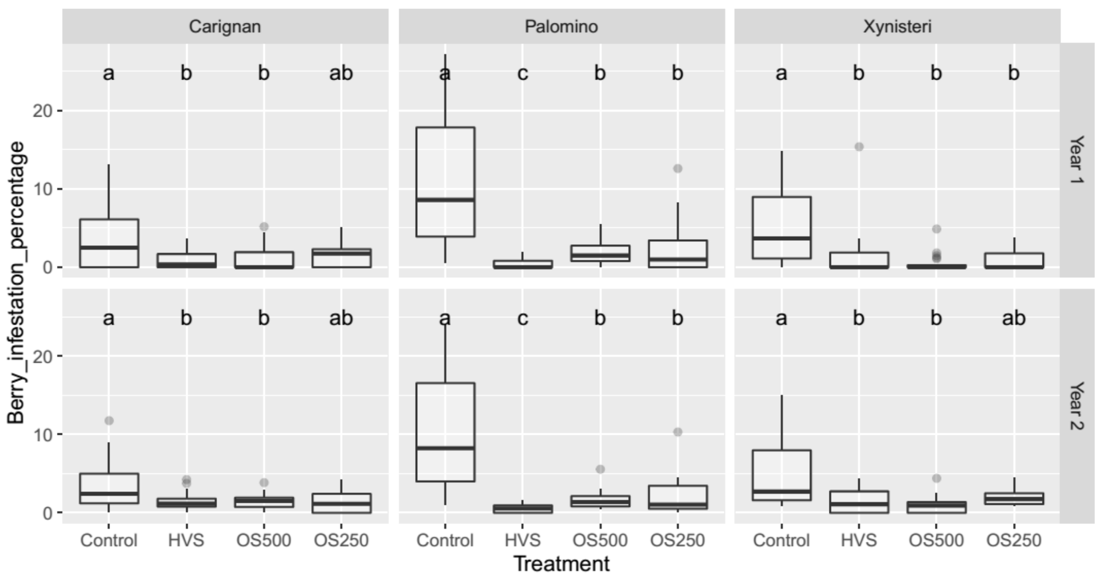

Figure 20: Infestation by the grape berry moth on bunches in Control, HVS, OS500 and OS250 treatments for the two study years. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually. See Results for details of the statistical analysis.

For Palomino, the median infestation percentage for the Control was slightly higher than 8% for both study years, which was significantly higher than all other treatments (Figure 20). On both years of the study, the infestation percentage remained below 0.6, 1.5 and 1.1% for HVS, OS500 and OS250, respectively. The infestation percentage in the HVS treatment was significantly lower than that for the two OS treatments (Table 12). The infestation level was similar between OS500 and OS250.

Infestation levels of grapes in the Control for Xynisteri were estimated at 3.7% for year 1 and 2.7% for year 2 (Figure 20). The infestation percentage remained below 1.1, 1.0 and 1.8% for HVS, OS500 and OS250, respectively, for both study years. In year 1, the infestation percentage was significantly higher in the control compared to the three sprayer treatments, with no significant differences between the three sprayers (Figure 20). In year 2, the infestation percentage was significantly higher in the control than HVS and OS500, while that for OS250 was intermediate and not significantly different than that of either the control or the other two treatments.

Table 12: Results of the statistical analyses for the effect of sprayer and year on infestation of grape berries by Lobesia botrana for three varieties: Carignan, Palomino, and Xynisteri.

Fixed effects*	***df***	***LRT***	***P*-value**	***LRT***	***P*-value**	***LRT***	***P*-value**
		Carignan		**Palomino**		**Xynisteri**	
Sprayer	3	13.26	0.004	64.65	<0.001	31.28	<0.001
Year	1	0.09	0.76	1.65	0.20	2.30	0.13
Sprayer: Year	3	1.87	0.60	0.71	0.87	9.45	0.02
Random effect for vine (st. deviation)		0.71		0.86		0.84	

Dispersion parameter	0.87	0.83	0.88
Residual degrees of freedom	151	151	151

*The standard deviation for the random effect (vine) and the residual degrees of freedom are provided for the full model that included as main effects sprayer, year and their interaction. See Materials and Methods and Results for details on statistical analyses.

3.5 Discussion

The current work investigated the effect of sprayer/volume rate on the infestation of grapes by the grape berry moth, *Lobesia botrana.*

The median coverage percentage for HVS remained above 80% for all three varieties (Figure 19). The median coverage percentage for OS500 ranged from 26 to 56%, while that for OS250 from 18 to 37%. The general trend of higher coverage for HVS than OS500 or OS250 was expected because of the higher volume of spray liquid applied with each sprayer (Table 11). Manktelow et al. (2004) showed that as the application volume increases, so does the absolute leaf deposit.

In the Michael et al. study (Chapter 2) the HVS resulted in the highest leaf deposit, followed by OS500 and OS250 in both a goblet and a trellis system. The deposition for the goblet training system was ca. 50% lower than that for the trellis system for all sprayers. Interestingly, the leaf deposit on leaves of vines trained as goblet was similar between OS500 and OS250, a finding corroborated by the coverage results for the goblet-trained Carignan in the current work.

Spray coverage between 20-50% is considered as adequate (Mangado et al., 2013), while coverage percentages beyond 50% can be defined as overspray. HVS led to coverage higher than 50% for virtually all leaves (Figure 19) of the three varieties. In contrast, the coverage pattern for OS500 and OS250 differed between the three varieties. The two sprayers resulted in similar median coverage in Carignan, which was close to 30% (Figure 19). In Palomino and Xynisteri, the median coverage for OS250 was consistently below 30%, and was significantly lower than that for OS500 (Figure 18 and Figure 19). Yet, in general, coverage for most leaves (with the exception of OS250 in Carignan) fell outside the 20 - 50% class. A positive feature of the very high coverage by the HVS is the low variability in coverage among leaves (Figure 19). Aiming for lower volumes, decreases mean coverage, which inevitably leads to higher variability. Further work needs to assess in more detail the potential effects of coverage variability in pest control.

Differences in coverage between the two vine growth stages were small for all three varieties, even though there was a significant effect for stage for Xynisteri with a *P*-value at 0.02 (Figure 18). Xynisteri has a more vigorous plant growth and dense foliage than Palomino, the other trellised variety in the study.

Infestation by the grape berry moth varied between the three varieties, from ca. 2.5% for Carignan, to 8% for Palomino and around 3.2% for Xynisteri for both study years (Figure 20). Spraying with HVS or OS500 decreased significantly the infestation damage by the grape berry moth for all three varieties (Figure 20). In contrast, OS250 spraying gave mixed results, as it was not different than either the Control or OS500 in both years in Carignan, and in year 2 in Xynisteri. In all other occasions, infestation of berries sprayed with OS250 was signficantly lower than berries in the Control.

The mixed results for OS250 suggest that under some circumstances, 250 L ha^{-1} might not provide adequate levels of control. However, because of the relatively low levels of infestation by the grape berry moth in control plots (around 2.5% for Carignan and 3.2% for Xynisteri), further work is needed to establish the effectiveness of OS250. In addition, even though there was a different pattern of statistical differences depending on the variety, the infestation percentage between different sprayer treatments did not differ by more than one percentage point (Figure 20).

Not many authors have investigated the influence of sprayer type and water volume on control of insect pests in vineyards. Wise et al. (2010) tested coverage of three different volumes with an airblast sprayer at 187, 468 and 935 L ha^{-1}. Coverage at 468 L ha^{-1} was higher compared to 187 L ha^{-1}, with intermediate coverage at 935 L ha^{-1}. The two lower volumes were evaluated for their effectiveness against the grape berry moth. Control of the moth was better at 468 L ha^{-1} compared to 187 L ha^{-1}.

Viret et al. (2003) tested six different spraying technologies regarding deposition and management of powdery mildew over two years in a flat area as well as in a steep vineyard on large experimental plots compared to unsprayed controls. The best disease control was achieved with the axial fan sprayer working each second row before bloom and each row after bloom and with the knapsack sprayer both calibrated at 400 L ha^{-1}. Also, it was shown that control of powdery mildew was better when deposit was more even on both leaf sides.

Gil et al. (2011) evaluated deposition, uniformity and control of different diseases at different crop stages by comparing the volume rate applied according to DOSAVIÑA with the conventional rate most generally used by farmers. The volume rate was reduced by an average 39.9% for DOSAVIÑA ®, with similar or even higher values of deposition and uniformity. The resulting reduction in pesticide use (average 53%) did not present any difference in disease control for the selected varieties.

The reduction in spray volume achieved through the use of modern spray technologies, such as air-assisted orchard sprayers results in a proportional reduction of pesticide amount, given that coverage is adequate (Mangado et al., 2013). Spray volume reduction provides a relatively straightforward road to achieving a 50% reduction in pesticide use by 2030, the arguably ambitious target of the Farm to Fork Strategy. However, as doses of most pesticides in the EU are based on kg of active ingredient per ha, lowering the spray volume without reducing the quantity of pesticide applied per ha does not decrease pesticide use.

As the registered and recommended product dosages against grapevine pests and diseases vary from country to country (Ruegg et al., 2001), the need for a harmonized approach from the crop protection industry is more than evident (Wolhauser, 2009). As indicated by Siegfried et al. (2007), spray deposit is a key element for successful control of pest and diseases. Water is only the carrier of the pesticides to the leaf surface. A precise calculation of the active ingredient depending on the leaf surface should be carried out. Recently, an expert working group following one of the recommendations of the EPPO workshop on harmonized dose expression for the zonal evaluation of plant protection products in high growing crops, established a Glossary of terms and a Guide for measurement of crop parameters. The two documents have been approved by the EPPO Working Party on Plant Protection Products in 2018 and will be used during the revision of the standard for dose expression (EPPO (2012); EPPO Activities).

Both the amount of pesticide and the applied volume during the spray application process should be calculated based on the canopy structure (Miranda – Fuentes et al., 2016; Koch, 2007; Cross et al., 2001; Barani et al., 2008; Gil et al., 2005). Decision support systems like DOSAVIÑA (Gil et al., 2019), give the opportunity to the farmer through a user-friendly web-based environment, to calculate the optimal volume rate and pesticide dose taking into account various parameters, such as the working pressure, forward speed, number and types of nozzles. Further work needs to assess the pest control effectiveness of spray applications which determine doses based on the canopy volume of vines.

3.6 Conclusions

The current study showed that adequate control of the grape berry moth *L. botrana* can be achieved in vineyards with volumes of 500 L ha^{-1} or lower. The use of 1000 L ha^{-1} in high volume applications resulted in over-coverage of leaf surfaces, without a biologically meaningful improvement in pest control. Subsequently, the current work demonstrates that it is possible to lower pesticide use in vineyards by 50%, without compromising the effectiveness of grape berry moth control, in line with similar findings for vine disease control (Gil et al., 2011). Lowering application volume to 250 L ha^{-1} resulted in non-significant differences in infestation levels from the control in two varieties, suggesting that further work is needed to establish the lowest volume and pesticide amount for sufficient control.

The present study used the same volume rate irrespectively of the growth stage and canopy volume of the vines. Further reduction in pesticide use can be achieved via calibrating volume rate to canopy volume, using tools such as DOSAVIÑA (Gil et al., 2019). Future work needs to evaluate the effects of volume rate adjustment to pest control. Furthermore, more work needs to be directed towards assessing the impact of the distribution of coverage of plant surfaces on pest control, as lowering the spray volume leads to a higher variability in spray coverage, which could impact pest control effectiveness.

GENERAL CONCLUSIONS / FUTURE PERSPECTIVES

The goal of the current dissertation was to test and evaluate the effectiveness of modern pesticide application technologies against vineyard pests and diseases, with a focus on lowering the volume rate and amount of pesticide used. The need to improve spray coverage of crops and reduce off-target losses while lowering pesticide use, as outlined in the EU Sustainable Use of Pesticides Directive (Directive 2009/128/EC), creates insurmountable problems for mountain viticulture. The changes are especially important for grape growers in Cyprus, where the banning of dust formulations of pesticides created an array of practical problems. Most vineyards in Cyprus are smaller than 1 ha in size, and are located on steep slopes or rocky hillsides. Furthermore, approximately 85% of vineyards lack irrigation facilities, requiring farmers to cover large distances and thus time for refilling their sprayers (Wise et al., 2010).

In the first study (Chapter 1), three sprayers/volumes were evaluated at the full development of the vine canopy to test whether use of newer technologies and lower volumes can control effectively the grape berry moth and downy mildew. The results showed that a High Volume Sprayer (HVS) connected to a spray gun at 1400 L ha^{-1} resulted in overspray of the treated surfaces, while a Common Sprayer (CS) at 250 L ha^{-1} resulted in non-adequate spray coverage. A motorized knapsack sprayer classified as a Low Volume Sprayer at 150 L ha^{-1} resulted in adequate spray coverage, with ca. 1/9 of the volume applied by the HVS. Adequate spray coverage can be achieved with volumes as low as 150 L ha^{-1}, in accordance with previous research (Holowniki et al., 2000; Chen et al., 2013). The results of the study also showed that spraying with the HVS at 1400 L ha^{-1} to runoff can lead to an exceedance of the dose of AI per ha (Table 1).

However, no significant differences in grape berry moth control were detected between the three sprayer treatments (Figure 4). Spray coverage is only one of several factors affecting control effectiveness, and factors such as timing of pesticide applications and the presence of resistance in the target pest may have affected control effectiveness.

Chapter 2 investigated leaf deposit and ground losses generated from spray application in a trellis and a goblet training system in mountain viticulture. Four treatments were examined: A spray gun calibrated at 1000 L ha^{-1} (HVS), a motorized knapsack sprayer at 200 L ha^{-1} (LVS), and a conventional orchard mist blower calibrated at 500 (OS500) and 250 L ha^{-1} (OS250). Normalized tracer deposit (d_N) was higher at higher volumes (Figure 10). When the amount of tracer used by each sprayer was standardized to 1 kg ha^{-1} with decreasing volume rate, especially for the three air-assisted treatments (Figure 11), there was a trend of increasing deposition, which shows the potential of low volume applications to replace high volume pesticide sprayings. Besides that, the HVS resulted in the highest normalized deposit on the ground (Figure 13), suggesting that runoff is excessive compared to the other types of sprayers.

The training system had an important impact on leaf deposit since d_N was twice as high for the trellis than for the goblet training system (Figure 10), possibly because of the spherical shape of the vines in the goblet system, in contrast to the trellis where the foliage is spread as a continuous wall. In addition, the narrower width of the trellised systems facilitated the penetration of the spray liquid.

Chapter 3 investigated spray coverage and pest control effectiveness against the grape berry moth by two different spray technologies and three volume rates: A spray gun (HVS) calibrated at 1000 L ha^{-1} and a conventional orchard mist blower calibrated at 500 L ha^{-1} (OS500) or 250 L ha^{-1} (OS250). Results showed that adequate control of the grape berry moth *L. botrana* can be achieved in vineyards with volumes of 500 L ha^{-1} or lower. The use of 1000 L ha^{-1} with HVS resulted in over-coverage of leaf surfaces leading to runoff, without a biologically meaningful improvement in pest control. Subsequently, this work showed that it is possible to lower pesticide use in vineyards by 50%, without compromising the effectiveness of grape berry moth control. The reduction of the volume and therefore the A.I by 75% (250 L ha^{-1}) did not result in significant differences in infestation levels from the control in two of the three varieties, suggesting that further work is needed to establish the lowest volume and pesticide amount for sufficient control.

In conclusion, the three studies showed that high volume sprayings (>1000 L ha^{-1}) result in excessive coverage of target surfaces and result to substantial runoff. Alternative technologies, such as the low volume motorized sprayer (LVS) at 250 L ha^{-1} (Chapter 1) or the airblast orchard sprayer at 500 L ha^{-1} (Chapter 3) give adequate coverage and potentially control of the grape berry moth.

The reduction in spray volume is associated with a proportional reduction of AI applied per unit area (Table 1). This allows the reduction of pesticide use, in accordance with the European Directive 2009/128/EC on the Sustainable Use of Pesticides. Using a reduced amount of water (and AI) to apply the pesticide following accurate procedures to determine the optimal volume rate allows the achievement of a high level of efficiency, and guarantees an optimal biological efficacy of the process (Gil et al., 2019).

Both the amount of pesticide and the applied volume during the spray application process should be calculated based on the canopy structure (Miranda – Fuentes et al., 2016; Koch, 2007; Cross et al., 2001; Barani et al., 2008; Gil et al., 2005). Decision support systems like Dosaviña (Gil et al., 2019), give the opportunity to the farmer through a user-friendly web-based environment, to calculate the optimal volume rate and pesticide dose taking into account various parameters, such as the working pressure, forward speed, number and types of nozzles.

As the registered and recommended product dosages against grapevine pests and diseases vary from country to country (Ruegg et al., 2001), the need for a harmonized approach from the crop protection industry is more than evident (Wolhauser, 2009). As indicated by Siegfried et al. (2007), spray deposit is a key element for successful control of pest and diseases. Water is only the carrier of the pesticides to the leaf surface. A precise calculation of the active ingredient depending on the leaf surface should be carried out. Recently, an expert working group following one of the recommendations of the EPPO workshop on harmonized dose expression for the zonal evaluation of plant protection products in high growing crops, established a Glossary of terms and a Guide for measurement of crop parameters. The two documents have been approved by the EPPO Working Party on Plant Protection Products in 2018 and will be used during the revision of the standard for dose expression (EPPO (2012); EPPO Activities).

Low volume applications need to be an integral part of EU policies for sustainable pest management. Follow up studies must assess the effectiveness of low volume sprayings against vine pests and diseases under different settings, i.e., environmental conditions and varieties. Furthermore, more work needs to be directed towards assessing the impact of the distribution of coverage of plant surfaces on pest control, as lowering the spray volume leads to a higher variability in spray coverage, which could impact pest control effectiveness. Last, unmanned aerial vehicles (drones) are a promising technology, especially for mountain viticulture. Future work needs to incorporate drone spraying effectiveness and off-target losses under different conditions, as the technology offers a huge potential for mountain viticulture.

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Supplementary Material

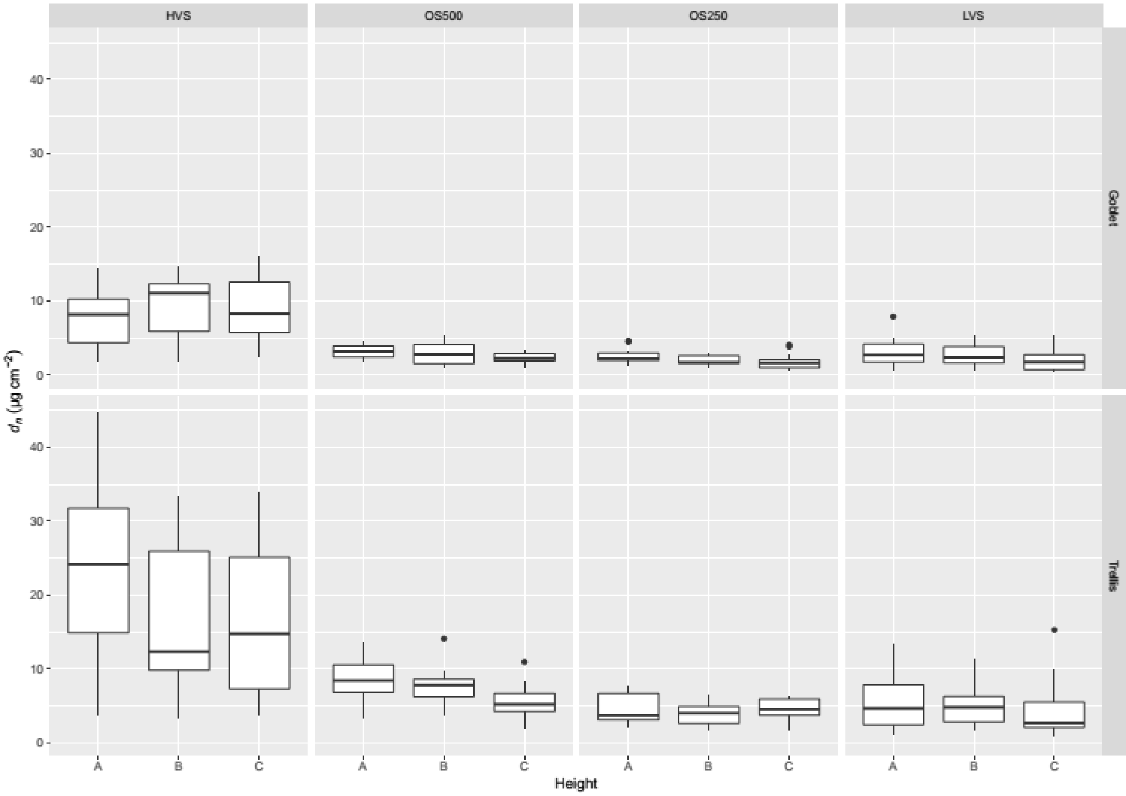

Figure S2.1: Interactions between sprayer and height

Normalized deposition (d_N) on (a) different heights of vines (A - lower – see Figure 10 for details) for the goblet and the trellis training systems. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually. See text for results of statistical analyses.

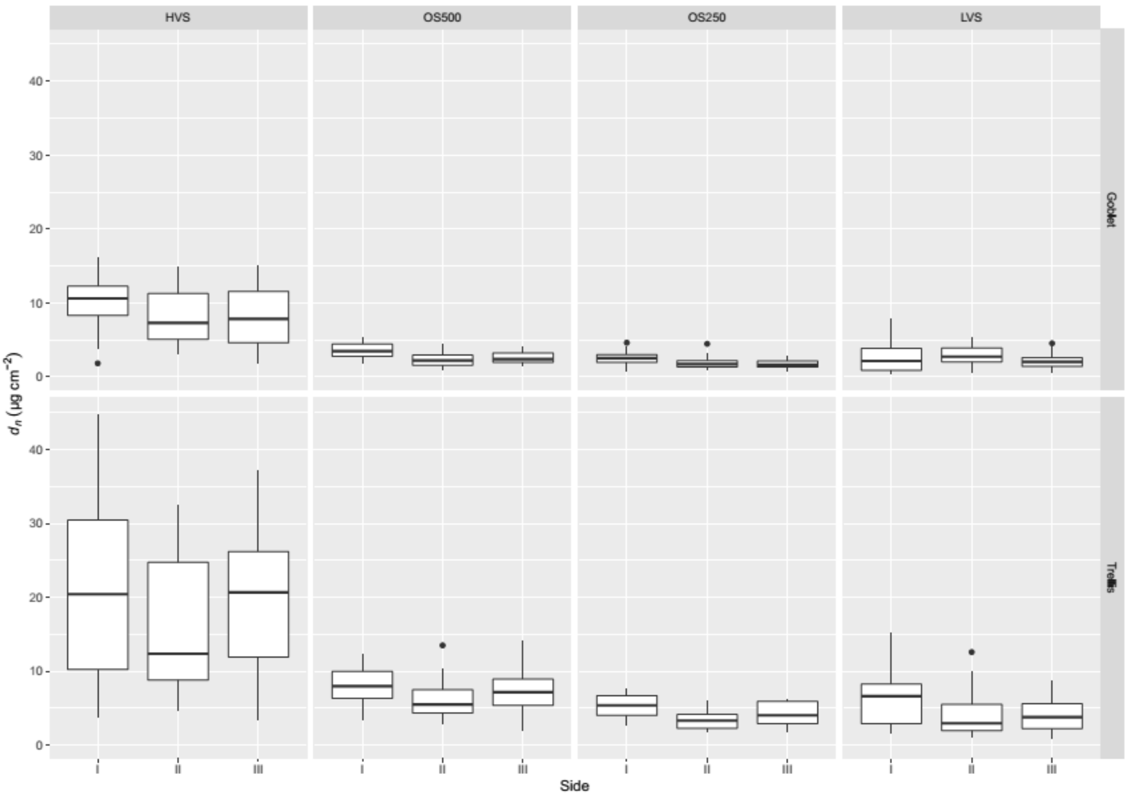

Figure S2.2: Interactions between sprayer and side

Normalized deposition (d_N) on different sides (II - interior part of the vine) for the goblet and the trellis training systems. Boxplots show the median for each treatment, box boundaries show the 25th and 50th percentile, while whiskers extend to 1.5 times the interquartile range (IQR). Points beyond 1.5 times the IQR are plotted individually. See text for results of statistical analyses.