Aculops lycopersici Tryon (Acari: eriophyoidea)

monitoring, control options and economic relevance in German tomato cultivation

Dissertation

zur Erlangung des Doktorgrades Dr. sc. agr.

der Fakultät für Agrarwissenschaften

der Georg-August-Universität Göttingen

vorgelegt von:

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geboren am 9. Mai 1990 in Bad Hersfeld, Deutschland

Göttingen im April 2023

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Tag der mündlichen Prüfung: 26.06.2023

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Summary

The tomato russet mite *Aculops lycopersici* Tryon (Eriophyoidea) is a damaging pest in commercial tomato production in many countries, including Germany. This mite feeds on the surface cells of leaves, stem and fruit. This feeding behaviour leads to plants and fruit taking on a russeted appearance, the plant dropping leaves, and in severe cases it can lead to the death of the plant. In dry and warm conditions, *A. lycopersici* populations are able to grow rapidly, and in high numbers they are able to cause extensive damage. Infestation is usually detected when first symptoms become visible, at which point a large population of *A. lycopersici* has already built up. Currently in Germany, the number and efficacy of available acaricides is limited. *A. lycopersici* is very small and it can move freely between the trichomes of tomato. The mite's small size serves to partially protect it from the beneficial arthropods who are usually larger and therefore struggle to navigate the trichomes when preying on this species. To date, research looking at *A. lycopersici* control has mostly taken place under laboratory conditions; comparatively little research that has been conducted under practical conditions. Prior to this work, no research had investigated the dynamics and spread of *A. lycopersici* populations in the widely-used layer cultivation method of tomato in detail. There was also room for improvement of the detection methods for *A. lycopersici*, such that infestations might be identified at an early timepoint. The main goal of this dissertation was to contribute to an improvement of *A. lycopersici* management in commercial tomato cultivation.

The first study, detailed in Chapter 1, addressed the lack of efficient early detection methods. In this study, repeated non-imaging fluorescence measurements were taken from the stems of tomato plants grown under semi-practical conditions. Via a machine learning algorithm, it was possible to differentiate between infested and healthy plants based on stem fluorescence. After 20 dpi (days post inoculation) correct classification rate was above 90 % and reached up to 99 % in consecutive measurements. In comparison, visual assessment of plants reached a correct classification rate of above 90 % after 22 dpi. A simple sticky tape imprint method revealed infestations much earlier but this method was considered too time consuming for large scale monitoring and instead could be used to verify suspected infestations. In addition

to testing non-imaging fluorescence as a detection method, this study further supported the hypothesis that population growth of *A. lycopersici* was accelerated on plants that were in a state of drought stress.

A second study (two trials), detailed in chapter 2, investigated (a) how *A. lycopersici* spread in layer tomato cultivation under practical conditions, given the opportunity to disperse unhindered and (b) how *A. lycopersici* respond to a barrier method that restricts their movement on the plant. The development of the barrier method that was implemented was partly based on the observations that were made in the first trial of this study. Under practical conditions, when small numbers of *A. lycopersici* arrive on a previously uninhabited plant, it takes between 4 and 6 weeks for symptoms to become visible. *A. lycopersici* populations will usually feed and grow in the area where they first arrive on the plant, until at some point the population begins to move upwards. This leads to many *A. lycopersici* individuals becoming trapped on the highest points of leaves and fruit trusses, where they begin to accumulate. The only remaining route to crawl upwards to colonise fresh, undamaged, plant material is via the stem. In the second trial of this study, the plant stem was blocked using insect glue that was applied weekly 15 cm below the tip of plants grown in layer cultivation. Only small numbers of *A. lycopersici* were able to reach the next higher level above a glue ring. This meant that the population was reset to a low number and it first had to grow for several weeks before plant damage occurred at this higher point on the stem. As a result, glue ring treated plants grew new plant material faster than the pace in which *A. lycopersici* damaged these plants. In the untreated control plant damage was significantly higher. Additionally, fruit damage was avoided entirely on the treated plants.

In a third study (chapter 3), a survey of 50 tomato producing farms in Germany was conducted. One aim of the survey was to examine whether there was a link between *A. lycopersici* incidence and severity and cultivation practices. A second aim was to capture the farmers' perspective on the impact of *A. lycopersici*, and to identify any countermeasures that might have established in practical cultivation. The survey revealed that *A. lycopersici* occurrence was not limited to specific areas of Germany; 33 of the 50 surveyed farms reported infestations. Twenty-four of the 33 farms reported repeat infestations, with *A. lycopersici* present every year following the year of first incidence. The yearly number of farms with first *A. lycopersici* incidence increased between 2014 and 2019. This study revealed that *A. lycopersici*

occurred significantly more often on farms implementing high intensity cultivation methods (a short cultivation break, heating during cold months, use of an artificial substrate and a large production area). Occurrence could not be linked to a single intensification factor due to autocorrelation. *A. lycopersici* was considered the most important pest by participating farmers in terms of plant protection effort exerted.

General introduction

Tomato (*Solanum lycopersicum* L.)

Tomato is by far the most popular vegetable in Germany. In the year 2019 the amount consumed per capita was 27.2 kg (including processed tomato) (BLE 2020). Most of the tomato consumed in Germany is being imported. The most important countries of origin for imported fresh tomato in 2019 were the Netherlands with 387,174 t, Spain with 180,142 t and Belgium with 57,607 t (BLE 2020). Between 2010 and 2021 the cultivation area in Germany has increased from 322 ha (BMEL 2021) to 399 ha (destatis 2022) and in the same timeframe also the national production has increased from 73,300 t to 104,206 t (BLE 2020; destatis 2022). In addition to being a tasty vegetable that comes in many different shapes and sizes tomato can be considered a healthy addition to a diet. For example, tomato contain vitamin C, vitamin E (Ramandeep et al. 2002) and vitamin A as well as phenolic acids, flavonoids, carotenoids and glycoalkaloids (Chaudhary et al. 2018).

In Germany, tomatoes are almost entirely produced in greenhouses or foil tunnels to protect the plants from frost and rain, and to provide the crop with an optimal microclimate that is needed to be able to produce adequate yields (BZL 2021). Cultivation in greenhouses ensures that the temperature is held between 22°C and 25°C during the day, this is the optimal temperature range for this plant (Shamshiri et al. 2018). This temperature range is higher than the average outside temperatures in Germany. The higher temperature in the greenhouses means that certain pests that are adapted to warmer climates are able to establish on the tomato crops. The whitefly for example (predominantly the species *Trialeurodes vaporariorum* but also the species *Bemisia tabaci*) are a pest commonly found in German tomato cultivation today (Crüger et al. 2002).

Aculops lycopersici (Tryon; Acari: Eriophyoidae)

A pest that has been spreading in German tomato cultivation in recent years, as the data presented in chapter 3 provides evidence of, is the mite *Aculops lycopersici* Tryon (Eriophyoidae). *A. lycopersici* is thought to originate from South America (Duarte et al. 2022). The reproduction rate of *A. lycopersici* is at its peak at temperatures of approximately 25°C

(Haque and Kawai 2003) and *A. lycopersici* therefore, like the before mentioned whiteflies *T. vaporariorum* and especially *B. tabaci*, is a pest that benefits from the warmer conditions found in tomato greenhouses.

A considerable amount of research has been conducted on Eriophyoids. For example, in their book titled "Eriophyoid Mites: Their Biology, Natural Enemies and Control", Lindquist et al. (1996) have collated information on an array of topics: the biology and morphology of eriophyoids, techniques on how to study these species in the field and in the laboratory, the natural enemies of these pests, the types of plant damage caused by eriophyoids and the different control strategies that can be used in different crops, host plant resistance to eriophyoids in addition to an overview of specific eriophyoids and their beneficial traits.

Eriophyoids are referred to as gall, rust or bud mites usually depending on the type of damage or symptoms they cause on host plants. Alongside spiders, ticks and scorpions, eriophyoids belong to the class Arachnida. Arachnida belongs to Chelicerata, a subphylum of the phylum Arthropoda (Zhang 2011). Eriophyoids are considered pests in many different crops and damage their host plants, usually by sucking activity on epidermal cells of plants (Lindquist et al. 1996). However, the damage would usually not reach a severity that would lead to the death of host plants, as it is the case for *A. lycopersici* on tomato (Lindquist et al. 1996).

A. lycopersici individuals are minute; they measure less than 200 μm, and as such *A. lycopersici* is one of the smallest herbivore species currently in existence (Sabelis and Bruin 1996). *A. lycopersici* is not just physically small: With a genome size of 32.5 Mb it has the smallest reported arthropod genome as Greenhalgh et al. (2020) showed by sequencing the whole genome of *A. lycopersici*. In their comprehensive study they show, that the reduction of genome size in the case of *A. lycopersici* is mainly due to the loss of introns: 80 % of the protein coding genes in *A. lycopersici* do not contain any introns. They further describe that the mite has lost coding genes responsible for their body structure. A direct result of this is that *A. lycopersici* have only four legs (two leg pairs; visible in Fig. 1), as compared to other Arachnida who have eight legs. Bailey and Keifer (1943) provide drawings and a description of the *A. lycopersici* lifecycle in addition to information on some morphological parameters of the species. *A. lycopersici* individuals undergo two nymphal stages before they reach the final adult stage. Adult individuals measure between 150 and 200 μm in length and have a body diameter of

around 50 µm. *A. lycopersici* individuals have feeding stylets that are between 10 and 15 µm long (Vervaet et al. 2021), these stylets allow individuals to feed only on the upper and lower epidermal cells of tomato plants which collapse as a consequence of the feeding activity (Royalty and Perring 1988).

Fig. 1: Two *A. lycopersici* individuals on the leaf surface of a tomato plant viewed under a stereomicroscope. © Pfaff 2019.

During sexual reproduction, males deposit spermatophores on the plant surface and these are subsequently taken up by females (Lindquist et al. 1996). Females can lay up to 57 eggs, usually close to Trichomes or next to leaf veins (Rice and Strong 1962) and a lifecycle can be completed within 5 days. The highest reproduction rate is reached at 25°C (Haque and Kawai 2003). Bailey and Keifer (1943) report that *A. lycopersici* has no dormant life stages; irrespective of life stage, *A. lycopersici* individuals die within a few days when exposed to temperatures below 0 °C. Anderson (1954) supplies further evidence in support of this.

Tomato and *A. lycopersici* interactions

A. lycopersici causes bronzing and a russeted appearance of leaves and stem as it feeds on surface cells and in severe cases *A. lycopersici* feeding can lead to the death of whole plants (Royalty and Perring 1988). More details on the damage caused to tomato plants can be found in the following chapters 1 and 2.

Fig. 2: Left: Trial plot of tomato plants with *A. lycopersici* symptoms in the lower and middle height of plants. **Right:** Mosaic pattern on tomato fruit caused by *A. lycopersici* feeding on surface cells. © Pfaff 2019.

In recent years there have been reports of a new virus, the *tomato fruit blotch virus* (ToFBV) in various countries; so far the virus has been reported in Australia, Italy and Brazil (Ciuffo et al. 2020; Nakasu et al. 2022). Recently it has been suggested that *A. lycopersici* may act as a vector for the virus (Tiberini et al. 2022). If this hypothesis is verified, *A. lycopersici* will not only impair yields in tomato cultivation with its direct tissue damage during feeding, but it would also indirectly impair tomato yields by acting as a virus vector.

The surfaces of cultivated tomato plants and their wild relatives are covered with different types of trichomes. Amongst these there is the so-called glandular trichome. Glandular trichomes have a small head that has the ability to produce sticky or toxic substances. Other

trichome types without head only pose a physical barrier for arthropods that attempt to move on the surface of tomato plants (Simmons and Gurr 2005). Simmons and Gurr (2005) adapted a classification of trichomes into seven different types that was previously proposed by (Luckwill 1943). The presence and concentration of the different types of trichomes vary between tomato species. Cultivated tomato for instance, lacks trichome type II (a non-glandular trichome) and trichome type IV (a glandular trichome), both of which are present in some of its wild relatives (Simmons and Gurr 2005). Despite the presence of trichomes on cultivated tomato plants, *A. lycopersici* seems to be able to reproduce on tomato plants just fine. The finding of wild varieties of tomato being less susceptible to *A. lycopersici* amongst other factors could potentially be related to the absence of certain Trichomes in cultivated tomato as compared to its wild relatives (Kitamura and Kawai, 2006, publication in Japanese, only English abstract was viewed). Given their small size, *A. lycopersici* individuals are not just unhindered by trichomes since they can move more or less freely between them (van Houten et al. 2013; Aysan and Kumral 2018), they have the added benefit that larger predatory mites that potentially prey on *A. lycopersici* often struggle with the mechanical barrier the trichomes pose (Brodeur et al. 1997). This protective benefit exists at least for some time, until tomato plants lose their trichomes at high *A. lycopersici* infestation rates (van Houten et al. 2013).

Trichomes are not the only line of defence tomato plants possess. Tomato plants have the ability to release, amongst other compounds, antinutritional proteins that suppress nutrient uptake or utilisation by feeding arthropods, and also proteins that have toxic effects on arthropods (Zhu-Salzman et al. 2008). Two signalling pathways, the jasmonic acid (JA) and salicylic acid (SA) signalling pathway, are responsible for upregulation of the gene expression coding for these proteins. The JA signalling pathway, for example, is triggered by the feeding activity of arthropods (Howe and Jander 2008). It has been shown that *A. lycopersici* has the ability to suppress the JA signalling pathway when feeding on tomato plants which, as a consequence, prevents the initiation of plant defence mechanisms, and eases the successful development of *A. lycopersici* on tomato plants (Glas et al. 2014). Eriophyoids in general secrete saliva into the plant while feeding - how exactly they suppress the signalling pathway – how the underlying process works, has yet to be understood (de Lillo et al. 2018). It is clear that *A. lycopersici*, with its short lifecycle and high reproductive rate, in conjunction with this ability to bypass the most important defence mechanisms in tomato plants, has the potential to cause

substantial damage to greenhouse tomato production. Bailey and Keifer (1943) theorise that tomato, as it is cultivated today, could not have been the original host of *A. lycopersici* since the plant often dies off within a short period of time after being colonised by *A. lycopersici*. for a pest such as *A. lycopersici* that is so highly dependent on agencies beyond its control for transport to new compatible host plants, killing the host plant would be highly unfavourable in nature (Bailey and Keifer 1943). Dispersal methods of *A. lycopersici* from plant to plant and within plants are described in more detail in chapter 2 of this dissertation. *A. lycopersici* may not have adapted to tomato as it is cultivated today, it may simply by chance have ended up on cultivated tomato, a plant that unfortunately happens to be highly susceptible to this pest (Bailey and Keifer 1943). Since in tomato cultivation tomato plants are proximate to one another, the dying off of host plants, and the rather immobile nature of *A. lycopersici* do not pose any great obstacles to keep *A. lycopersici* populations from thriving in the artificial environment commercial tomato production provides.

A. lycopersici in commercial cultivation

In commercial cultivation, there are several challenges that require innovative solutions. There is the lack of suitable monitoring and early detection tools or methods. In practice, recognition of an *A. lycopersici* infestation coincides with symptom recognition on plants. Due to its small size, *A. lycopersici* is usually not recognised before first symptoms occur. First symptoms in practical cultivation however, do usually not occur before at least four to six weeks have passed, following a small number of *A. lycopersici* individuals reaching a tomato plant (as observations in conducted trials indicate: e.g., chapter 2). In other studies first symptoms were observed after five weeks (Pijnakker et al. 2022a). Early symptoms, such as light chlorosis on leaves or light grey and brown colouring on the stem, are easily overlooked. Later, more obvious symptoms may be misdiagnosed by inexperienced growers, for instance stem and leaf browning might be mistakenly attributed to the fungus *Phytophthora infestans* (Crüger et al. 2002). Late detection costs time and prevents early countermeasures from being taken. Several monitoring and quantification approaches are described by Perring et al. (1996). These approaches however, are more suitable for research and trial purposes than for large scale implementation in practical cultivation. Until now there are no efficient *A. lycopersici* monitoring methods available for practical tomato cultivation that go beyond improved

sampling plans (Moerkens et al. 2018). Reliable, precise and fast monitoring methods, especially for detection but also for quantification of a pest are crucial when it comes to successful implementation of integrated plant protection measures. An early and reliable detection might increase the chances of successful control, this is especially important in the case of *A. lycopersici* infestations, which usually advance rapidly.

Lack of applied research for *A. lycopersici* in tomato cultivation

This dissertation presents the results of applied research, trials were designed accordingly. Most of the trials presented were carried out under practical greenhouse growing conditions with large plants, large plots and over longer time periods. Even though these trials cannot always offer highest numbers of replicates or being replicated in time, their usefulness and their relevance is high as they closely mirror commercial production. This allows for conclusions to be drawn with more certainty regarding relevance in practice. When assessing the latest review on *A. lycopersici* by Vervaet et al. (2021) it becomes clear that there is a lack of applied, up-to-date research that offers immediate solutions to the increasing damage caused by *A. lycopersici*. One area that remains uncovered are cultivation and hygiene methods, and their potential effects on *A. lycopersici* pest pressure - they are not described in Vervaet *et al.* (2021) and they do not seem to have been investigated to date. Another area are the cited studies in Vervaet et al. (2021) that consider chemical control agents which are, with one exception (Haji et al. 1988), either laboratory studies that were carried out under highly controlled conditions (Abou-Awad and El-Banhawy 1985; Royalty and Perring 1987), or come with a very scarce description of methodology which in turn makes the results difficult to interpret (Kashyap et al. 2015). Similarly, the cited studies that consider biological control in Vervaet et al. (2021) are laboratory studies (de Moraes and Lima 1983; Osman and Zaki 1986; Park et al. 2010, 2011a; Xu 2011; Al-Azzazy et al. 2018; Tixier et al. 2020), or leave unanswered on how many plants the experiment was conducted and whether plants were grown under practical growing conditions (Kawai and Haque 2004). More recent studies, that have been conducted under semi-practical conditions, and that have well-described methods, make a valuable contribution toward closing this gap in the field of biological control of *A. lycopersici* (Pijnakker et al. 2022a, b; Vervaet et al. 2022; Castañé et al. 2022). The mentioned research activities conducted under controlled (laboratory) conditions are an important and much

needed contribution to many questions surrounding *A. lycopersici* and its control and these studies should by no means be considered redundant. However, these laboratory studies fall short of covering the last step towards solutions for commercial production.

In light of the substantial economic damages *A. lycopersici* can wreak on commercial cultivation, one might question why the majority of the research carried out to date has been laboratory-oriented. The reasons for this focus can possibly be seen as falling into two different categories. The first category summarises the special requirements that come with running *A. lycopersici* trials under practical conditions. As mentioned earlier, *A. lycopersici* is one of the smallest plant-feeding animals and can survive on many different plant species in the nightshade family. *A. lycopersici* can disperse via clothes, tools or air currents and there is therefore a relatively high chance that cross-contamination will occur – that individuals will spread to other trials with compatible host plants, or within trials between control and test plots. In applied research that looks at plant feeding arthropods, or larger mites such as *Tetranychus urticae*, common approaches are to apply distinct numbers of individuals of a known age and with a distinct sex ratio to trial plants. Such preconditions are very difficult to maintain with pests like *A. lycopersici*. Handling such small animals in high numbers, and especially distinguishing their sex requires experience, a lot of time, and special laboratory equipment. A common approach in research trials investigating plant protection issues with plant feeding arthropods is to count pest individuals on plants in the different treatments of a trial. Later on, this count data can be correlated to the observed plant damage in the respective treatments and both measures combined confirm the effect the specific treatment had. Additionally, the counts lay the groundwork for the development of damage- and treatment-thresholds. For *A. lycopersici* on tomato, these counts are either too time consuming for sufficiently large trials under practical conditions (Pfaff et al. 2020), or they only allow a rough estimation of pest numbers (Moerkens et al. 2018) that likely will not be precise enough to reveal differences in the effect of treatments or methods that are smaller in scale.

The second category concerns the conditions in applied agricultural and horticultural research in general. Success in research today is often measured in terms of the quantity of publications, and the impact factor of the journal publications appear in. These measures are relevant for both research institutes and for the personal résumé of the researcher. To produce these publications the financial hurdles are significantly lower for trials in small climate chambers

or laboratories because they do not require maintaining large greenhouses or fields and the workforce or technique to actually cultivate plants under practical conditions. This likely is one of the reasons for why there seems to be a decrease in the number of universities providing the possibility to conduct in-depth applied research on crop protection in agriculture and horticulture (von Tiedemann 2021).

Scope of the dissertation

All of the trials conducted for this dissertation were aimed to fill some of the gaps in applied research, and thereby support the development of practical solutions for the problematic tomato pest *A. lycopersici*.

Part of the reason why *A. lycopersici* has such a high damage potential is that after first symptoms become visible, damage can progress quickly throughout the tomato crop as several greenhouse trials have shown (e.g. described in chapter 2) and it often leads to host plants dying off (Crüger et al. 2002). In light of this the question arises whether an early detection of *A. lycopersici* infestation, perhaps even before the development of symptoms visible to the naked eye, would be possible. The time won by an early detection could be used for hygiene measures that reduce further spread of the mites, for local introduction of large numbers of predatory mites, or for the targeted and local spraying of acaricidal compounds. Since there were no efficient monitoring tools available for *A. lycopersici* detection in commercial tomato cultivation (Pfaff et al. 2020), a non-imaging spectral analysis of tomato plants and a simple sticky tape method were evaluated for their potential in detecting *A. lycopersici* infestations in a greenhouse experiment with potted tomato plants. The trials and results are presented and discussed in chapter 1.

Only a limited number of acaricides are available that work against *A. lycopersici*, and the efficacy of beneficial arthropods to combat *A. lycopersici* infestations is limited too (limitations described in detail in chapter 2 and 3). In light of this, it was recognised that alternative countermeasure approaches against *A. lycopersici*, or mitigation of damage caused by *A. lycopersici* are needed and such countermeasures are explored in this dissertation. In all the greenhouse trials conducted under practical cultivation at the trial station in Braunschweig, as well as on commercial farms with *A. lycopersici* presence, it was noted that *A. lycopersici* individuals gather in very large numbers at the highest tips of tomato leaves or fruits (own

observation). The accumulation of *A. lycopersici* in these areas has already been described in literature (Anderson 1954). This effect is believed to be a result of a tendency in *A. lycopersici* to move linearly upwards (Kawai and Haque 2004), likely when feeding conditions worsen or population densities increase. Two greenhouse trials under practical conditions were conducted, first to further investigate this movement behaviour and secondly to exploit this behaviour in an attempt to reduce the damage *A. lycopersici* causes. In this case a barrier based on insect glue was applied weekly to the stem of tomato plants. The experiments and results are described and discussed in chapter 2.

By voices of practical tomato cultivation, plant protection counselling and through grey literature a picture of *A. lycopersici* was drawn that showed the pest was spreading through Germany in recent years. It was claimed that *A. lycopersici* more often caused significant damage in commercial tomato cultivation. This said, the extent and the speed of the spread was not clear. For instance, it was unclear whether *A. lycopersici* incidence and/or severe damage were limited to certain cultivation practices or farm types. It was also not entirely clear how countermeasures are conducted and how successful different countermeasures are. To contribute data from commercial cultivation and provide answers to at least some of these questions, a survey amongst farms with commercial tomato cultivation was conducted in the first half of 2019. The results of the survey are described and discussed in Chapter 3.

Mitespotting – Approaches for *Aculops lycopersici* monitoring in tomato cultivation

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This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant number 2816ERA01L.

This chapter is published in:

Experimental and Applied Acarology **80**, 1–15 (2020).

https://doi.org/10.1007/s10493-019-00448-3

Mitespotting - Approaches for *Aculops lycopersici* monitoring in tomato cultivation

1.1 Abstract

Aculops lycopersici is a major pest in tomato cultivation worldwide, and lately its relevance in German tomato cultivation has increased markedly. *A. lycopersici* causes damage to tomato plants by feeding on the surface of leaves, stem and fruits and can lead to the loss of whole plants. Given the small size of the pest, *A. lycopersici* infestation may go unnoticed for quite a length of time. When discovered symptoms can be easily confused with those of diseases. In addition to these issues *A. lycopersici* has a very high reproduction rate. In this study, fluorescence measurements were performed on the stem of *A. lycopersici* inoculated potted tomato plants and these were compared with a visual bare eye assessment and a sticky tape imprint method for classification of these plants as either infested or healthy. The best correct classification rate was achieved with sticky tape, but this method is time intensive, which makes it unsuitable for large scale monitoring in practice. Classification based on a ridge regression performed on stem fluorescence measurements was at least as good as the classification based on the visual assessment, and detection was robust against symptoms of drought stress. In a second trial the specificity of stem fluorescence measurements for *A. lycopersici* against *Trialeurodes vaporariorum* was successfully tested. The fluorescence method is promising as this method allows for high automation and thereby has the potential to increase monitoring efficacy in practice considerably. The relevance of the tested monitoring methods for practical tomato cultivation and the next steps to be taken are discussed.

Keywords

Aculops lycopersici; tomato russet mite; detection; fluorescence; spectroscopy; monitoring

1.2 Introduction

The tomato russet mite, *Aculops lycopersici* (Tryon), an eriophyoid mite (Acari: Eriophyidae), is considered a pest of several Solanaceae crops (Perring and Farrar 1986). *A. lycopersici* is currently found throughout the world in both tropical and temperate regions. In the more distant past, there have only been minor reports of *A. lycopersici* in Germany. In recent years the economic impact of eriophyoid mites such as *A. lycopersici* has increased (Duso et al. 2010). Following this 2010 publication it appears the number of reported *A. lycopersici* infestations also continued to increase in Germany. *A. lycopersici* causes bronzing and a russeted appearance of leaves and stem as it feeds on surface cells (Royalty and Perring 1988). In contrast to several other eriophyoid mites *A. lycopersici* has a superficial lifestyle and does not induce or inhabit galls on the plant tissue (Van Leeuwen et al. 2010). *A. lycopersici* feeding on the surfaces of tomato plants damage the upper and lower epidermis. Where epidermal cells are destroyed, tomato plants form a thickened layer of callous with a high lignin content (Royalty and Perring 1988). The feeding may also result in curling of leaf edges and later in dropping of leaves (Capinera 2001).

Several factors make *A. lycopersici* a problematic pest. It has a high reproduction rate and at less than 0.2 mm in length, it is relatively small (Haque and Kawai 2003). Only a limited number of plant protection agents are registered for use against *A. lycopersici* intomato cultivation in Germany, currently this is Abamectin and Metarhizium anisopliae (BVL 2019). Although a number of studies have shown certain predatory mites, such as *Amblyseius fallacis* and *A. swirskii* (Brodeur et al. 1997; Park et al. 2010, 2011b), have an effect on *A. lycopersici*, the practical implementation of biological control is limited (van Houten et al. 2013).

In practice recognition of *A. lycopersici* infestation coincides with symptom recognition on plants. Early symptoms such as light chlorosis on leaves or light grey and brown shades on the stem are easily overlooked. Later, more obvious symptoms may be misdiagnosed, for instance stem and leaf browning might be mistakenly attributed to the fungus *Phytophthora infestans* (Crüger et al. 2002). As yet there are no efficient *A. lycopersici* monitoring methods available for practical tomato cultivation. Yet reliable monitoring methods especially for detection but also quantification of a pest are crucial when it comes to successful implementation of integrated plant protection measures. Reliable monitoring decides whether

a measure taken against a pest will be successful or not. Perring et al. (1996) describe several sampling methods, which either involve destruction of plants or have been designed for research purposes and in terms of costs and time consumption are not suitable for upscaling towards an application in commercial tomato cultivation. A more promising approach with regard to the requirements of commercial cultivation is, for instance, the analysis of volatile organic compounds (VOC) of trichomes or, in other words, changes in plant smell to detect *A. lycopersici* (Takayama et al. 2013).

In recent years, also spectroscopic methods and their potential use for biotic stress detection in plant breeding and plant production have become a topic of interest in horticultural and agricultural research. For plant protection in tomato cultivation, several possible applications have been investigated to date, for example: early detection of mealybug infestation using a non-imaging spectrometer in a spectrum of 400 to 1000nm (Canário et al. 2017), the application of hyperspectral imaging for early blight and late blight disease detection (Xie et al. 2015) or the application of hyperspectral imaging for differentiation between healthy and gray mold diseased tomato leaves (Xie et al. 2017). The idea of utilising fluorescence for the detection of *A. lycopersici* already led to a US-patent (Takayama et al. 2016). However, until now detailed research has not been published in this area. As blue light-induced chlorophyll fluorescence is a product of photosystem II, changes in fluorescence under the influence of *A. lycopersici* infestation are to be expected. Photosystem II is a 'measure of efficiency of photosynthesis' and the resulting fluorescence can act as a quantitative measure of plant stress (Kancheva et al. 2007). Based on this effect fluorescence measurements on the stem of infested and of healthy tomato plants were conducted in this study. The decision to perform spectroscopic measurements on the stem of tomato plants and not on leaves was based on several factors. *A. lycopersici* symptoms are often described, for instance by Capinera (2001), as first occurring on the lower part of tomato plants. In layer cultivation, which is common in most modern greenhouses in Germany, old leaves in the lower part of plants are removed weekly. Thus leaves, which possibly harbour *A. lycopersici* and which could develop symptoms, are removed. In consequence the stem is the only remaining organ in the lower part of plants that is permanently exposed to *A. lycopersici* feeding and also the bottleneck for *A. lycopersici* populations when moving upwards on plants towards younger uncolonised plant tissue. As drought stress can have direct and indirect, short- and long-term effects on chlorophyll

fluorescence (Fracheboud and Leipner 2003), a drought stress treatment was also included to see whether the water supply level interferes with the results of the fluorescence measurements. Hence, this study aims to evaluate the stem fluorescence approach to detecting of *A. lycopersici* infestations. The efficacy and specificity of *A. lycopersici* infestation detection and an additional confirmation method based on sticky tape imprints were tested and their potential for application in practical cultivation is discussed.

1.3 Materials and methods

Experimental design

Ahead of the trial, 96 plants of the cultivar Bocati F1 (Enza Zaden) were sown on 9. December 2016 and singled into 10-cm pots on 28. December 2016. The plants were kept at a constant 24±1.5 °C and 23±1.5% relative air humidity on tables in greenhouse chambers with concrete floors. On 31. January 2017, when the average total plant height had reached 25 cm, half of the plants were randomly selected and inoculated with 10 adult *A. lycopersici* at the internode of the first leaf above the cotyledons, resulting in 48 control and 48 inoculated plants.

The *A. lycopersici* individuals used for this trial were taken from a rearing on tomato plants at 25 °C and a 16/8 day/night cycle. The mites for the rearing were originally recovered from tomato plants in a private garden near Brunswick (Germany). The plants were watered every 48 h. For 24 of the 48 control plants and 24 of the 48 inoculated plants, artificial drought stress was induced until the point of clearly visible light wilting in leaves before every watering for the duration of the trial. The remaining control and inoculated plants received sufficient water. The amount of water for the plants with low water supply was adjusted according to the moisture of the soil in each pot. Soil moisture was measured using the Field Scout TDR 100 System (Spectrum Technologies, Aurora, IL, USA). Previous to the trial it was investigated on separate tomato plants which measured moisture level coincided with light wilting before watering. In this way, the amount of water each plant received could be adjusted individually according to the respective soil moisture measurement. The second trial for evaluating specificity was conducted at the beginning of 2018. The trial consisted of 12 plants in total and was run with the same temperature and relative air humidity levels as the previous trial. Each

plant was located in a separate bugdorm-2120F tent (MegaView Science, Taichung, Taiwan). Plants were inoculated on 18. January 2018 in four diferent treatments, three plants with 10 adult *A. lycopersici* each, three plants with 30 adult *T. vaporariorum* each and three plants with 10 adult *Tetranychus urticae* each. Three plants remained pest free as a control (n = 3 per treatment). The *T. vaporariorum* individuals used for this trial resulted from a rearing on tomato, The *T. urticae* individuals resulted from a rearing on strawberry (*Fragaria ananassa*) and were transferred to tomato 2 months previous to the trial inoculation. Both rearings were kept at 20 °C with a 16/8 day/night cycle.

Sampling procedure

All plants remained at their initial position throughout the trial to avoid and minimize unwanted spread of mites. Sampling and measurements were conducted at 48-h intervals on 13 dates in total from 4 days post inoculation (dpi) till 28 dpi. The sampling procedure consisted of fluorescence measurements on the stem of the tomato plants, and visual assessment of plants and of sticky tape imprints that were taken from the stem of the tomato plants. For the fluorescence measurements the spectrometer STS-VIS-L-50-400- SMA (Ocean Optics, Duiven, The Netherlands) with a resolution of 1024 wavelengths in the spectrum of 350–800 nm was used. The light source was a blue LLS-455 LED (Ocean Optics), emitting blue light with a peak at the wavelength of 450 nm. Light source and spectrometer were connected to the RE-BIFBORO-2 (Ocean Optics) bifurcated optical fibre with a fibre bundle diameter of 1 mm. The spectrometer was operated with Ocean Optics software. For the so-called dark measurements, it was ensured that no light reaches the spectrometer sensor. In this way they only show signal noise and artefacts originating from the spectrometer itself. To reduce signal noise and remove possible artefacts generated by the spectrometer the dark measurements were subtracted from the fluorescence measurements prior to analysis. Fluorescence measurements were taken on the stem of all plants prior to watering. The fibre optic was held 2 mm away from the stem and 5–10 cm above the inoculated stem section. To ensure the optic was held at a constant distance from the stem, a black plastic cone was attached to the end of the fibre optic. The plastic cone simultaneously prevented surrounding light from interfering with the fluorescence measurement process. The measurement was taken within 1 s of exposure of the plant tissue to the blue light (with a wavelength of 450 nm). To minimise

interference of daylight or artificial lighting, the measurements were performed before sunrise and with minimal artificial lighting in the surroundings. The fibre optic and the plastic cone were disinfected after each measurement to avoid cross-contaminating the control with *A. lycopersici* when switching between inoculated and control plants. For the visual assessment, *A. lycopersici* symptoms on stem and leaves were estimated with the bare eye. Symptoms were classified into three classes, (1) healthy; (2) light (stem showing light to grey/rust brown discoloration, leaves showing light discoloration/ light chlorosis); and (3) strong (stem showing strong, rust brown, coloration and strongly reduced trichome coverage, leaf browning, necrosis and leaf deformation apparent). In order to detect possible contamination of control plants with *A. lycopersici* and to monitor and quantify the population growth of *A. lycopersici* on the inoculated plants, sticky tape imprints were taken. The imprints were taken from a mid-section of the stem of each plant and measured on average 2 cm^2. Each imprint was later analysed under a stereomicroscope and the individuals of *A. lycopersici* trapped on the imprints were counted. In the specificity trial fluorescence measurements on the stem of each plant were conducted weekly for 5 weeks from 31. January 2018 till 5. March 2018. The measurements were conducted in the same way as described for the previous evaluation trial. To check whether the applied pests established on the plants, individuals were counted on 2 days. *T. vaporariorum* larvae, *T. urticae* adults and — up to a certain number that proved establishment on the plant — *A. lycopersici* adults were counted using a magnification lens where necessary.

Data treatment and calibration model development

To test whether water supply had an effect on the number of *A. lycopersici* individuals captured on the sticky tape imprints at the different sampling dates generalized linear mixed effects models with negative binomial family were fitted using the R-package glmmTMB (Brooks et al. 2017). Water supply, time since inoculation (dpi) and the two-way interaction were fitted as fixed effects and plant ID as a random effect to account for the repeated measurements of each plant. Significance of model parameters was assessed using Wald χ^2 test and ANOVA type 3 sums of squares using the R-package 'car' (Fox and Weisberg 2019). Pearson residuals were inspected visually to check model assumptions. A Tukey post hoc test for comparison of the estimated marginal means between sufficient and low level of water supply at each dpi

was conducted with the R-package 'emmeans' (Lenth 2021) and the confidence intervals and the estimated means of the model are reported. The dark measurements were subtracted from the fluorescence measurements to remove signal noise and possible artefacts generated by the spectrometer. When exposing plants to blue light (450 nm wavelength) during fluorescence measurement, the plant fluorescence is triggered in an area from 660 to 780 nm. Hence this wavelength range was used as 'fluorescence window' for further analysis. Analysis of the fluorescence data was performed using R (R Core Team 2019). The fluorescence data was smoothed and derived by applying the Savitzky-Golay filter of the R-package 'prospectr' (Stevens and Ramirez-Lopez 2013) with a window size of 15 and a polynomial regression of the third order to further remove underlying signal noise. To normalize the data and ensure comparability between spectra measured for the main test and the following specificity test, every single measured fluorescence signal for each wavelength at each sampling date was divided by the average fluorescence at 685.117 nm from all measurements of the respective test. The normalized fluorescence data for the 13 sampling dates was then plotted separately and inspected visually. For each sampling date 32 measurements from inoculated plants and 32 measurements from control plants were selected randomly and separated into a model-training dataset. The remaining 16 measurements from inoculated plants and 16 measurements from control plants from each sampling date became the model test dataset. It was ensured that in both train and test dataset for both inoculated and also control plants 50 % of the plants were plants with a sufficient water supply level and 50 % were plants with a low water supply level. A ridge regression was fitted to the training dataset using the R package 'glmnet' (Friedman et al. 2010). Based on this model a prediction was made for the test-dataset. Ridge regression is a method which is especially useful for data with many explanatory variables (in this case the different wavelengths) relative to the number of samples. It prevents overfitting via inclusion of the correction factor, λ (lambda), as a penalty term which shrinks the coefficient estimates of unimportant predictor variables towards zero. This means that a limited 'desensitisation' to the training data allows for a possible better fit to the test data (Hastie et al. 2009). In addition to ridge regression a partial least squares regression (PLS), a lasso regression and a principal components analysis (PCA) in combination with a least discriminant analysis (LDA) were applied to classify the data. The best model was selected based on correct classification rate and stability of classification. In the following, the

developed ridge regression calibration model was used to classify all measurements for all dates. Receiver Operator Characteristic (ROC) curves differentiating between train-, test- and complete dataset from 6 to 28 dpi based on the ridge regression were investigated. To evaluate the specificity and transferability of the ridge regression model, it was also applied to the measurements conducted in the specificity trial.

1.4 Results

Sticky tape imprints for *Aculops lycopersici* counts

The average count of *A. lycopersici* individuals on inoculated plants increased over time (Fig. 1) with a maximum at 26 dpi for plants with low water supply and at 28 dpi for plants with sufficient water supply. At early sampling dates (4–8 dpi) the average number of individuals was similar between plants with sufficient vs. low water supply. From 10 dpi onwards until 28 dpi a significant interaction between water supply and dpi indicated a higher number of *A. lycopersici* on the plants with low water supply in comparison to plants with sufficient water supply (mixed effect model: water supply, P=0.14; dpi, P <0.0001; water supply×dpi, P =0.0085).

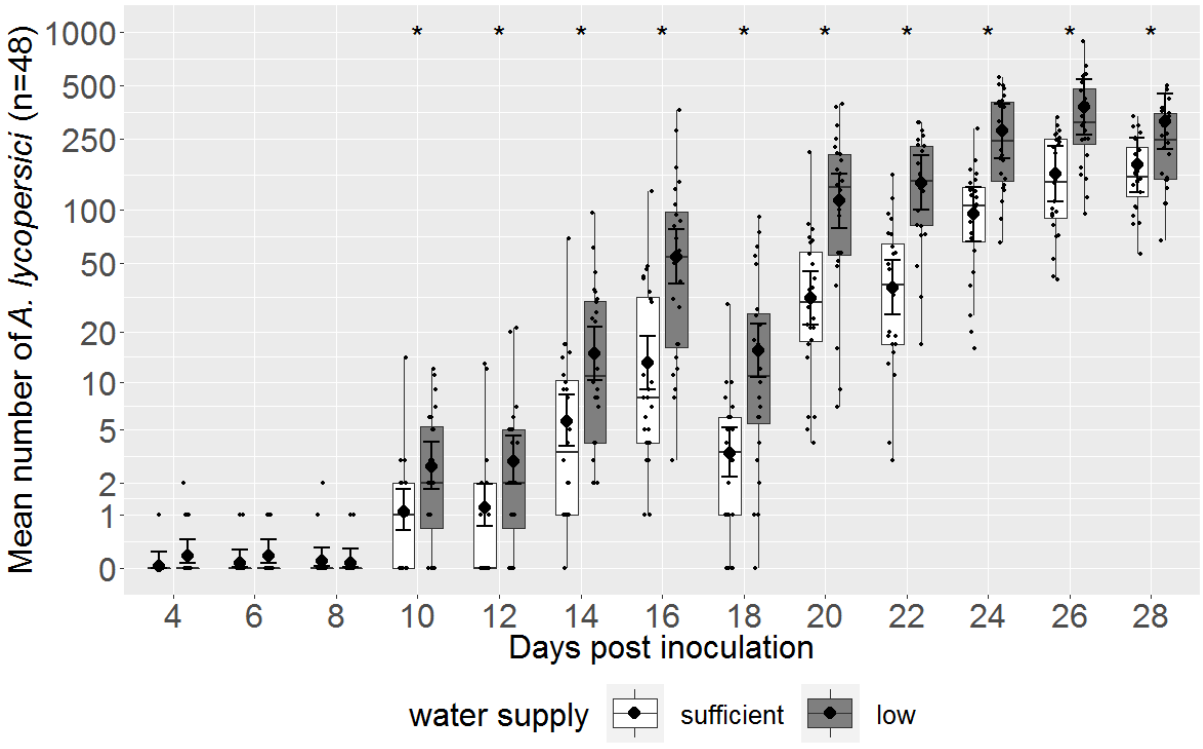

Fig. 1 Comparison of the sticky tape imprint count of *A. lycopersici* individuals per plant on

inoculated plants with sufficient (white boxplot, n=24) and low-level (grey boxplot, n=24) water supply at the individual sampling dates expressed in days post inoculation (dpi). Small black dots display *A. lycopersici* counts per plant. The lower whisker of the boxplot ends at the lowest *A. lycopersici* count within 1.5 ×the lower quartile range, the upper boxplot whisker ends at the largest count within 1.5×the upper quartile range. The horizontal middle line of the boxplot displays the median. The large black dot displays the estimated marginal mean and the 95% confidence interval obtained from the model. The stars at the top indicate significant differences between the water supply treatments at the respective dpi according to Tukey test. Note, the Y-axis has a logarithmic scale to better visualise low counts.

Visual evaluation of the fluorescence spectra

The mean fluorescence spectra separated between control plants and inoculated plants are displayed in Fig. 2.

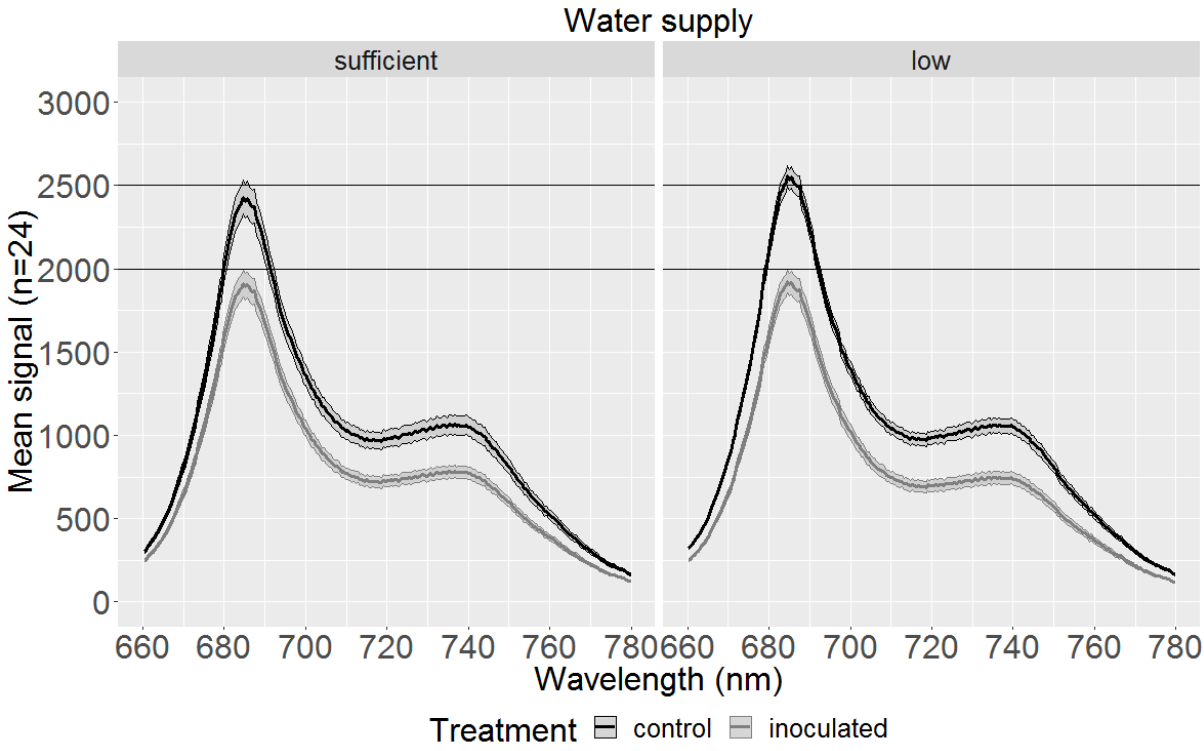

Fig. 2 Comparison of the average stem fluorescence 24 days post inoculation (dpi) between control plants (black line, n = 24) and inoculated plants (grey line, n =24) separated between plants that had sufficient water supply on the left and plants with low water supply on the right. The means of the raw measurements are displayed (i.e., these data have not been treated or normalized). Strength of the light signal as processed by the spectrometer is displayed on the Y-axis. Grey bands around the curves show the 95% confidence interval of the mean. The sensor sensitivity of the spectrometer varies across the wavelength spectrum. As a result, the comparability between signal strength across wavelengths is limited for the displayed fluorescence measurements.

Classification of plants based on the stem fluorescence measurements

The correct classification rate resulting from the fluorescence data between 660 and 780 nm was the highest and most stable when calibration was based on the ridge regression (see Fig. S2 in the Appendix Chapter 1 for comparison). ROC curves for each sampling date from 6 to 28 dpi differentiating between train-, test- and complete dataset from 6 to 28 dpi are displayed in Fig. S3 in the Appendix Chapter 1. The classification of all plants for the sampling dates from 4 till 28 dpi when applying the ridge regression to the stem fluorescence data is shown in Fig. 3. Whereas at first the model does not seem to differentiate between control and

inoculated in the beginning, from 16 dpi onwards the classification improves noticeably. From 16 dpi onwards inoculated plants mostly have a predicted probability of above 0.5 for *A. lycopersici* presence, whereas for control plants it mostly remains below 0.5. The classification for inoculated and control plants across the sampling dates appears to be similar for plants with sufficient water supply and plants with low water supply. This indicates, that the water supply level did not interfere with the chosen analysis and differentiation between inoculated and control based on fluorescence data obtained from the stem. For instance, from 18 dpi onwards the correct classification rate based on the fluorescence measurements was above 80%. The exact classification rates are displayed in Tab. 1, the exact classification rates based on the fluorescence measurements, displayed separately for sufficient and low water supply, can be found in Tab. S1 in the Appendix Chapter 1.

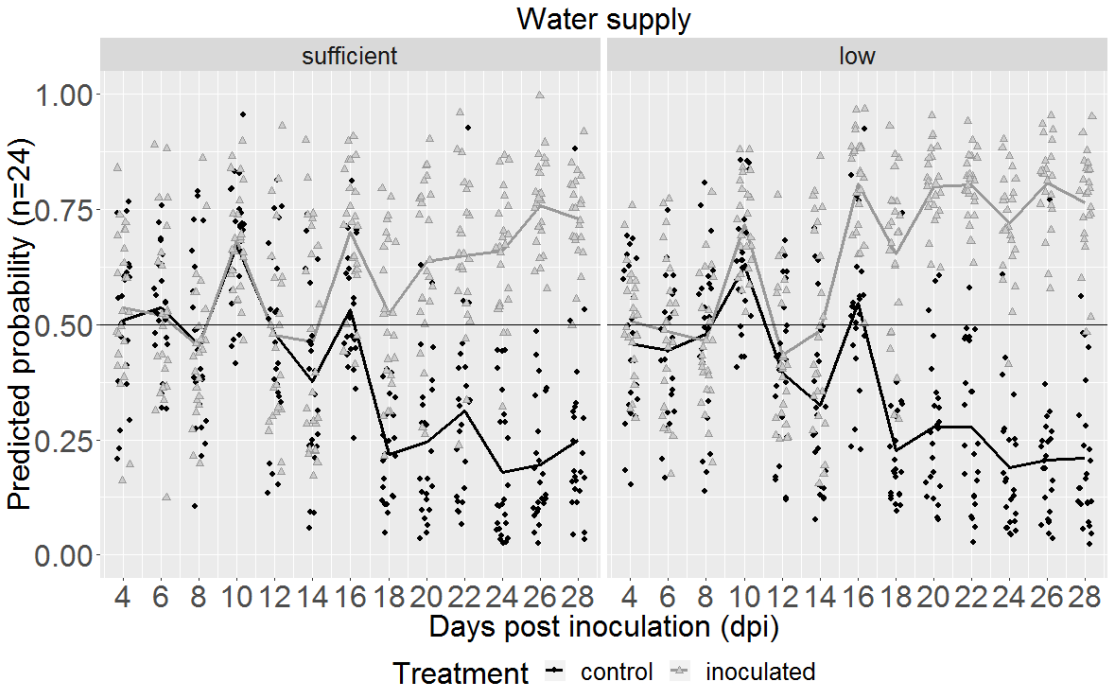

Fig. 3 Classification of all plants at the various days post inoculation (dpi) in control (black symbols and line) and inoculated (grey symbols and line) based on the developed calibration model for the fluorescence data. The predicted probability for *A. lycopersici* presence based on the ridge regression is shown on the Y-axis. Each symbol in the graph represents one plant on a specific sampling date. The average predicted probability is shown by the lines (n=24 per treatment). Samples with a predicted probability>0.5 are classified as inoculated plant. Samples with a predicted probability <0.5 are classified as control plant. The black dots and grey triangles indicate the factual class of all individual plants. Plants in the left panel received sufficient water, those in the right panel received low water.

Comparison between stem fluorescence, visual assessment and sticky tape imprints

The correct classification rates of the ridge regression based on stem fluorescence measurements, of the visual assessment of symptoms on the whole plant and of the sticky tape imprints are compared in Table 1. For visual stem assessment plants were counted as "infested" when the stem was classified '2' or '3'. For leaf assessment the plant was counted as infested if it was classified at least '3' For a plant to be classified as infested in the visual assessment it was sufficient if either the stem or leaves reached the respective threshold. For the sticky tape imprints a plant was classified as infested when at least one *A. lycopersici* individual was found on the imprint of the plant. The table shows that no *A. lycopersici* individuals and fittingly also no visible symptoms were found on the 48 control plants

throughout the trial. The highest correct classification rates based on the stem fluorescence measurements were obtained from 18 to 28 dpi and range from 81 to 99 %. From 20 dpi onwards, the correct classification rate was consistently above 90 %. The best results in the classification based on the visual assessment of stem and leaves was reached between 20 and 28 dpi and ranged from 75 to 100 %. From 22 dpi onwards the correct classification rate was constantly above 90 %. The highest correct classification rate was achieved with the reference method: sticky tape imprints taken from the stem of the tomato plants. From 14 dpi onwards the classification rate was constantly above 90 % and from 20 dpi onwards it was consistently at 100 %. In this method, a plant was classified as infested when at least one *A. lycopersici* individual was found on a sticky tape imprint.

Table 1 *Comparison of monitoring methods* Displayed is the number of correctly identified samples in the inoculated and control group and the correct classification rate at each dpi to monitor *A. lycopersici* development on the plants for classification based on 1) ridge regression with the fluorescence measurements, 2) visual assessment of symptoms on plants and 3) sticky tape imprints that were taken from the stem of plants and were evaluated for *A. lycopersici* presence under a stereo-microscope. Measurements were taken from 96 plants, of which 48 were inoculated with *A. lycopersici* and 48 were kept as control plants. The grey columns state the percentage of correctly classified plants by the respective method.

	fluorescence classification			visual (symptom) classification					sticky tape classification		
	inoculated (n=48)	control (n=48)	correct classification rate	inoculated (stem, n=48)	inoculated (leaves, n=48)	control (stem, n=48)	control (leaves, n=48)	correct classification rate	inoculated (stem, n=48)	control (stem, n=48)	correct classification rate
dpi	class. correct:	class. correct:	% correct:	class. correct:	class. correct:	class. correct:	class. correct:	% correct:	class. correct:	class. correct:	% correct:
4	25	24	51	0	0	48	48	50	5	48	55
6	24	23	49	0	0	48	48	50	7	48	57
8	16	28	46	0	0	48	48	50	4	48	54
10	47	9	58	0	0	48	48	50	32	48	83
12	17	30	49	0	1	48	48	51	28	48	79
14	20	39	61	0	2	48	48	52	47	48	99
16	45	20	68	0	7	48	48	57	48	48	100
18	31	47	81	16	6	48	48	69	42	48	94
20	44	43	91	18	17	48	48	75	48	48	100
22	45	44	93	30	26	48	48	91	48	48	100
24	44	47	95	39	31	48	48	95	48	48	100
26	48	47	99	44	39	48	48	97	48	48	100
28	46	44	94	48	43	48	48	100	48	48	100

Specificity evaluation of the stem fluorescence measurements

The specificity of the fluorescence measurements was evaluated in a second trial over 5 weeks. The results of five measurement dates are displayed in Fig. 4. The separation between *A. lycopersici*-infested plants and the other treatments is distinct from 24 dpi onwards. Healthy, *T. vaporariorum*-infested and *T. urticae*-infested plants are classified closer to each other than to the *A. lycopersici*-infested plants. Of note is that *T. urticae* did not establish on any of the three *T. urticae* inoculated plants. Of the 10 individuals applied to each plant, only 8, 3 and 6 individuals were found again when the plants were checked at 21 dpi and these numbers decreased even further when the plants were checked at 35 dpi. *T. vaporariorum* and *A. lycopersici* numbers, on the other hand, increased on all of the inoculated plants. On the *T. vaporariorum*-infested plants 82, 55 and 66 larvae were found at 21 dpi and these numbers increased further when the plants were checked again at 35 dpi. On the *A. lycopersici*-infested plants at least 40 *A. lycopersici* were found per plant at 21 dpi and at 35 dpi all plants were heavily infested with *A. lycopersici*, showing strong symptoms.

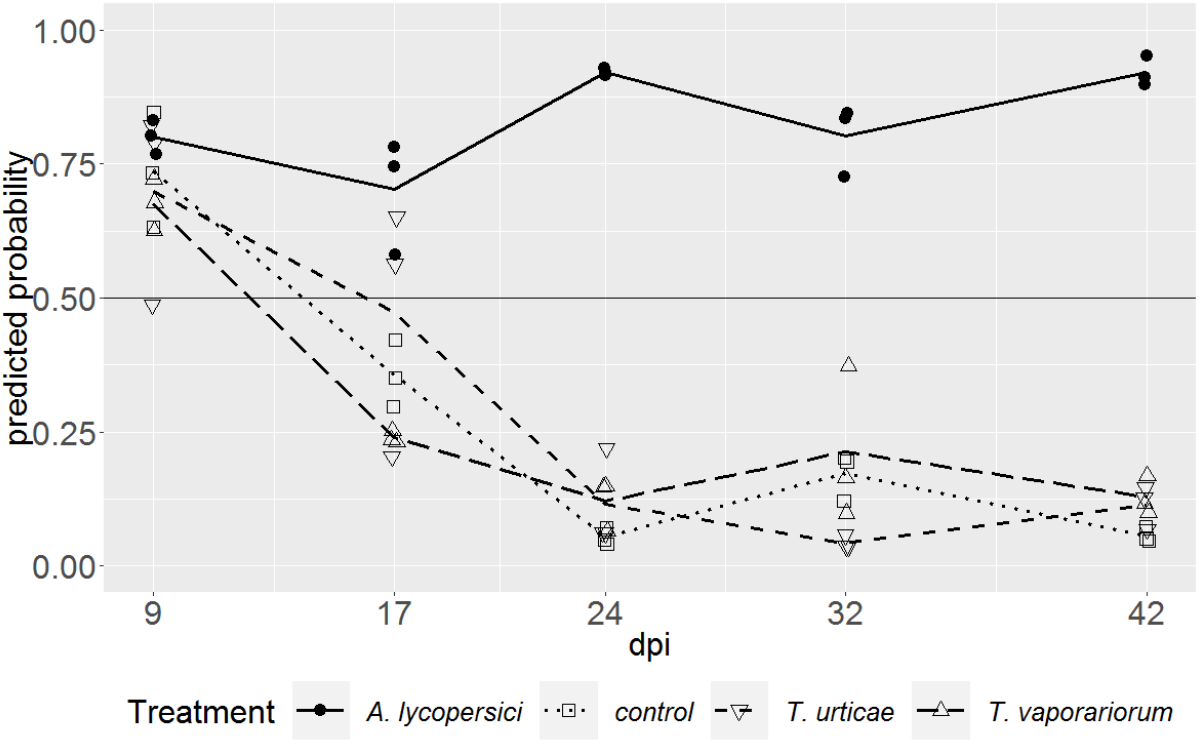

Fig. 4 Classifcation of specifcity based on stem fuorescence measurements from 9 to 42 days post inoculation (dpi). Predicted probability of *A. lycopersici* presence resulting from ridge regression is displayed on the Y-axis. Each dot represents one plant on a specifc sampling date. The lines indicate the average predicted probability per treatment (n=3 per treatment), solid= *A. lycopersici*, dotted=healthy, dashed=*T. urticae*, dot-dashed=*T. vaporariorum*. If a plant has a probability lower than 0.5 it is considered free of *A. lycopersici*, if it has a probability above 0.5 it is considered infested by the model.

1.5 Discussion

Results of the classification into either 'healthy' or 'infested' based on the fluorescence measurements were compared with a visual assessment of plants and a count of A. *lycopersici* individuals on sticky tape imprints that were taken from the stem of the tomato plants. In a follow up trial, the specificity of fluorescence measurements for *A. lycopersici* was investigated by testing it simultaneously against plants infested with *T. urticae* and plants infested with *T. vaporariorum*. It was possible to differentiate *A. lycopersici* infested plants from healthy plants based on stem fluorescence measurements. With the applied ridge regression, correct classification rates above 90 % were achieved from 20 dpi onwards (Tab. 1). The decision to

rely on the ridge regression was made as it performed better, with higher classification stability compared to other methods (PLS, lasso regression, PCA+ LDA, displayed in Fig. S2 in the Appendix Chapter 1).

The trial was conducted in a highly stable and controlled environment on potted plants. Given that the lighting, temperature and substrate conditions are highly variable in practical cultivation, a higher variance in the induced fluorescence on healthy plants is to be expected under practical conditions. Also, the fluorescence differences seem to be quantitative and not qualitative, as the inspection of the curves of the average fluorescence in Fig. 2 revealed that there were no shifts of fluorescence peaks into other wavelength areas but a parallel decrease in signal strength across the whole wavelength area. Thus, differentiation between healthy plants and *A. lycopersici*-infested plants could be more difficult. The process of inducing fluorescence only works if photosystem II is not oversaturated by light. In an additional trial under practical conditions the signal strength of the induced fluorescence was strongly reduced under bright daylight and almost undetectable if the measured plant tissue was exposed to direct sunlight (data not shown). This circumstance requires the measurements to be conducted either at night or under stable and low light conditions during dawn / dusk. In Fig. 3 two peaks are visible at 10 and 16 dpi, both for plants receiving sufficient and low water supply. At these two peaks, many more plants were classified as inoculated compared to the proximate sampling dates. Although the definitive explanation for these peaks could not be identified, perhaps there was exposure to increased artificial lighting in the morning prior to the measurements, resulting in partial saturation of photosystem II. This emphasises once again that stable light conditions are required and that comparisons between measurements can only be made if they were taken under comparable light conditions. Although stability of light conditions is important for the fluorescence method, this may not be the case for detection methods using volatile organic compounds (VOC) (Takayama et al. 2013). With the VOC method, however, other environmental factors could interfere with the analysis of VOCs as this has not been investigated so far.

As stated in the introduction, the stem in contrast to the leaves remains throughout the season and therefore is potentially permanently exposed to *A. lycopersici* feeding. For this reason, this site was selected for the measurements. If an automated measurement system were to be developed there are likely mechanical benefits in taking measurements at the stem of tomato

plants as it is more or less at the same position and orientation for all plants in a modern greenhouse. The local reduction of fluorescence of plant tissue as observed in this experiment, is not necessarily due solely to the feeding of *A. lycopersici* but could also be the result of several biotic and abiotic stressors as it resembles the photosynthetic activity in the sampled area. Given that other arthropod pests occur in tomato cultivation it was cross-checked in an additional trial whether the two-spotted spider mite *T. urticae* or the greenhouse whitefly *T. vaporariorum* could interfere with fluorescence results. This was not the case for *T. vaporariorum*, most likely due to the fact that the damage caused by *T. vaporariorum* just like the damage caused by *A. lycopersici* only results in local reduction of fluorescence. As the feeding of *T. vaporariorum* is restricted to leaves (Capinera 2001) a reduction of fluorescence cannot be measured at the stem. *T. urticae* did not establish on the inoculated tomato plants, therefore it cannot be said with certainty that interference would not occur. Given that *T. urticae* damage is, as is the case for *T. vaporariorum*, generally limited to leaves (Capinera 2001) it is highly unlikely that the presence of this organism would interfere with stem fluorescence measurements. To further validate the hypothesis that direct local surface damage at the measured spot is required for an influence on fluorescence, follow-up tests should be carried out with other common tomato pests. Several fungal pathogens cause symptoms on leaves, but few cause symptoms on the stem of tomato plants similar in appearance to those induced by an *A. lycopersici* infestation. By choosing the stem for measurements, it is less likely that *A. lycopersici*-caused fluorescence reduction can be confused with fluorescence reduction caused by fungal pathogens. Nevertheless, investigation into fungal pathogens and whether their occurrence can influence a classification of plants into *A. lycopersici*-infested or healthy based on fluorescence measurements at the stem should be conducted. The same procedure of specificity analysis should be performed for the approach of VOC analysis investigated by Takayama et al. (2013).

Another important aspect to consider is that a relatively small part of the plant surface was measured in this study. This aspect is also relevant for VOC detection methods (Takayama et al. 2013). The optical fibre that was used had a diameter of 1 mm, resulting in measurement of an area of 0.79 mm^2 per sample. The small size of the measured area makes the measurement vulnerable to even small local physiological or morphological surface changes on the plant stem. To increase reliability and reduce measurement variance it would be advisable to either

increase the measured area or work with several measurement points per plant. This study was based on a non-imaging sensor. If an attempt was made to increase the plant stem surface on which the classifications are based, one possible approach would be to conduct fluorescence imaging using a hyperspectral camera. In a fluorescence image, larger surface areas could be evaluated for their fluorescence patterns with the consequence that the representativity of the obtained data for the classification of the plant could be increased. Although the quality and reliability of the classification based on an imaging system have the potential to increase, the downside of an imaging system would be the high acquisition costs. Use of such an imaging system would result in substantially higher costs compared to the non-imaging system applied in this study.

One potential confounding factor, which was considered in this study, was the occurrence of drought stress and the impact that it could have on fluorescence measurements. Drought stress is a frequent source of abiotic stress in practical cultivation depending on the way of cultivation. Drought stress as a common abiotic stressor can have an effect on photosystem II (Fracheboud and Leipner 2003) and thus potentially affect the fluorescence signal emitted by the plant. To account for this a drought stress factor was included in the trial. In the analysis of the fluorescence measurements, *A. lycopersici*-inoculated plants with low vs. sufficient water supply performed similar to one another, as did the control plants with low vs. sufficient water supply (for detailed classification rates see Tab. S1 in the Appendix Chapter 1). Although a direct interference of the water supply levels with the fluorescence signal was not detected, it was observed that the *A. lycopersici* populations on plants experiencing drought stress reached significantly larger sizes compared to the populations of mites on plants receiving sufficient water (Fig. 1). This observation confirms the findings of Ximénez-Embún et al. (2017) and emphasises the importance of supplying sufficient water consistently throughout the season as one measure to counteract *A. lycopersici* outbreaks.

The sticky tape imprints performed best in the classification into infested or healthy plants (Tab. 1). In previous tests it was shown that even very small rates of infestation could be revealed with the sticky tape imprints and that the number of individuals found on the imprints correlated well with the number of individuals found on the sampled plant surface (Appendix Chapter 1, Fig. S1). Sticky tape imprints were a suitable reference method for retrieving infestation status and population density during this trial. Already at 4 dpi,

A. lycopersici individuals were identified on 5 of the 48 inoculated plants using the sticky tape method. From 14 dpi onwards the correct classification rate was above 90% and, from 20 dpi onwards it was 100%. However, similar to the detection and quantification methods described by Perring et al. (1996) and in contrast to fluorescence measurements, the detection method based on sticky tape imprints is very time consuming as every imprint has to be checked for the presence of *A. lycopersici* individuals with a stereo-microscope. It took roughly 4 s for each stem fluorescence measurement to be taken. In contrast the process of taking a sticky tape imprint including the screening for *A. lycopersici* at a stereomicroscope took roughly 1 min. Another factor to keep in mind is that the plants used in the trial were relatively small in comparison to plant heights of several meters that are reached in practice. Given that *A. lycopersici* populations are unlikely to distribute evenly across a large plant, several imprints would need to be made per plant to achieve comparable representativity and this would multiply the monitoring time required yet again. In summary, although the sticky tape imprint method has high detection success, this method seems suitable only for verification of suspected infestations, not for continuous monitoring in practical cultivation. As for correct classification based on visual symptoms it must be noted that the plants were examined with a level of diligence that is not feasible for practical cultivation as such examination is simply too time intensive. This is a crucial factor to be considered if the attempt is made to compare and relate the efficacy of the various detection methods of this study to practical cultivation. In practice, similar to the sticky tape imprint method, visual examination of all plants in a tomato crop is not feasible. In contrast, the analysis of the spectrometric data provided in this study can form the basis for a standardised automated monitoring. If the measurement can be conducted by a robotic arm directing the optical fibre towards the plant stem and taking the measurement, a large number of plants could be automatically monitored within one night, even after the time taken for the machine to move between plants has been taken into account. In the results, the plants with the highest predicted probability are those most likely infested with *A. lycopersici*. It is then left up to the grower to decide which and how many plants should be checked more closely for *A. lycopersici*—visually, with the described sticky tape imprints or, for example, with the binomial sampling plan developed by Moerkens et al. (2018). Application of these methods in this way could potentially improve monitoring efficacy in practice considerably. A similar path in which plants are ordered based on their probability of

infestation could be taken for the analysis of VOCs as investigated by Takayama et al. (2013) if there ever were attempts to further develop this method.

1.6 Conclusion

This study has shown that it is possible to differentiate between healthy and *A. lycopersici* infested tomato plants based on stem fluorescence. The level of correct classification based on stem fluorescence was comparable to, or better than, the classification derived from visual assessment. An increase in area measured has the potential to increase the correct classification rate and should be considered in future studies. The classification based on fluorescence showed a stable outcome when possible interference factors such as drought stress or other common tomato pests were included. Further research is required which investigates specificity of the applied method with regard to fungal pathogens. From a current point of view, the adaption of stem fluorescence measurements for *A. lycopersici* detection in practical tomato cultivation seems possible. Tests with plants grown under conditions approximating practical cultivation and approaches to automate the sampling are essential next steps. The cost of implementing an automated sampling system will pay of if the value of crops that are saved by this procedure outweigh the procedure's cost. In order to evaluate this distinct economic threshold, more data on yield loss caused by *A. lycopersici* and the frequency of its occurrence in tomato cultivation is necessary. In any case, by adding the detection of *A. lycopersici* to the pool of existing spectroscopic applications for the screening of plant health, the development of commercial automated plant-health screening systems becomes increasingly attractive. Any such system that allows time saving detection of *A. lycopersici* infestation directly benefits tomato growers faced with *A. lycopersici* infestations in their crop.

1.7 Acknowledgements

Tina Drechsler, Kathrin Burlak, Dörte Achilles, Elke Jeworutzki, Rowan Titchener

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Observation and restriction of *Aculops lycopersici* dispersal in tomato layer cultivation

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Funding organisation:

This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant number 2816ERA01L.

This version of the article has been accepted for publication after peer review in: ***Journal of Plant Diseases and Protection*** and reflects the state of the accepted manuscript but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The publication is available online at: https://doi.org/10.1007/s41348-023-00817-6

Observation and restriction of *Aculops lycopersici* dispersal in tomato layer cultivation

2.1 Abstract

The tomato russet mite *Aculops lycopersici* has become a challenging pest in tomato production in the EU. The number of available acaricides is low and the efficacy of biological control is limited. With this study we aim to understand better the unhindered dispersal dynamics, and develop a method to reduce dispersal on plants.

To better understand the dynamics of *A. lycopersici* dispersal in layered tomato cultivation under practical conditions, a first trial was carried out. The trial confirmed that first *A. lycopersici* symptoms in practical cultivation usually occur in the lower or the middle third of tomato plants and then move upwards on plants. It was observed that plants, for a limited period of time often are able to grow new healthy leaves in the same pace as existing leaves, mostly in the lower and middle part of the plant are damaged by *A. lycopersici*. This is possible due to the fast growth rate of hybrid tomato varieties in layer cultivation. To test if the observed effect can be supported by further slowing down the upwards movement of the pest, a second trial was conducted. Here, the stems of inoculated tomato plants were blocked weekly for *A. lycopersici* by applying a ring of insect glue 15 cm below the tip of the plants. This stem blockage severely impaired the only active dispersal mode of *A. lycopersici*: walking. The growth of new plant material, when the method is applied, is able to exceed the speed with which *A. lycopersici* destroys plant material in layered tomato cultivation. This resulted in significantly less plant damage and prevented fruit damage on all treated plants. The approach of manipulating the plant stem and thereby restricting the movement of the mite on tomato plants could potentially be exploited for plant protection purposes under practical conditions.

Keywords:
Aculops lycopersici, tomato russet mite, pest dispersal, protected cultivation, control method, glue ring

2.2 Introduction

The tomato russet mite *Aculops lycopersici* (Tryon; Acari: Eriophyoidea), is a vagrant eriophyoid mite that is considered a pest in different Solanaceae crops (Perring and Farrar 1986). *A. lycopersici* is currently found throughout the world in both tropical and temperate regions. Before 1999, there were only minor incidents of *A. lycopersici* in Germany. However, in recent years the mite has occurred more frequently in tomato production in Germany (Merz 2020) and other parts of the EU. This observation is in line with reports of an increased economic impact of eriophyoid mites in general in several regions of the world (Duso et al. 2010). *A. lycopersici* causes bronzing and a russeted appearance of leaves and stem as it feeds on surface cells (Royalty and Perring 1988), damaging the upper and lower epidermis of the tomato plants (Royalty and Perring 1988). The feeding can result in curling of leaf edges, followed by the dropping of leaves (Capinera 2001) and in severe cases it leads to the death of the whole plant (Keifer et al. 1982).

A. lycopersici is a problematic pest due to its high reproduction rate, relatively small body size with a maximum length of 0.2 mm (Haque and Kawai 2003), and the fact that a limited number of plant protection agents are authorised for this pest in Germany (BVL 2022). Despite studies that show a certain effect of predatory mites against *A. lycopersici* (Brodeur, Bouchard, and Turcotte 1997; Park, Shipp, and Buitenhuis 2010; Park et al. 2011), the efficacy of these natural enemies in practical conditions is small (van Houten et al. 2013). More recent studies show very promising results for different predatory mites under semi-practical conditions but have yet to be confirmed with trials in practical cultivation (Pijnakker et al. 2022a, b; Vervaet et al. 2022; Castañé et al. 2022). In practise, recognition of *A. lycopersici* infestation coincides with recognition of symptoms on plants which first usually appear in the lower part of plants (Bailey and Keifer 1943; Capinera 2001). Especially early symptoms, such as light chlorosis on leaves, or light grey and brown shading on the stem, are easily overlooked. Later developing and more obvious symptoms can be misdiagnosed as a fungal pathogen by unexperienced farmers or workers.

So far, there is not one ideal strategy to combat *A. lycopersici* outbreaks in tomato cultivation. One reason for this is that too little is known about *A. lycopersici* dispersal patterns in modern layered tomato cultivation. As a pest that is unable to fly and that walks only at a low speed,

A. lycopersici makes use of further techniques to disperse and reach new host plants (Sabelis and Bruin 1996; Michalska et al. 2010). Michalska et al. (2010) categorised the dispersal modes of eriophyoid mites into active dispersal, such as walking to uncolonized plant surfaces or to secondary plants in direct contact to the already colonized plant, and passive dispersal such as transferral via air currents, phoresy (i.e. using moving vectors as transport vehicle) or raindrop splashes (Jeppson et al. 1975). The described dispersal modes have been studied in different eriophyoid mites, but not yet specifically in *A. lycopersici*. However,they are assumed to apply for *A. lycopersici* too (Capinera 2001). According to Sabelis and Bruin (1996), aerial dispersal is considered the most important mode under natural conditions.

In the artificial greenhouse environment, *A. lycopersici* faces distinct conditions for dispersal that are very different to those they encounter under natural conditions. While dispersal via raindrops can be disregarded entirely, active dispersal via walking short distances in relative comparison is likely to be of higher importance to reach new host plants, as all neighboured plants are hosts, or uncolonized plant surfaces within one plant. In addition, dispersal via phoresy is likely with several different airborne tomato pests (Michalska et al. 2010) or beneficials, but also via humans working on the crop with clothes or tools that can function as a carrier medium (Capinera 2001). Dispersal via air currents will also occur in greenhouses, as ventilation is often used to reduce air humidity (Kittas and Bartzanas 2007). Whether dispersal can be triggered via droplets or airstreams produced in the application process of pesticides has not been looked into until today.

For within-plant dispersal *A. lycopersici* individuals tend to move upwards and aggregate on the highest tips of different plant organs such as leaves or fruits. The logical explanation for this is that, since plants grow upwards, younger uncolonized plant tissue is available at higher locations. This behaviour is also assumed to facilitate dispersal via air currents (Sabelis and Bruin 1996).

Tomatoes are commonly cultivated in a system called layer cultivation. This cultivation method produces a high dynamic in the tomato crop and makes the recording of pest dispersal challenging. Nevertheless, layer cultivation currently is the most common cultivation method.

That almost none of the relevant studies cited in the latest *A. lycopersici* review by Vervaet et al. (2021) were conducted under practical growing conditions underlines the relevance of the

trials conducted for this study: In a first trial, the unhindered movement of *A. lycopersici* was assessed by closely monitoring the corresponding symptom development throughout a tomato crop and within plants growing in layer cultivation in the typical double row arrangement (dispersal trial). Based on the observations from this first trial, in a second trial tomato plants were grown in layer cultivation and it was investigated whether interfering with *A. lycopersici* within-plant dispersal by blocking the stem using insect glue would reduce pest damage (glue ring trial). The glue ring trial was based upon the hypothesis that walking is the most important within-plant dispersal mode; the glue ring at the stem aimed to interfere with this mode of dispersal.

2.3 Materials and Methods

Dispersal trial: setup, greenhouse conditions and cultivation work

For the dispersal trial, 160 plants of the cultivar Baylee F1 (Enza Zaaden) were sown on the 14th of March in 2017 and singled into pots with a 10 cm diameter on the 22nd of March. The plants were planted into four adjacent soil-greenhouses on the 19th of April. Each greenhouse contained 40 plants divided into two double rows (a and b), each consisting of 2 x 10 plants (Fig. 1 left). This setup resulted in 8 separate double rows. The in-row distance between plants was 50 cm, the distance between the two single rows of a double row was 100 cm, the distance between the centre of the double rows was 200 cm. The minimum temperature of the greenhouses was set to 18.0 °C and whenever the temperature fell below this level the heating system was activated. Roof windows were opened at temperatures above 22.0 °C and side windows opened above 24.0 °C. There was no active cooling or humidity control in the greenhouses.

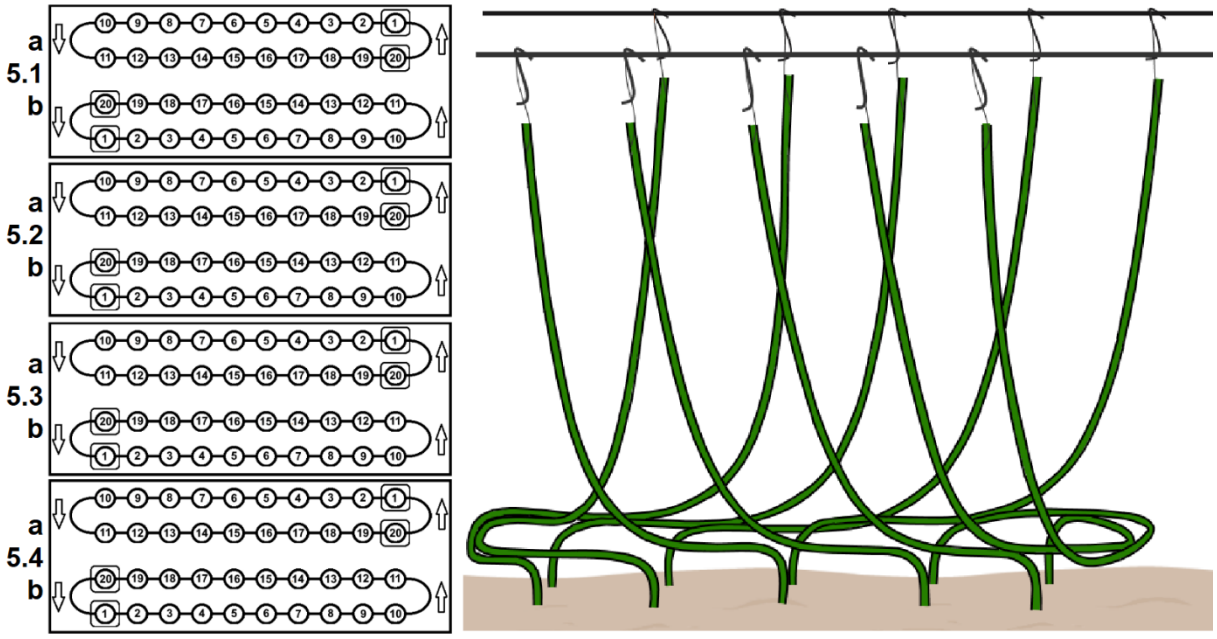

Fig. 1: Left: birds-eye-view of the dispersal trial showing all eight double rows in the four consecutive greenhouses. Each number displays one plant, each double row consists of 2 x 10 plants. The squares indicate plants that were inoculated with *A. lycopersici*. Arrows indicate the direction in which the plants were layered and thereby in which direction the vertical leafy part of the plants were moving as the plants grew. **Right:** schematic display of tomato plants in layer cultivation viewed from the side. To allow better visualisation, plant stems are shown without leaves. The illustration shows a double row with 2 x 5 plants.

When provided with good growing conditions, modern hybrid tomato varieties can reach a stem length far beyond 10 m within one growing season. Since greenhouses are not high enough to allow for a vertical stem of 10 m, and to keep the fruit zone at a workable height, tomato plants are usually grown in "layer cultivation". To resemble practical conditions, layer cultivation was also chosen for the two trials of this study. A detailed schematic of layer cultivation is depicted in Fig. 1. As the tomato plants grow at the top, additional growing thread is unwound from the metal hooks depicted in Fig. 1 (right), and the plants are lowered to give room for more growth while the vertical leafy part of the plants, together with the hooks, move along the horizontal wire at the top. Before the lower part of the plant is aligned horizontally it is defoliated and fruits are harvested. At the end of the double row, plants are simply hung on the other wire and from that point on they move in the opposite direction as they grow further. This switch explains why the stems curve at the bottom, at the end of each

double row. In the results section for the dispersal trial, the single row closer to the viewer is termed the "front row" and the one behind is termed the "back row".

On the 19th of June, two plants in each double row on one side of the greenhouse (Fig. 1 – Left) were inoculated with 30 adult *A. lycopersici* individuals of unidentified sex at the stem in the middle height of the leaf area, roughly 125 cm above ground. The inoculation was repeated on the 26th of June, 7 days past (first) inoculation (dpi), and again on the 6th of July, 17 dpi, to ensure inoculation success and sufficiently high pest pressure. The *A. lycopersici* individuals used in this trial had been reared on tomato plants. The mites in the rearing originated from a private garden near Brunswick (Germany). Cultivation work such as winding the tomato plants around the twines, removing side shoots, defoliation and harvest was always started at the inoculated plant 1 in each double row. Between each double row, gloves were disinfected to avoid that *A. lycopersici* individuals were carried from one double row to another. Cultivation works (removing side shoots, unwinding growing thread, removing leaves at the bottom, harvesting fruits) were conducted twice per week.

Dispersal trial: symptom assessment

A. lycopersici-typical symptoms were assessed two times per week for each plant, and in total 33 times over a period of 16.5 consecutive weeks. The first assessment was conducted on June 19th directly before the first inoculation. The vertically aligned and leafy part of each plant was divided into three different heights (low, mid and high) and scored separately into classes 1: healthy; 2: light symptoms (stem showing light grey/rust brown discoloration, leaves showing light discoloration/light chlorosis); and 3: strong symptoms (stem showing strong, rust brown, coloration and strongly reduced trichome coverage, leaf browning, necrosis and clear leaf deformation). To make sure that the symptoms did not derive from other causes, symptoms were investigated closely with magnification lenses where necessary and presence of *A. lycopersici* on symptomatic plant organs was checked frequently on a sample basis with sticky tape imprints. Due to the size of the plants, the plant numbers and the practical growing conditions, the dispersal data was derived solely from symptom development. Detailed and comprehensive probing for *A. lycopersici* presence or counting individuals would have been too time intensive. This is especially due to the heterogenic distribution of *A. lycopersici* within plants and within single plant organs. To ensure that symptoms were assessed independently,

the results of the previous assessment were not on hand to the person conducting the assessment.

Glue ring trial: setup, greenhouse conditions and cultivation work

For the glue ring trial, 48 plants of the cultivar Baylee F1 (Enza Zaaden) were sown on the 11th of March in 2019 and singled into pots with a 10 cm diameter on the 21 of March. The plants were planted into two adjacent soil-greenhouses on the 24th of April. Each greenhouse contained 24 plants in total divided into 6 plots. Each plot consisted of 2 x 2 plants in a double row as shown in Fig. 2. The climate control measures in this trial were identical to those implemented in the dispersal trial.

Fig. 2: One of two greenhouse chambers of the glue ring trial at an early stage.

The plants were grown in layer cultivation. Defoliation and harvest were conducted once per week, prior to the sampling of leaves and after winding tomatoes around the twines and removing of side shoots. Winding and shoot removal were done twice per week and separately from defoliation and harvest. Gloves were always changed after work was finished

at each plot. To quickly produce symptoms, all plants were inoculated, each with (equally) highly infested tomato leaves (estimated as having at least 2000 *A. lycopersici* individuals per leaf) on the 19th of June. The infested leaves were placed on a leaf between the middle and the upper third of the plants. To test whether the inoculation had been successful, all plants were checked for *A. lycopersici* individuals seven days post inoculation, with sticky tape imprints taken from the stem as described in Pfaff et al. (2020). On the 22nd of July, 33 dpi, inoculation with *A. lycopersici* was repeated but this time the inoculation site was 10 cm below the tip of each plant, on the youngest fully unfolded leaf so as to simulate *A. lycopersici* reaching the highest parts of tomato plants. The applied *A. lycopersici* individuals were taken from the same rearing as those used for the dispersal trial. The trial consisted of two treatments, each with six plots of four plants. The treatments were (1) positive control without countermeasures against *A. lycopersici*, and (2) treatment with an insect glue ring applied weekly to the stem of the tomato plant at the same day the sampling was carried out. As soon as the first symptoms of *A. lycopersici* were visible, 5 glue rings were applied evenly over the height of every plant to the stem of the plants, followed by a weekly glue ring application 15 cm below the tip of the plants. The applied insect glue was "Temmen Insektenleim" (Temmen GmbH, Hattersheim, Germany). On average the glue covered a height of 2 cm on the plant stem. This glue is the adhesive that is usually used on coloured sticky traps. It was specifically chosen for this trial as it does not change viscosity when exposed to heat, and it maintains a high level of adhesiveness over time. *Macrolophus pygmaeus*, a predatory plant bug used for biological control of several insect pests, was introduced into the greenhouses on the 29th of April to mimic practical growing conditions and enable potential phoresy such that *A. lycopersici* could overcome the glue barrier.

Glue ring trial: symptom assessment

The total number of leaves and the number of leaves showing *A. lycopersici*-typical symptoms were counted weekly over a period of 84 days, between the 17th of June and the 9th of September. With this data it was possible to calculate the proportion of symptomatic leaves on every plant. In this trial, in contrast to the dispersal trial, the severity of the observed symptoms was not noted. The seven-day growth of 12 plants in the glue ring trial (one plant per plot in both treatments) was measured over a period of four weeks.

Statistical analysis

Statistical analysis was performed with the Software 'R' (R Core Team 2021; version 4.1.0).

To test whether the working direction in the dispersal trial had an effect on time until symptom development, a Kaplan-Meyer curve was fitted, followed by a log-rank test conducted using the 'survival' R-package (Therneau 2021). For comparison, plants 3, 4 and 5 (in working direction) were compared with the plants 18, 17 and 16 (not in working direction). The plants were selected for their equal "in-row" distance to the inoculated plants. The direct neighbours to the inoculation plants, 2 and 19, were left out as the chance that these plants would have been quickly colonised by walking *A. lycopersici* individuals was assumed to be high. It was not distinguished between different plant heights, only the timestamp of the first symptom on a plant was considered in the analysis. The survival analysis was chosen because not all plants developed symptoms. The plants that did not develop symptoms were still included as censored data points in the analysis.

To test whether the weekly application of a glue ring to the stem of tomato plants had an effect on the proportion of leaves damaged by *A. lycopersici*, generalised linear mixed effects models with beta family were fitted using the 'glmmTMB' R-package (Brooks et al. 2017). The interaction between glue ring treatment and sampling date was fitted as a fixed effect, plant ID nested in plot, nested in double row, nested in greenhouse was fitted as random effect to account for the trial structure. To account for the repeated measurements over time, for each plant an (AR1) autocorrelation structure was fitted since this model had a considerably lower AIC (Aikaike information criterion) in comparison to the model without AR1 when fitted with restricted maximum likelihood (REML). The AIC allows a relative comparison of the goodness of fit between models by penalising models with higher numbers of independent variables. Significance of model parameters was assessed using Wald χ^2 test and ANOVA type 3 sums of squares using the 'car' R-package (Fox and Weisberg 2019).

A Tukey post-hoc test for comparison of the estimated marginal means between the symptomatic leaf proportion in the glue ring treatment and in the positive control at each sampling date was conducted using the 'emmeans' R-package (Lenth 2021).

2.4 Results

2.4.1 Dispersal trial

Time until first symptom development on inoculated plants

Eleven out of 16 plants showed first symptoms on the 27th of July, 38 days post (first) inoculation (dpi). Of the five exceptions, one of the two plants in the double row b in greenhouse-chamber 5.2 showed first symptoms 42 dpi on the 31st of July. The remaining four inoculation plants of the double rows a and b in the greenhouse-chamber 5.1 showed first symptoms between 101 dpi on the 28th of September, and 123 dpi on the 20th of October. The first symptoms developed in an area of 5 cm around the inoculation spot on the stem on each plant, partly on leaves growing in this area.

Height at which first symptoms occurred

Of the 160 tomato plants in the dispersal trial, 12 plants remained free of *A. lycopersici* symptoms. Of the remaining 148 plants, 143 plants showed first *A. lycopersici* symptoms either in the lower or the middle third of the plant, or in both of these heights at the same time. Five plants showed first symptoms either at the same time in the middle and the high third of the plant, or in all three heights at the same time, as shown in Fig. 3.

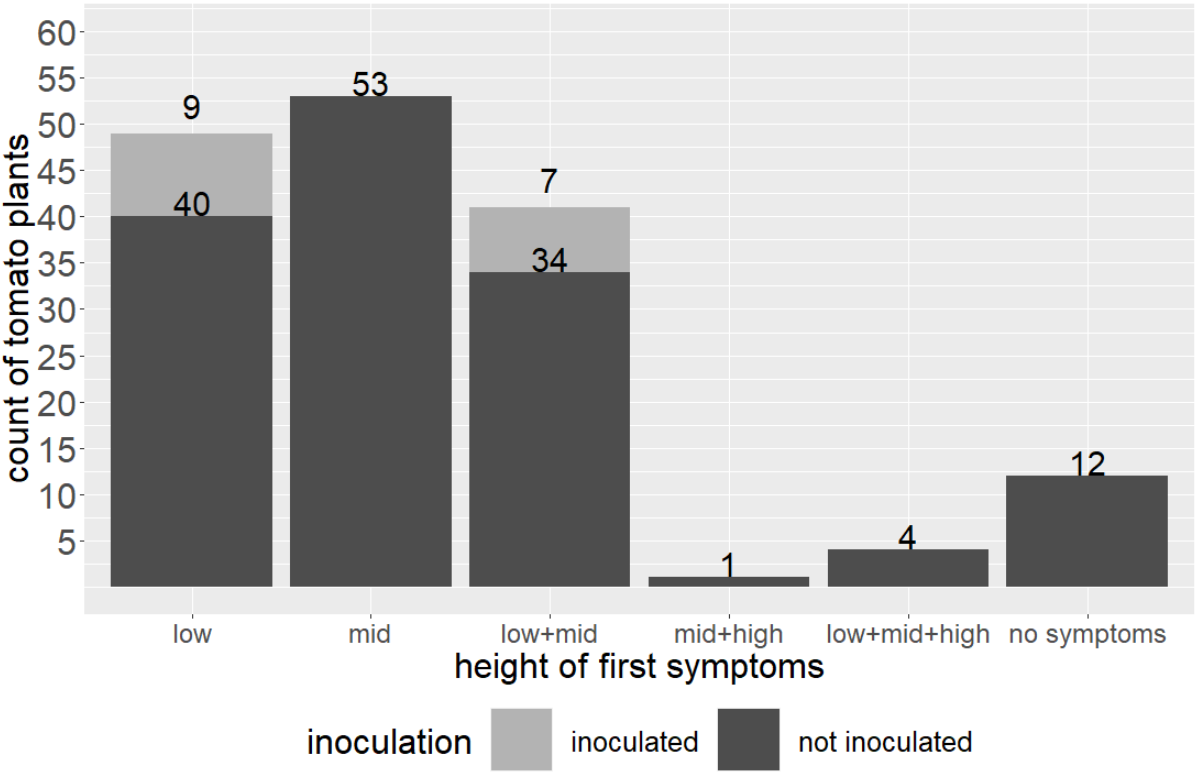

Fig. 3: Count of tomato plants on the Y-axis, and the height at which they showed first *A. lycopersici* symptoms displayed on the X-axis for inoculated plants and plants that were not inoculated. None of the plants showed first symptoms just in the high third or combined in the low and the high third (n = 160, of which 16 were inoculated plants).

Influence of working direction on symptom development

Three out of the 24 plants in working direction did not produce any symptoms and eleven plants did not produce strong symptoms. Two of the 24 plants not in working direction did not produce any symptoms and nine plants did not produce strong symptoms. A Kaplan-Meyer curve followed by a log-rank test revealed that there was no significant difference between the group of plants in working direction (plants 3, 4 and 5) and the group of plants not in working direction (plants 16, 17 and 18) in the time until first symptoms ($p = 1$) and strong symptoms ($p = 0.4$) occurred (Fig. S1 & S2 in the supplementary information).

"In-row-dispersal" vs "inter-row-dispersal" to neighbouring plants in double rows

It was observed that in the surroundings of the inoculated plants with the numbers 1 and 20, the in-row neighbouring plants 2, 3, 19 and 20 produced strong and lasting symptoms slightly earlier compared to the neighbouring plants in the corresponding single row within the double

row. Since the plants in the single row of the inoculation plants 1 and 20 move in the opposite direction to the corresponding single row, the inter-row neighbouring plants of the inoculation plants changed frequently as opposed to the in-row neighbouring plants 2 and 19 which remained the same. Thus, a valid statistical comparison is difficult. Nevertheless, this phenomenon can be observed more or less prominently in most of the double rows displayed in Fig. 4.

Tomato growth versus speed of symptom development

Inspecting the symptom development across plant height levels and time shown in Fig. 4, it appears that the symptoms on multiple plants visible at specific heights either decreased in severity or disappeared entirely in those heights (for instance plant 20 in double row 5.1_a, plants 1-6, 10, 11 and 13-20 in 5.2_a, plants 1, 2, 5, 12-14, 17 and 19 in 5.3_b and plants 1, 3-6, 8, 10, 11 and 16 in 5.4_a). Not visible in the chosen symptom display of Fig. 4 but nevertheless observed: plants that only showed symptoms in the high or middle third, showed them in the middle and lower third in following sampling dates, whereas the high or middle third of the plants was observed to be symptom free. Also, in some cases the middle or high third of the plants remain symptom free over several sampling dates while in the lower third of the plants symptoms were apparent.

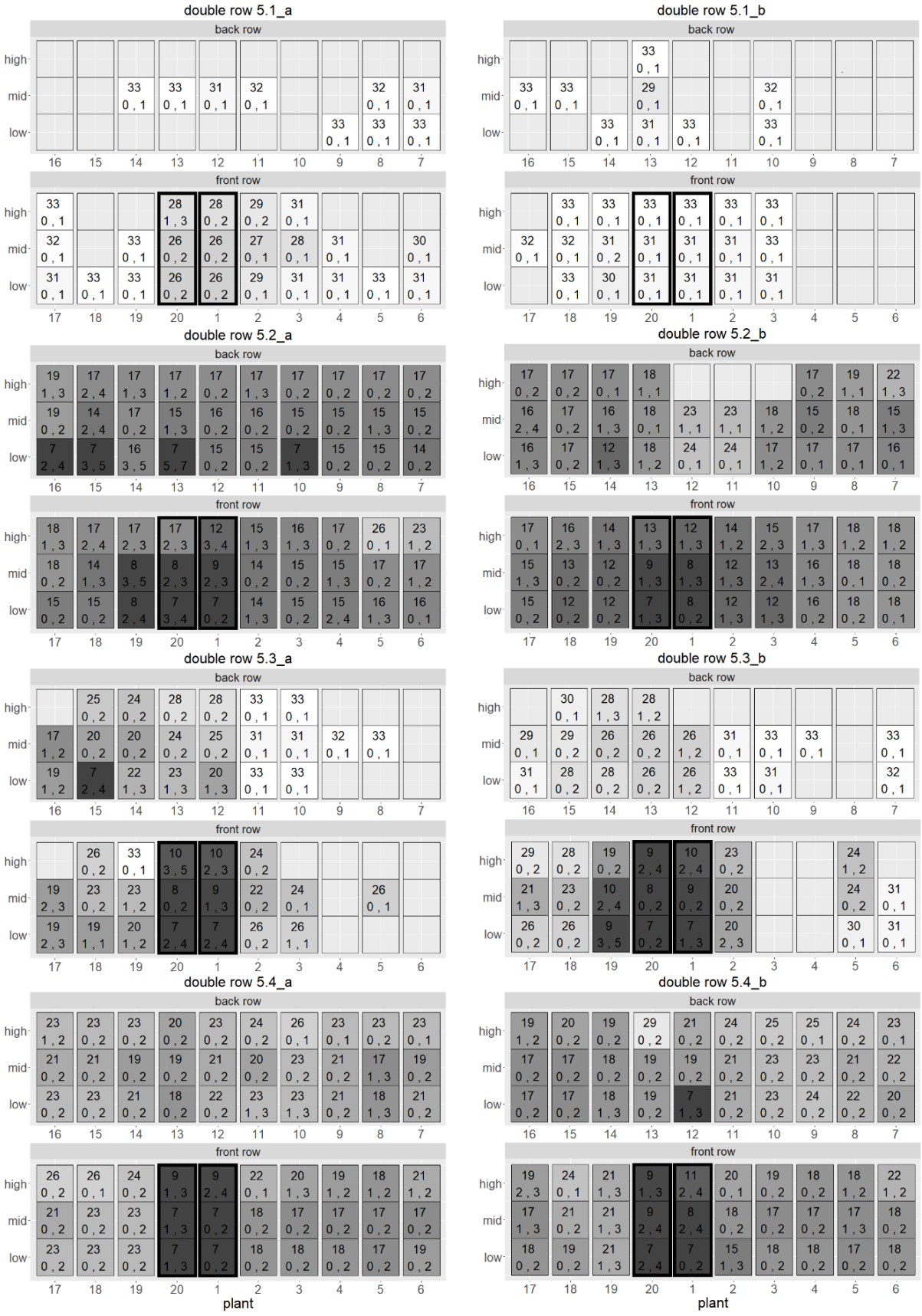

Fig. 4: Symptom development in the vertical leafy part of plants, displayed separately for the eight double rows, each labelled as front and back row (and shown above each other). Each

tile stack displays a single plant, with its plant number displayed underneath. Each plant is stacked threefold, divided into the heights in which symptoms were sampled (low, mid, high). For a better understanding, refer to Fig. 1 and the respective section on "layer cultivation". Numbers in the tiles display: the assessment post inoculation at which first symptoms occurred in that height on that particular plant (upper number), the number of times symptom intensity decreased (lower left number) and the number of times symptom intensity increased (lower right number) from one sampling date to the next. Empty tiles indicate that the plant remained free of symptoms. The shading of the tiles corresponds to the assessment date at which first symptoms occurred (Dark: early sampling date, light: late sampling date). Plants 1 and 20, marked with a bold black frame, were inoculated with *A. lycopersici*.

A. lycopersici upward movement on plants

A. lycopersici populations tended to move upwards on the plants. This behaviour led to *A. lycopersici* individuals accumulating on the highest points on different plant organs. These accumulations closely resemble pollen or dust debris (Fig. 5). The observed accumulations remained in these high spots until the particular leaves or fruits were removed.

Fig. 5: Picture of tomato leaves covered with *A. lycopersici* accumulations at the highest points. Upper left and right: picture taken in greenhouse. Lower left and right: magnified picture taken with stereomicroscope (magnification factor not noted). Pictures: A. Pfaff 2017.

2.4.2 Glue ring trial

A. lycopersici induced symptom development

At the first four sampling dates, 2 days prior to inoculation until 19 days post (first) inoculation (dpi) there were no significant difference in terms of the proportion of symptomatic leaves between plants that received a weekly glue ring and plants that received no treatment (positive control) (Fig. 6). From 26 dpi until 82 dpi the proportion of symptomatic leaves in the glue ring treatment was significantly lower compared to the positive control (post hoc test p-values: 26 dpi: 0.00122; 33 dpi: 1.14e-7; 40 dpi: 2.01e-14; 47 dpi: < 2e-16; 54 dpi: < 2e-16; 61 dpi: 9.07e-14; 68 dpi: 3.74e-6; 75 dpi: 1.02e-6; 82 dpi: 2.5e-11). From 40 dpi to 82 dpi, the median proportion of symptomatic leaves in the positive control ranged between 20-40%, whereas there were virtually no symptoms in the plants which received the glue ring treatment. At 68 and 75 dpi, symptoms increased temporarily in the glue ring treated plants, with the median ranging from 5-10% symptomatic leaves but still remained significantly lower compared to the positive

control. There is a slight decreasing trend in the proportion of symptomatic leaves in the positive control and the glue ring treatment at 75 and 82 dpi. Of the 24 plants in the positive control, 18 showed fruit damage. No plant in the glue ring treatment developed fruit damage.

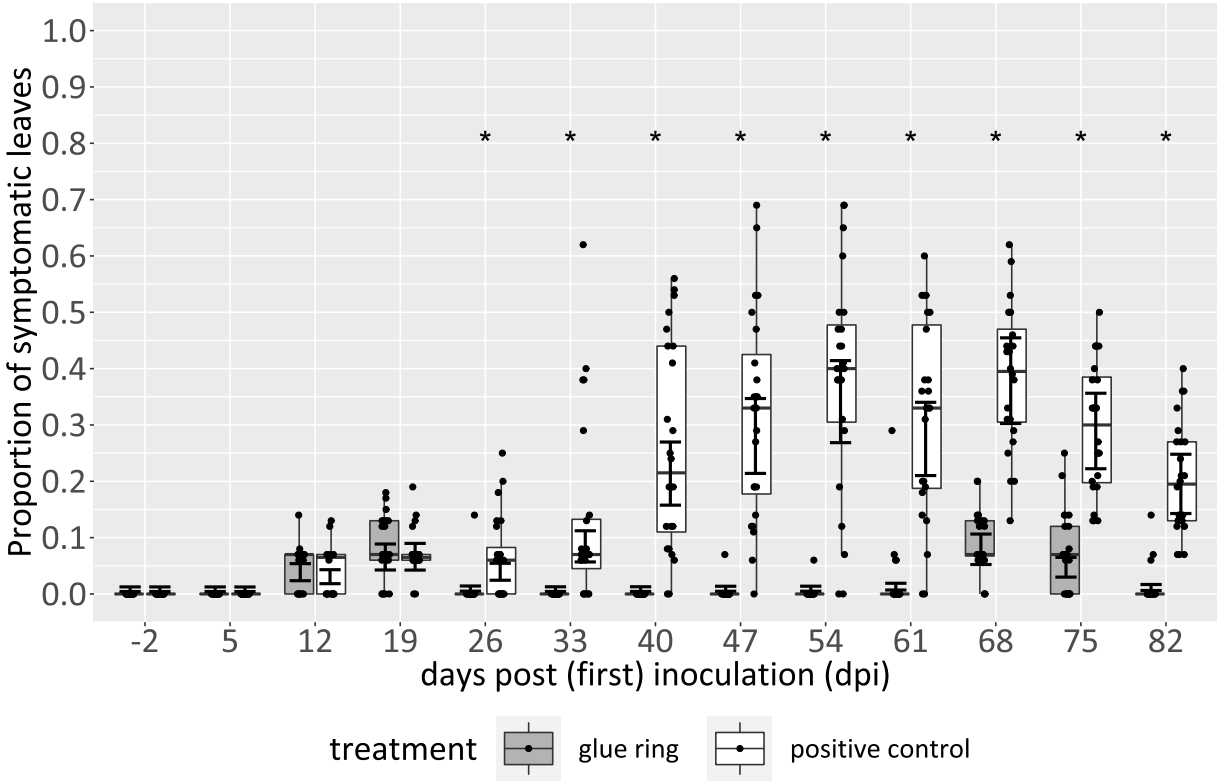

Fig. 6: Comparison of the proportion of symptomatic leaves per plant for plants that had a weekly glue ring applied to their stem (n = 24, grey box) and plants that received no treatment (n = 24, white box) over a period of 84 days. Each dot represents one plant at the sampling date. Boxplots display lower and upper quartiles (boxes), and the lowest and highest proportion of symptomatic leaves within 1.5 x the lower and higher quartile range (whisker). The horizontal line in each boxplot displays the median. The black error bars display the 95% confidence intervals obtained from the generalized linear mixed model. The stars at the top indicate significant differences between the treatments at the particular sampling dates according to a post-hoc Tukey test.

Fig. 7 shows tomato plants that received a glue ring treatment on their stem. *A. lycopersici* individuals accumulate as they are prevented from reaching the higher parts of the tomato plant. There are clearly visible symptoms below the glue ring.

Fig. 7: Two tomato plants that received a glue ring treatment on their stems. In the pictures symptoms caused by *A. lycopersici* are visible below the glue ring.

Growth rate of tomato plants

The seven-day growth was relatively constant throughout the measurement period in July and August. On average the tomato plants grew 25.47 cm every 7 days (Fig. 8).

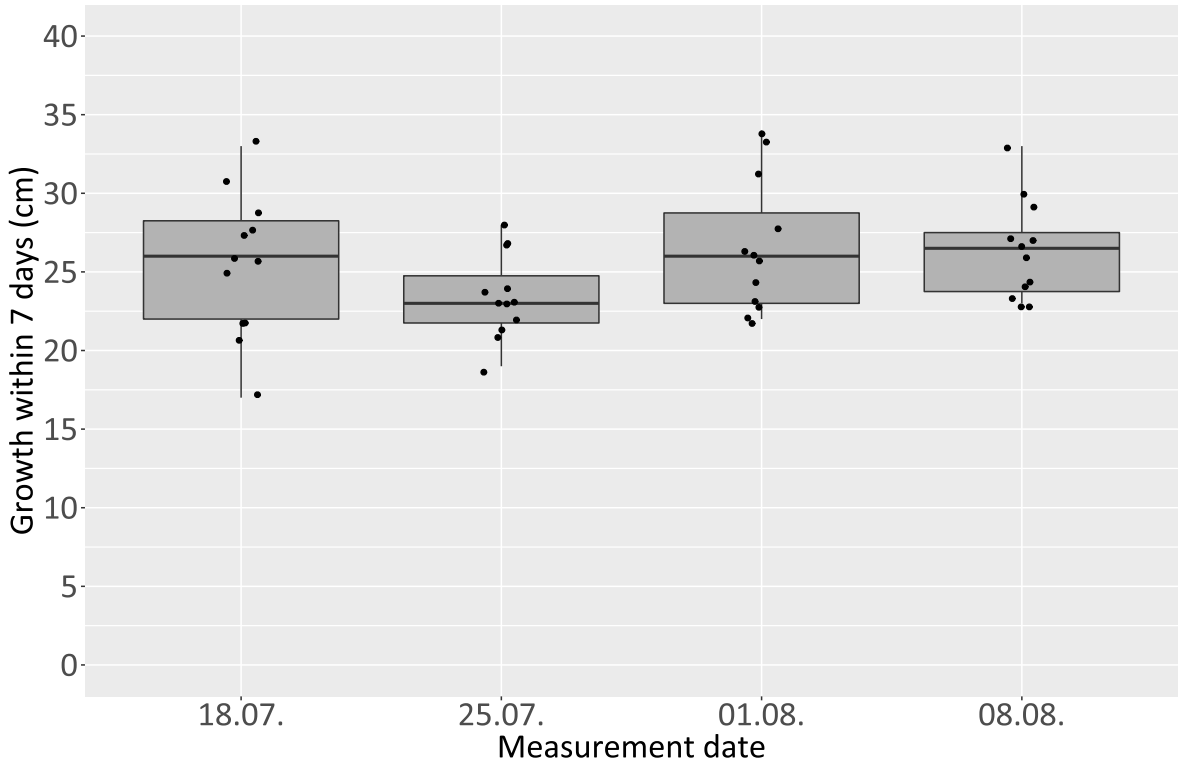

Fig. 8: Weekly plant growth (in cm, n = 12) across five weeks of observation starting with the 18th July (29 dpi). Each dot represents one plant at the sampling date. Boxplots display lower and upper quartiles (boxes) and the lowest and highest proportion of symptomatic leaves within 1.5 x the lower and higher quartile range (whisker). The horizontal line in each boxplot shows the median.

Beneficial insects

The glue rings remained free of the introduced *M. pygmaeus*. *M. pygmaeus* individuals were found in high numbers on all plants, both on plants that had received the glue ring treatment and on those that had not. No count was performed, so it is not possible to conclude whether *M. pygmaeus* showed a preference for plants that had or did not have a glue ring.

2.5 Discussion

In the dispersal trial under practical conditions, following inoculation with a small number of *A. lycopersici* individuals (< 30), first symptoms became visible 38 dpi. Other trials conducted under practical conditions with low inoculation numbers at the same trial station also

supported the conclusion that it takes at least four to six weeks for first symptoms to develop (data not shown). This finding corroborates observations in other studies, for example Pijnakker et al. (2022a) reported that first symptoms appeared after five weeks. First symptoms on inoculated plants always developed directly at, and surrounding the inoculation sites. This indicates that small numbers of *A. lycopersici* arriving on a new plant tend to feed and propagate at the arrival site before they move upwards, likely when population density has increased. This assumption and the assumption that a large population of *A. lycopersici* is required to produce significant plant damage, are the fundamental preconditions upon which the following glue ring trial was developed.

The absence of a significant difference in symptom development between plants in working direction and those not in working direction in this trial indicates that in the close proximity of double rows, transport via gloves and tools (as reported in Capinera (2001)), seems to have played a less important role as compared to the other possible dispersal modes. However, since the total number of plants with 2 x 10-plants double rows was relatively small it cannot be excluded that *A. lycopersici* individuals were transported by workers from plant 1 to, for instance, plants 16, 17 and 18 that were classified as "not in working direction".

From visual inspection of the dispersal trial results, it is clear that in-row neighbouring plants tended to develop strong and lasting symptoms earlier than inter-row neighbouring plants. One likely reason for this is that between in-row neighbouring plants not only leaves but also stems touch as soon as they are aligned horizontally at the bottom (Fig. 1, right) whereas with the plants in the other single row of a double row only leaf contact exists. Additionally, the vertical leafy parts in the two single rows of a double row move in opposite directions, resulting in a much shorter time of plant-to-plant contact as compared to permanent in-row neighbour plants. It can be assumed that this shorter period of contact means less mites were able to move to the inter-row neighbouring plants. Another reason for this effect in this particular trial could be the larger inter-row planting distance (1 m) compared to the in-row planting distance (0.5 m), although especially the in-row distance between the vertically leafy part of plants can vary throughout the season. As there are no stable parameters due to the plant movement in layer cultivation, it was not possible to test this observation in a statistical model.

In almost all 148 plants, first symptoms were observed in the lower and/or middle third section of the plant. This finding corroborates reports in the literature (Bailey and Keifer 1943; Capinera 2001) that first symptoms usually occur in the lower half of cultivated tomato plants; here proven for layer cultivation. Three hypotheses can explain this phenomenon. I: the leaves in the lower and middle part of tomato plants in layer cultivation are usually completely unfolded – in contrast to the leaves in the upper part of the plant, the leaves at the lower and middle section of the plant have reached their maximum size. This means there is more plant and leaf contact to neighbouring plants in the lower and middle part of the plant. This increased contact makes it easier for *A. lycopersici* populations to transfer onto the lower and middle thirds of neighbouring plants. II. The time initial infestations require to produce symptoms to tomato plants is slower than plant growth. As it was shown in the dispersal trial, the average time until first symptom development in warm months on hybrid tomato plants after inoculation with small numbers of *A. lycopersici* individuals (<30) was at least 38 days. On average plants grew 25 cm every seven days (Fig. 8). Thirty-eight days equates therefore, to approximately 135 cm of growth. In consequence even when a small number of *A. lycopersici* individuals reach the highest parts of a plant, the symptoms appear 135 cm below the tip. III. *A. lycopersici* makes use of the fact that the lower parts of the plant stems come into contact as soon as the plants are layered, to infest new plants. In this situation *A. lycopersici* colonize new plants mainly from the lowest defoliated regions, damaging lower leaves first.

For a limited period of time, it seems there was an equilibrium between the pace at which new symptoms occurred, and the growth of the plant, or even a temporary period of time where the plant was able to "grow free of symptoms". *A. lycopersici* individuals that reached the leaves in the lower half of the tomato plants, and populations that built up on these leaves were removed in the frequent defoliation and harvest process in the lower part of plants. This removal happens within a timeframe of four weeks, considering a time window based on the leaf wall height (180 cm divided by two for the lower half) and the growth rate at the trial station (25 cm/7 days). What is happening in layer cultivation is, metaphorically speaking, a race for height between the growing plant and the *A. lycopersici* population that is damaging the plant. Sooner or later this race is usually won by *A. lycopersici*, in its pace depending on different environmental factors, such as drought stress (Pfaff et al. 2020), or temperature and humidity (Haque and Kawai 2003). Due to the exponential population growth of *A. lycopersici*,

at some stage the destruction of photosynthetically active leaf-area ultimately progresses faster than new leaves can grow, slowing down growth even further.

Given the limited number of tools that exist to control *A. lycopersici*, a review of the dispersal modes of *A. lycopersici* and the distinct behaviour patterns this mite shows, reveals possible weaknesses that can be exploited to better control infestations. As reported in the literature (Michalska et al. 2010), and observed in this study (Fig. 5), *A lycopersici* has a strong tendency to move upwards on plants. Possible triggers for this could be reaching a certain population density and / or reaching a plant damage threshold that lowers the quality of the feeding site. When looking at upward within-plant dispersal and movement in layered tomato cultivation, the role of walking as the only active dispersal mode, seems to be of high importance in the combined movement of larger populations. The accumulation of *A. lycopersici* individuals at high points (Fig. 5) indicates that this behaviour probably is due to an instinct, such as non-compass negative gravitaxis as described in Grob et al. (2021), rather than being a cognitive decision to search for less populated surfaces with better feeding conditions. The tendency to move upwards seems to be of such a linear nature that when moving upwards on the stem of tomato plants, *A. lycopersici* tend to avoid leaves that point downwards. In the conducted trials, downward pointing leaves seemed to show symptoms less often or at a later stage compared to leaves that had a more horizontal or upward orientation. Also, once they arrived at high points, the *A. lycopersici* accumulations seemed to remain in these locations. As a consequence, the majority of these "stranded" mites did not manage to disperse aerially, or they failed to attach to carrier vectors (phoresy). This observation underlines the importance of the stem layer cultivation as the only plant organ that allows completely unhindered and dead-end-free upward movement on tomato plants.

Considering the described "race" between plant growth and *A. lycopersici* population growth, and with the assumed importance of the plant stem in mind, it was decided to test the effect of blocking the stem of tomato plants against mites' upward movement. This way the hypothesis of walking being the most important within-plant dispersal mode in layered tomato cultivation was put to the test. Despite there being plant contact via leaves within and between plants, in the glue ring trial the blockage of the stems strongly limited the upward-movement of the *A. lycopersici* populations. Only small numbers of individuals managed to reach the higher sites on the plants, they were of a magnitude that was incapable of creating

considerable plant damage. As the glue rings were applied weekly 15 cm below the tip of the plants, the rings were on average 25 cm away from each other. For every new stage above a certain glue ring the *A. lycopersici* population size was reset to the low number of individuals that reached this particular height. This prevented unlimited population growth, and consequently severe damage to the plants and the fruit in the glue ring treatment compared to the plants in the positive control. Cultivation work in the glue ring trial was done either on the lower part of the plant (defoliation and harvest) or on the higher part (winding of tomato plants and removal of side shoots) to avoid the uncontrolled transport of mites via gloves or tools from lower to higher parts of the plants. To test under more controlled conditions how the scenario of a small number of mites (<30) reaching higher parts of the plants would influence symptom development, all plants were inoculated 10 cm below the tip on the 22nd of July 33 dpi for a second time. The temporary increase in the proportion of symptomatic leaves in the glue ring trial observed at 68 dpi and 75 dpi before it decreased again at 82 dpi is most likely the result of this secondary inoculation. The effectiveness of glue rings around tomato stems under greenhouse conditions (where there was air movement due to window ventilation and with *M. pygmaeus* presence as a potential travel vector) against *A. lycopersici* can be considered additional evidence for the relatively higher importance of walking for the pest's dispersal and population build up as compared to passive dispersal modes.

Despite significant differences in symptom development, there was no collapse or death of complete plants in the positive control of the glue ring trial. On the contrary, this did occur in the dispersal trial. Even though there was severe damage in the positive control of the glue ring trial, the absence of plants completely dying off might indicate that pest pressure could have been higher. Towards the end of the sampling period the outside temperatures were lower and the humidity higher than the usual in this time of the year (data not shown), which might have influenced the development of the *A. lycopersici* populations negatively. A repetition of the glue ring trial under dry conditions should be performed. Comparable trials should be conducted in greenhouses with different environmental and climatic conditions, and with different tomato cultivars and *A. lycopersici* populations to allow for an estimation of efficacy under a broader range of practical conditions.

Despite the fact that the glue ring treatment successfully prevented *A. lycopersici* damage, there are some downsides to this method. The insect glue that was applied to the stems was chosen

for its durable stickiness – a high level of adhesiveness remains even when the glue is exposed to changes in temperature. This stickiness becomes a problem however, when the glue ends up on fruit. In addition to this, the applied insect glue is not selective for *A. lycopersici* but would also trap beneficial arthropods such as predatory mites that are applied against *A. lycopersici*. That said, it is known that *A. lycopersici* benefits from trichomes as these hinder the movement of predatory arthropods, but not *A. lycopersici* itself (van Houten et al. 2013). For this reason, the use of predatory mites in tomato production has been limited to date. Fortunately, in this particular trial, the introduced *M. pygmaeus* seemed to be unaffected by the glue rings. In any case, non-sticky alternatives that are semi-permeable for beneficial arthropods should be investigated. Potential alternatives could be substances containing acaricidal compounds, for example Sulphur. Sulphur for instance, is somewhat effective against *A. lycopersici* (Royalty and Perring 1987) and is sprayed in practice against *A. lycopersici.* Therefore, a ring at the stem containing or consisting of concentrated sulphur might be feasible although negative effects on predatory mites might occur with this compound as well.

2.6 Conclusion

Small numbers of *A. lycopersici* cause first damage at the initial infestation site on plants and apparently begin to move upwards when a certain amount of plant damage or population size is reached. This typical behavioural pattern can be used as a starting point for a control strategy, particularly in tomato layer cultivation. A good example of how this strategy can be realised under practical growing conditions is the use of physical barriers, as demonstrated with glue rings around tomato stems. The results suggest a closer look at *A. lycopersici* dispersal interference and the stem as a major route for within-plant movement of *A. lycopersici*. If this method provides equally good results across a wide range of environmental conditions and with a workable substance, this method will be a useful addition to integrated plant protection in tomato crops against this challenging pest.

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Survey on *Aculops lycopersici* and operational factors potentially affecting successful pest management among 50 tomato producers in Germany

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Funding organisation:

This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant number 2816ERA01L.

This version of the article has been accepted for publication after peer review in: ***Journal of Plant Diseases and Protection*** and reflects the state of the accepted manuscript but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The publication is available online at: https://doi.org/10.1007/s41348-023-00840-7

Survey on *Aculops lycopersici* and operational factors potentially affecting successful pest management among 50 tomato producers in Germany

3.1 Abstract

Aculops lycopersici (Acari: Eriophyoidea) is a pest in tomato cultivation worldwide. In recent years, the number of reports of *A. lycopersici* infestations in tomato have increased in Germany. In the first half of 2019, a survey of 50 tomato producing farms was conducted to assess the occurrence of *A. lycopersici* and the impact this pest has on tomato cultivation in Germany. The participating farms represented ~ 3.5 % of the 1448 farms in Germany with protected tomato production in 2019. Total tomato production area considered in the survey was 131.8 ha which corresponds to ~ 34 % of the 385.63 ha of protected tomato production area in Germany in this year. *A. lycopersici* presence was reported by 33 of the 50 surveyed farms, within the last 5 years. Amongst these 50 participants it was the pest with the highest relative importance in terms of plant protection effort exerted. *A. lycopersici* occurrence was reported more frequently from production systems with a higher intensification. For instance, heating in cold months and a larger production area were considered intensification factors in this study. However, due to autocorrelation between intensification factors it was not possible to link increased occurrence to specific factors. As the intensification factors favouring *A. lycopersici* occurence are more prevalent in integrated production, those farms faced *A. lycopersici* occurrence more often than the organic growers in this study. Plant protection strategies often combine broad treatments of sulphur with local abamectin treatments, removal of infested plant material and the introduction of natural enemies.

Keywords

Aculops lycopersici; distribution; Germany; plant protection; farmers perception; survey

3.2 Introduction

Pest regimes in modern agriculture and horticulture are in a process of constant change (Kolar and Lodge 2001). The occurrence of new pests is facilitated by changes in climate (Hellmann et al. 2008), or by changes in production and cultivation methods, which especially allow airborne pests to spread swiftly and establish in new regions when favourable conditions are met. Another driver in this process is local and international trade and travel, which spreads less mobile and non-airborne pests into new regions (Kolar and Lodge 2001). Greenhouse production of tomato in Germany and its pest regime is not exempt from this. Currently the greenhouse cultivation area of tomato in Germany measures 385 ha (Destatis 2020). Among the pests that occur in tomato production in Germany, the tomato russet mite *Aculops lycopersici* (Tryon) is currently spreading and establishing on tomato production sites across the country.

The tomato russet mite *Aculops lycopersici* (Acari: Eriophyoidea), an eriophyoid mite, is considered a pest of several Solanaceae crops (Perring and Farrar 1986). *A. lycopersici* is currently found throughout the world in both tropical and temperate regions. Before 1999 *A. lycopersici* was rarely reported as a pest on tomato in Germany (Merz 2020). In recent years the economic impact of eriophyoid mites such as *Aculus scchlechtendali*, *Calepitrimerus vitis* and *A. lycopersici* has increased worldwide (Duso et al. 2010), as well as in Germany in the case of *A. lycopersici* (Merz 2020). *A. lycopersici* causes bronzing and a russeted appearance of leaves and stem as it feeds on surface cells, leading to the death of leaves and even complete plants since they no longer are able to photosynthesise (Royalty and Perring 1988). In contrast to several other eriophyoid mites, *A. lycopersici* has a superficial lifestyle and does not induce or inhabit galls on plant tissue (Van Leeuwen et al. 2010).

Several factors make *A. lycopersici* a problematic pest. It has a high reproduction rate, and at less than 0.2 mm in length, is very small in size (Haque and Kawai 2003). There are no plant protection products authorised for use specifically against *A. lycopersici* in tomato cultivation in Germany (BVL 2022). Authorised products against mites in general have been shown to be limited in efficacy, or are expected to be limited in efficacy due to them being contact acaricides (Vervaet et al. 2021). Although a number of studies have shown that certain predatory mites, for example *Amblyoseius fallacis* and *A. swirskii* (Brodeur, Bouchard, and Turcotte 1997; Park,

Shipp, and Buitenhuis 2010; Park et al. 2011) feed on *A. lycopersici*, the practical implementation of biological control is limited (van Houten et al. 2013). More recent studies show very promising results for different predatory mites under semi-practical conditions but have yet to be confirmed with trials in practical cultivation (Pijnakker et al. 2022a, b; Vervaet et al. 2022; Castañé et al. 2022). In practice, recognition of *A. lycopersici* infestation coincides with symptom recognition on plants. Early symptoms such as light chlorosis on leaves, or light grey and brown shades on the stem are easily overlooked. Later, more obvious symptoms may be misdiagnosed. For instance stem and leaf browning might be mistakenly attributed to the fungus *Phytophthora infestans* (Crüger et al. 2002) in practice and by people unexperienced with the pest *A. lycopersici*. As yet there are no efficient *A. lycopersici* monitoring methods available for practical tomato cultivation (Pfaff et al. 2020).

Whenever new or invasive pests occur and negatively impact the production of food, in this case tomato production in greenhouses, information on the frequency of occurrence and case specific data from farms are essential for estimating economic damage potential. When farms with *A. lycopersici* presence are identified, a closer look at cultivation techniques and factors such as substrate, crop rotation, climatic conditions, applied pesticides or introduced beneficial arthropods might reveal intervention strategies that can be exploited to reduce the impact of *A. lycopersici*. The experience of farmers may help to better understand the pest dynamic of *A. lycopersici*, and could aid in the development of efficient countermeasures. To obtain this information, the presented survey of farms with tomato production was carried out.

3.3 Materials and Methods

Survey details

A survey was developed and integrated into the professional survey platform https://umfrageonline.com and the domain "tomatenschaedlinge.org" was created to redirect participants to the survey on *umfrageonline*. In December 2018, a link to the survey was forwarded to the official plant-protection consultation services of the federal states, to private plant protection consultants and to grower organisations, with the request to disseminate the

survey to tomato growers. In this way, an undocumented number of tomato-producing farms were contacted via E-Mail and invited to fill out the questionnaire. Aside from being offered the possibility to receive information on the results of the survey, no form of payment was offered to growers for participating in the survey. Due to a rather small number of participants by the end of February 2019, the decision was made to switch to phone interviews. The contact addresses of growers were obtained using google search engine. The following search terms were used: "Tomatenproduktion in [federal state]" or "Tomatenanbau in [federal state]" for each of the 16 federal states of Germany to achieve a spatial distribution of participants across Germany.

Criteria for integrating farms in the analysis:

- Production of tomato located in Germany
- At least 50 m² tomato cultivation area
- Production for commercial purposes
- The questionnaire was completed (all mandatory questions answered)

The questionnaire consisted of five parts:

- General information on participant and farm
- Crops, cultivars, cultivation methods
- Confirmed pests and diseases of tomato on the surveyed farm
- Impact of and measures against *A. lycopersici*
- Agreement on use of data for scientific purposes only

Determination of the sample size

Prior to the study, it was considered that with the workforce conducting the study, it would be realistic to reach up to 100 valid participants. This number also took into account the fact that many factors could potentially limit the number of growers who might take part, for instance the fact that it may be difficult or time-consuming to identify and locate potential farms that could participate, that without monetary incentives there may be a limited motivation for the invited growers to participate, and finally it was recognised that it may be difficult to determine the representativeness of the recruited participants beyond a rough estimation. In light of this, additional questions were included in the questionnaire with the ability produce insight into farmers perception and strategies for the pest of interest that go beyond a quantitative analysis approach.

Statistical analysis

Statistical analysis was conducted where feasible; Fisher exact tests were used for categorical data, a non-parametric Wilcoxon rank sum test to test for dependence between tomato production area and *A. lycopersici* presence, and a linear model was used to check whether there was a correlation between production area and length of break between tomato seasons. An exact binomial test was used to test whether an initial infestation increased the likelihood that there would be continuous *A. lycopersici* presence in subsequent consecutive years (Clopper and Pearson 1934). All functions used in these analyses are part of the statistical software R (R Core Team 2021; version 4.1.0). For correction of multiple testing in the Fisher exact tests the "fdr"-method (Benjamini and Hochberg 1995) was applied.

3.4 Results

Acquisition of participants and metadata on participating farms

A total of 83 tomato producing farms had responded to the invitation to participate by the 5th of August 2019. Of the 29 online respondents, 17 were excluded for not having completed the survey. Of the 54 farms contacted via phone, 38 farms agreed to participate and all of them completed the survey. Twelve valid online respondents plus 38 valid phone respondents resulted in a total of 50 valid participants becoming subject to the following analyses, half of what initially was aimed for. For the growers contacted via E-Mail, the calculation of a response rate is not possible as the total number of recipients contacted is not known. For the growers contacted via phone the response rate was 70.4 %. Germany is divided into 10 regions by the first digit of the postal code and into 99 regions by the first two digits of the postal code. The distribution of participating farms separated by first digit is shown in Tab. 1. In Fig. 1 separation is based on the combination of first and second digit of the postal code. On the first digit-level it was possible to cover all 10 areas with participants.

Tab. 1: postal code areas with quantity of participants

Postal code (first digit)	1	2	3	4	5	6	7	8	9	0	∑
participants	2	4	8	7	5	4	4	7	5	4	**50**

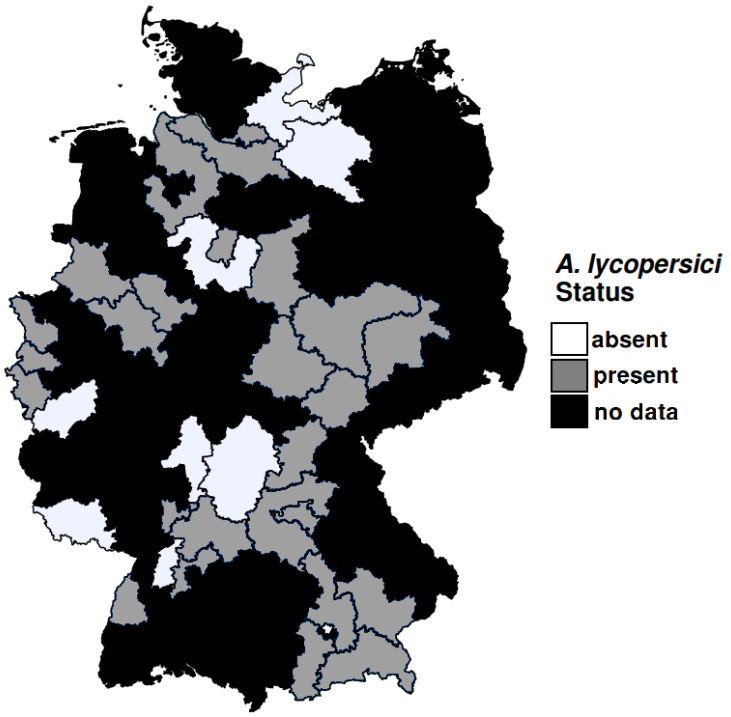

Fig. 1: Map of Germany, divided into 99 regions by the first two digits of the postal code. If *A. lycopersici* was found at least in one of the participating farms in a region, the region is marked grey. If it was found in none of the participating farms in one region, it is marked white. Black regions indicate that there were no participants from this region (note this does not mean, that there are no tomato-producing farms in this particular region). White only means, that the respective pest was not found in the participating farms in the particular region, not that it does not occur in any of this region's farms.

The 50 farms that participated in the survey represent 3.5 % of the total number of 1448 farms with tomato production in Germany in 2019 (Destatis 2020). Together the participating farms account for 131.8 ha tomato production area. The total area used for tomato production in Germany in 2019 was 385.63 ha, thus the surveyed farms account for 34.2 % of this total area (Destatis 2020). The mean tomato production area of the 50 participating farms was 2.64 ha. The median production area was 0.2250 ha.

Of the 50 participating farms, 27 (54 %) were producing integrated (i.e., committed to the guidelines of integrated pest management) with an average cultivation area of 47.457 m² and 23 farms (46 %) were producing organic (at least following Regulation (EU) 848/2018), with an average cultivation area of 1593 m². The sizes of the participating farms are visualised in Fig. 2.

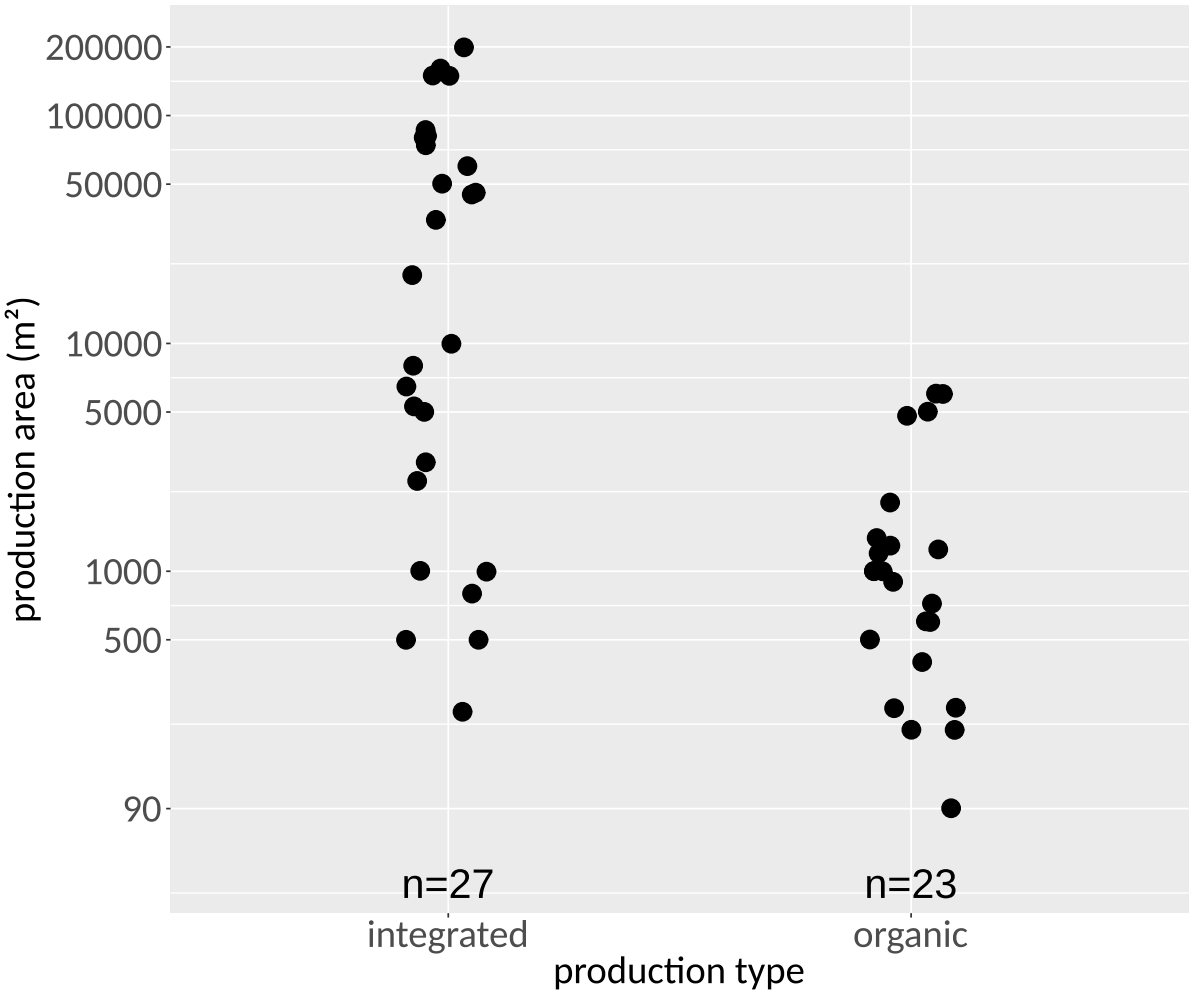

Fig. 2: Sizes of the participating farms displayed in m² on the y-axis divided by production type displayed on the x-axis. To improve visualisation, the y-axis has been log-transformed.

Of the 50 participants, 29 participants (58 %) stated that they had attended an apprenticeship in horticulture or agriculture. Twenty participants (40 %) stated that they had completed a university degree in horticulture or agriculture. Four participants (8 %) stated that they had both an apprenticeship and a university degree and five participants (10 %) stated that they had not finished a degree in horticulture or agriculture.

Of the 50 participants 48 (96 %), stated the sources that they use to obtain information about plant protection. Experience exchange with other growers, and specialist literature were each stated 43 times (89.6 %), followed by websites on plant protection with 41 mentions (85.4 %), and plant protection courses which received 35 mentions (72.9 %). Eight participants (16.7 %) stated that they rely on decision-support-systems (DSS) for plant protection. Thirty-one participants (64.6 %) indicated that they were interested in the utilisation of DSS, and 17 (35.4 %) stated that they were not interested in using DSS.

Employees on participating farms

It was assumed, that the employee background, familiarity and experience with and in tomato farming might have an influence on detection and / or treatment success against *A. lycopersici*. Twenty-eight participants (46 %) stated that the majority of employees doing cultivation work are permanently employed. Of these 28 participants, 21 participants (75 % of 28) stated that the employees are regularly offered training sessions on the topic of plant protection. Twenty-one participants (42 %) stated that the majority of employees doing cultivation work are seasonal employees that had already worked on the farm previously. Of these 21 participants, 14 participants (66.7 % of 21) indicated that regular training sessions on the topic of plant protection are offered to the employees. Only one participant (2 %) stated that most of the employees doing cultivation work were seasonal employees that had not worked on the farm previously, and that no training sessions on the topic of plant protection were offered to employees. Fisher exact tests were used to test whether there was a correlation between *A. lycopersici* incidence and employee demographics. No significant correlation was found between the two.

Relative importance of pests and diseases on participating farms

Participants were asked to name the most important pests (Tab. 2) and diseases (Tab. 3), in both cases results are displayed separately for farms with *A. lycopersici* presence and farms without *A. lycopersici* presence. Looking at most important pests *A. lycopersici* reached the highest relative importance in farms with *A. lycopersici* presence as well as in the overall ranking (farms with and without *A. lycopersici* presence combined). *Tuta absoluta* was considered important on 11 of 32 farms (34.4 %) with *A. lycopersici* presence, making *T. absoluta* the third most important pest in this particular group of farms. On the contrary, none of the

nine farms without *A. lycopersici* considered *T. absoluta* important. For the group of farms without *A. lycopersici* presence, seven from nine farms (77.8 %) consider whiteflies important, making it the most important pest for this group of farms with a relative importance of 50 %.

With regard to diseases, *Botrytis cinerea* was the most important disease among farms with *A. lycopersici* presence mentioned by 13 of these farms (40.6 %), whereas for farms without *A. lycopersici* presence *Phytophthora infestans* was the most important disease (Tab. 3). *P. infestans* was the only disease according to a Fisher exact test (followed by a correction for multiple testing) that showed a significant difference in importance between the two groups of farms, farms with, and farms without *A. lycopersici* presence.

Tab. 2: Farmers were asked to name the three most important pests in their tomato cultivation and rank them in decreasing order. This table shows the relative importance of the different pests. Calculation of the relative importance was as follows: naming a pest first resulted in three points for the respective pest, second in two and third in one. The sum of points for each pest was divided by the total sum of points within the respective group of farms (1: *A. lycopersici* present, 2: *A. lycopersici* absent, 3: total) and multiplied by 100 (results shown are rounded). The number of times the different pests were named appears in brackets next to the relative importance value.

Pest	*A. lycopersici* present, relative importance, n = 32	*A. lycopersici* absent, relative importance, n = 9	Adjusted *p*	Total %, n = 41
Aculops lycopersici	40.5 (27)	0.0 (0)	-	32.8 (27)
whiteflies	24.8 (17)	50.0 (7)	1 (0.2623)	29.6 (24)
Tuta absoluta	17.0 (11)	0,0 (0)	0.8275 (0.08275)	13.8 (11)
aphids	6.5 (6)	25.0 (3)	1 (0.3436)	10.0 (9)
spider mites	6.5 (5)	16.7 (3)	1 (0.6625)	8.5 (8)
Golden twin-spot moth	1.3 (2)	0.0 (0)	1 (1)	1.1 (2)
leaf miner fly	1.3 (1)	5.6 (1)	1 (0.3951)	2.1 (2)
fruit fly	0.0 (0)	2.8 (1)	1 (0.2195)	0.5 (1)
nematodes	0.6 (1)	0.0 (0)	1 (1)	0.5 (1)
thrips	0.6 (1)	0.0 (0)	1 (1)	0.5 (1)
caterpillar	0.6 (1)	0.0 (0)	1 (1)	0.5 (1)

Tab. 3: Farms were asked to name the three most important diseases in their tomato cultivation and rank them in decreasing order. This table shows the relative importance of the different diseases. For calculation of the relative importance values refer to the caption of Tab. 2. The number of times the different diseases were named appears in brackets next to the relative importance value.

Disease	*A. lycopersici* present relative importance n = 24	*A. lycopersici* absent relative importance n = 16	adjusted *p*	total % n = 40
Gray mold (*Botrytis cinerea*)	30.6 (13)	15.8 (8)	1 (1)	24.5 (21)
Late blight (*Phytophthora infestans*)	8.3 (5)	43.4 (13)	**0.0030** (**0.0003**)	22.8 (18)
Tomato leaf mold (*Cladosporium fulvum*)	18.5 (10)	27.6 (7)	1 (1)	22.3 (17)
Powdery mildew	27.8 (12)	9.2 (3)	0.6118 (0.0556)	20.1 (15)
Fusarium wilt (*Fusarium oxysporum*)	2.8 (2)	0.0 (0)	1 (0.5077)	1.6 (2)
pepino mosaic virus	5.6 (2)	0.0 (0)	1 (0.5077)	3.3 (2)
Early blight (*Alternaria solani*)	0.0 (0)	2.6 (1)	1 (0.4)	1.1 (1)
tomato mosaic virus	0.0 (0)	1.3 (1)	1 (0.4)	0.5 (1)
Bacterial canker (*Clavibacter michiganensis*)	2.8 (1)	0.0 (0)	1 (1)	1.6 (1)
Crazy roots (*Agrobacterium rhizogenes*)	1.9 (1)	0.0 (0)	1 (1)	1.1 (1)
Verticillium wilt (*Verticillium sp.*)	1.9 (1)	0.0 (0)	1 (1)	1.1 (1)

Initial occurrence and persistence of *A. lycopersici* on participating farms

Thirty-three farms reported that *A. lycopersici* was present at some time on their farm in the five years preceeding 2019. Thirty-two of those farms were able to report the year that *A. lycopersici* was first noted. Of those 32 farms, 26 farms reported that the first occurrence was between 2014 and 2018, with a peak of nine reports of first occurrences in the year 2018 (Fig. 3).

On 24 of the 33 farms (72.7 %) *A. lycopersici* was present in every year following the year of the first occurrence i.e., only nine farms (27.3 %) reported *A. lycopersici*-free seasons after the year of first occurrence. An exact binomial test revealed that an initial infestation increased the chance for continuous *A. lycopersici* presence in all consecutive years ($p = 0.01$). The production area of these nine farms ranged from 500 to 81000 m². The mean production area was 16855 m² and the median production area was 1200 m². Seven of these nine farms (77.8 %) were heated during the colder months, and the cultivation break between tomato sets on these farms ranged between one and 29 months.

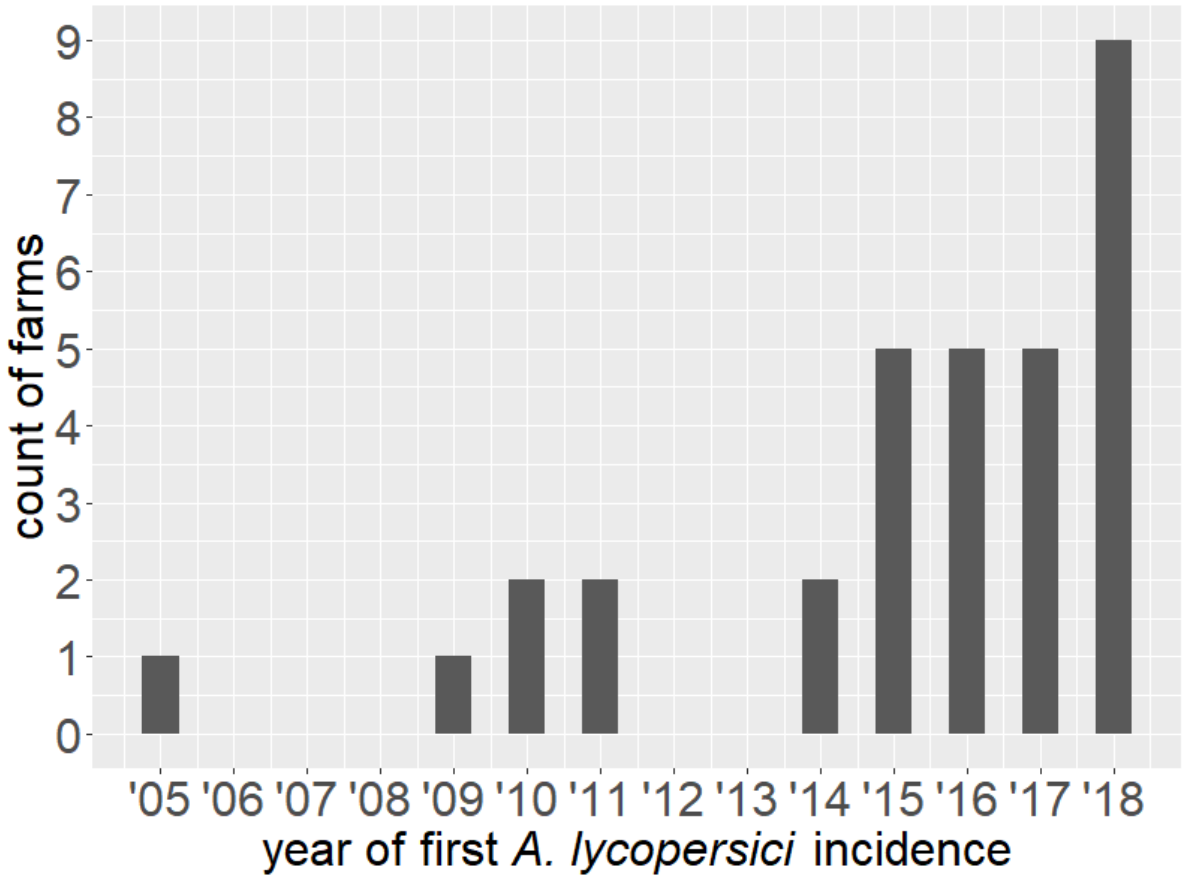

Fig. 3: First year of *A. lycopersici* occurrence on the farms that reported *A. lycopersici* presence. The y-axis shows the count of the farms and the x-axis shows the year.

Yield impact of *A. lycopersici*

Of the 33 farms affected by *A. lycopersici*, 12 farms (36.4 %) reported a negative impact on yield despite plant protection measures. Nine of those farms (75 %) reported a specific yield loss. The yield loss reported by these farms ranged between 0.5 % and 15 %, and on average amounted to 5.89 %. Twenty-one farms (63.6 %) reported no negative impact on yield considering plant protection measures taken.

Farm and cultivation parameters and possible links to *A. lycopersici* presence

In Fig. 4, a large tomato cultivation area and a short cultivation break seem to co-occur. A non-parametric Wilcoxon rank sum test revealed that there was a dependency between *A. lycopersici* presence and cultivation area ($p < 0.00$). A linear model showed that cultivation break was a significant predictor for cultivation area ($p < 0.00$) with a coefficient of -0.1068 at

an adjusted R^2 of 0.2799. *A. lycopersici* did not exclusively occur on farms with short cultivation breaks, but all 20 of the farms (40 %), with a cultivation break between tomato sets of less than 3 months, with one exception, reported *A. lycopersici* presence. Similarly, all 23 farms (46 %) with a cultivation area of 4800 m² or larger, with one exception, reported *A. lycopersici* presence. The latter is also reflected by the total combined cultivation area of the 33 farms with *A. lycopersici* presence of 129.09 ha which account for 33.3 % of the German tomato production area. The combined cultivation area of the 17 participating farms without *A. lycopersici* presence sums up to 2.71 ha, representing approximately 0.7 % of the German tomato cultivation area. The mean cultivation area of the participating farms with *A. lycopersici* presence was 3.911 ha and of those without was 0.159 ha.

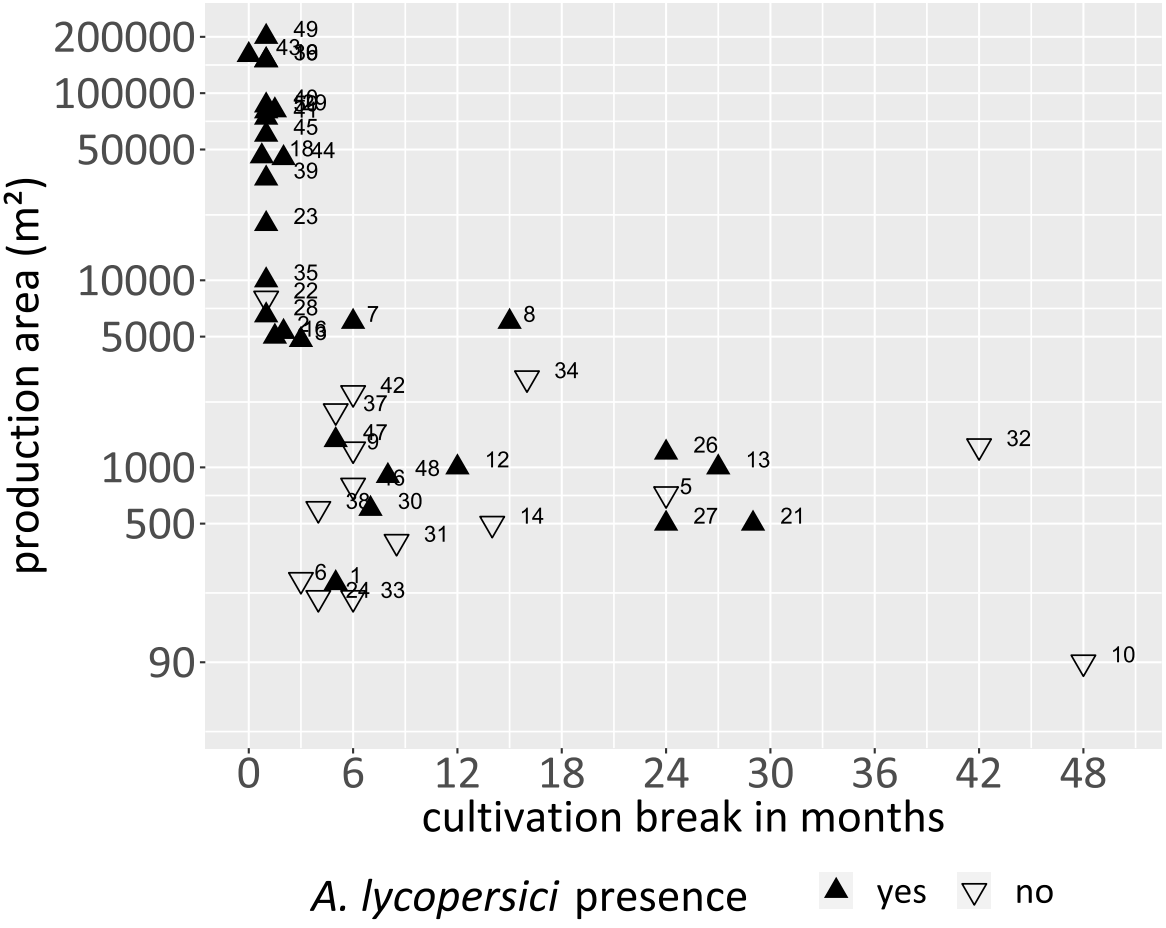

Fig. 4: *A. lycopersici* presence on the different farms. Each symbol represents one farm. The tomato production area is displayed on the y-axis and the cultivation break between tomato sets on the x-axis. To aid visualisation of small values, the y-axis has been log-transformed. The numbers next to the symbols indicate the IDs of the specific farms. Forty-five of 50 farms

are displayed; five farms with production areas between 250 and 5000 m² did not state the length of their cultivation break.

Tab. 4 shows that the farm production type – whether the farm was integrated or organic - was a statistically significant predictor for *A. lycopersici* presence according to a Fisher exact test for independence ($p < 0.00$).

Tab. 4: *A. lycopersici* presence/absence on integrated and organic farms. A Fisher exact test for independence of *A. lycopersici* presence and production type revealed production type was a significant predictor of *A. lycopersici* presence ($p < 0.00$).

		Integrated	Organic	Total count of farms
A. lycopersici presence	yes	23	10	33
	no	4	13	17
Total count of farms		27	23	**50**

Tab. 5: Percentage of substrate types shown separately for farms with (n = 33) and farms without (n = 17) *A. lycopersici* presence in addition to the total frequency of *A. lycopersici* presence (n = 50).

Substrate	*A. lycopersici* present (n = 33)	*A. lycopersici* absent (n = 17)	Total frequency
natural soil	42.4 % (14)	82.4 % (14)	56 % (28)
rock wool	27.2 % (9)	0.00 (0)	18 % (9)
coir substrate	15.1 % (5)	5.9 % (1)	12 % (6)
perlite	12.1 % (4)	0.00 (0)	8 % (4)
turf mixture	0.00 (0)	5.9 % (1)	2 % (1)
turf + woodfibre	3.0 % (1)	0.00 (0)	2 % (1)
natural soil + compost + coir substrate	0.00 (0)	5.9 % (1)	2 % (1)

Fifteen of the 29 farms (51.7 %) growing tomato in natural soils, and 19 of the 21 farms (90.5 %) not growing in natural soils reported presence of *A. lycopersici* in their production systems (Tab. 5). The mixture of natural soil, compost and coir substrate was considered a natural soil

in this analysis. According to a Fisher exact test, *A. lycopersici* presence depended on whether or not the tomatoes were grown in natural soil ($p < 0.00$).

Plant residues are removed at different time points and intervals in the participating farms (Tab 6). A Fisher exact test did not reveal any significant difference regarding *A. lycopersici* occurrence both before and after correction for multiple testing.

Tab. 6: Frequency of removal of plant residues for farms with (n = 32) and without (n = 17) *A. lycopersici* presence and the combined total (n = 49).

Removal	*A. lycopersici* present (n = 32)	*A. lycopersici* absent (n = 17)	Total proportion (n = 49)
immediately	46.9 % (15)	47.1 % (8)	46.9 % (23)
weekly	18.8 % (6)	23.5 % (4)	20.4 % (10)
End of season	25.0 % (8)	11.8 % (2)	20.4 % (10)
remain	3.1 % (1)	5.9 % (1)	4.1 % (2)
two times per season	6.3 % (2)	0.0 % (0)	4.1 % (2)
monthly	0.0 % (0)	5.9 % (1)	2.0 % (1)
varying	0.0 % (0)	5.9 % (1)	2.0 % (1)

There was no significant effect on the presence of *A. lycopersici* depending on whether farms cultivate in a crop rotation or not (Tab. 7).

Tab. 7: Presence and absence of *A. lycopersici* on farms that cultivate tomato in a crop rotation and farms that do not cultivate in a crop rotation. Fisher exact test for independence of *A. lycopersici* presence and crop rotation: $p = 0.13$.

		Crop rotation		
		yes	no	Total count of farms
A. lycopersici presence	present	13	20	33
	absent	11	6	17
Total count of farms		24	26	**50**

Of the 24 farms that grow tomato in a crop rotation with other crops, 21 farms provided information on the rotation crops (Tab. 8). None of the mentioned rotation crops acted as significant predictors for the presence of *A. lycopersici.*

Tab. 8: Frequency of crops grown in a rotation with tomato, shown separately farms where *A. lycopersici* was present (12 of 13 farms reported their rotation crops) and farms where *A. lycopersici* was not present (9 of 11 farms reported their rotation crops). Shown is the frequency of the mentioned rotation crops for 17 of 19 organic farms with crop rotation, and four of the five integrated farms with crop rotation (second number in brackets).

Rotation crops	Frequency of crops, *A. lycopersici* present (12 farms)	Frequency of crops *A. lycopersici* absent (9 farms)	total frequency
lettuce	6 (3)	5 (0)	14
cucumber	6 (2)	5 (0)	13
lamb's lettuce	4 (2)	3 (0)	9
sweet pepper	3 (1)		4
runner beans	2 (0)	2 (0)	4
red radish		3 (0)	3
kohlrabi	0 (1)	2 (0)	3
spinach	1 (1)	1 (0)	3
eggplant	2 (0)		2
celery		1 (0)	1
potted herbs	0 (1)		1
radish	0 (1)		1
winter greening	1 (0)		1
courgette		1 (0)	1

Participants were asked whether they had reared fresh plants in the last five years on the farm, or whether they purchased them externally. Of the 33 farms with *A. lycopersici* presence, 23 farms (69.7 %) received plants from external nurseries, six (18.2 %) had reared fresh plants on the farm, and four farms (12.1 %) had both on-farm reared and purchased plants in the last five years. Of the 17 farms without *A. lycopersici* presence seven farms (41.2 %) had received plants from external nurseries, 6 (35.3 %) had reared fresh plants on-farm, and 4 farms (23.5 %) had both on-farm reared and purchased plants in the last five years. A Fisher exact test revealed that there was no difference between farms in terms of the presence/absence of *A.*

lycopersici, depending on whether the farm had received plants from a nursery or had reared fresh plants on-farm in the last five years ($p = 0.14$).

Heating during the colder months was a statistically significant predictor of *A. lycopersici* presence according to a Fisher exact test for independence ($p < 0.00$; Tab. 9).

Tab. 9: *A. lycopersici* presence/absence on farms that do and farms that do not heat during the colder months. Fisher exact test for independence of *A. lycopersici* presence and heating: $p < 0.00$.

		Heating		Total count of farms
		yes	no	
A. lycopersici presence	yes	27	5	32
	no	8	9	17
Total count of farms		35	14	**49**

Most of the farms that did not heat during the colder months reported first symptoms in August and September, around one month later compared to the farms that heat. Farms that heat reported the first symptoms of the season in July and August (Fig. 5).

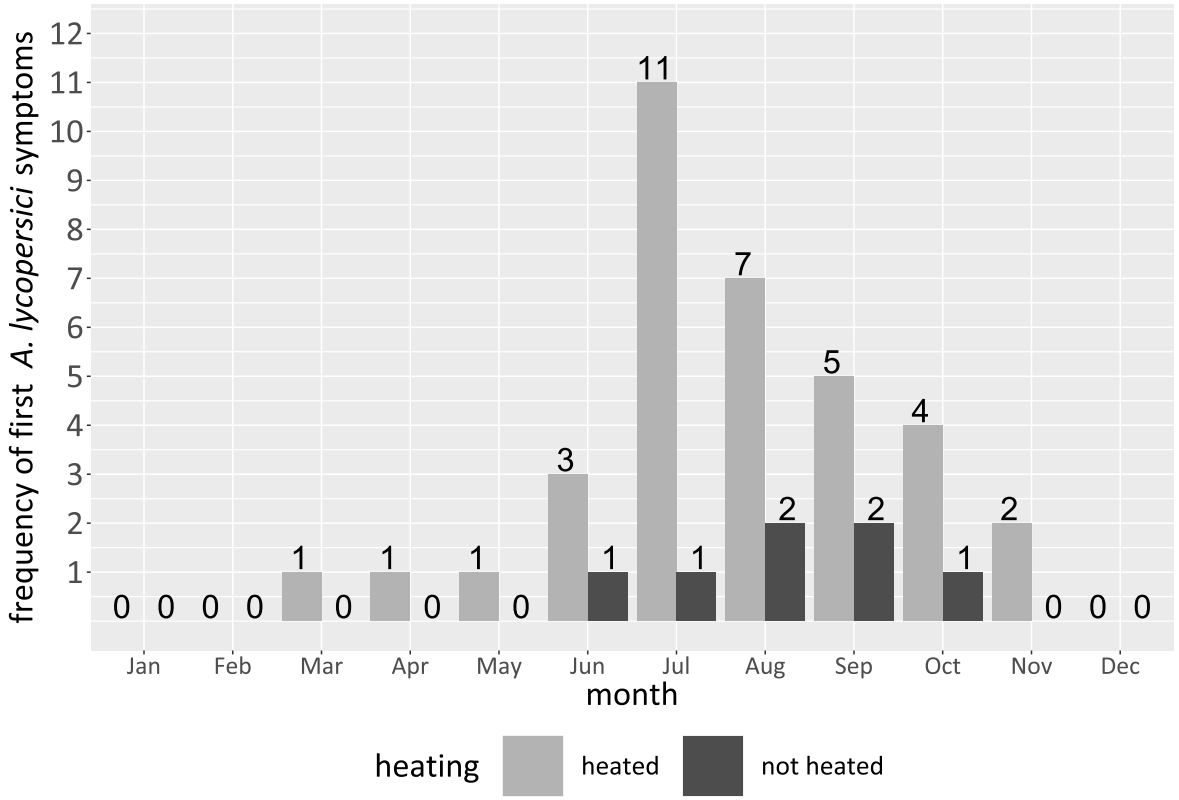

Fig. 5: Month when first *A. lycopersici* symptoms of the year were usually observed on farms with *A. lycopersici* presence (participants were able to name multiple months). The information is provided for both farms where the greenhouse was heated during the colder months and farms where the greenhouse was not heated.

Utilised Countermeasures against *A. lycopersici* infestation

Farms with *A. lycopersici* presence were asked to select known countermeasures against *A. lycopersici* from a list, indicating those they implement in their own control strategy against the pest. Each countermeasure was used at 14 (42.4 %) to 18 (54.5 %) of the 33 farms (Tab. 10). No significant differences were found between farms that reported that *A. lycopersici* caused significant yield loss, and those that did not report yield loss.

Tab. 10: The countermeasures that farms with *A. lycopersici* presence have taken to combat the pest. Farm IDs that are in bold show that the farm reported a negative impact on yield caused by *A. lycopersici*. Fisher exact test with 'false discovery rate' (fdr) (Benjamini and Hochberg 1995) correction for repeated sampling found no significant difference in the frequency of the different countermeasures used between farms that reported a negative impact on yield and those that did not. P-values not enclosed in brackets are 'fdr' corrected; p-values in brackets are the values prior to correction.

Farm ID	Acaricides	Sulphur spray	Sulphur vaporizer	Removal of symptomatic leaves	Removal of whole plants	Predatory mites	No measures
1		x				x	
2			x				
4		x		x	x	x	
15	x						
21	x			x	x		
26		x		x	x		
27					x	x	
35	x		x	x			
40	x	x	x	x	x	x	
41	x	x	x	x	x	x	
45	x	x	x	x	x	x	
48	x			x	x	x	
3						x	
7						x	
8		x	x	x			
12						x	
13							x
16	x		x	x	x	x	
17		x	x			x	
18	x	x	x				
19	x	x	x	x	x		
20	x						
23	x		x		x	x	
28							x
29		x	x		x		
30				x			
36	x			x	x	x	
39	x	x	x	x	x		
43	x	x	x	x	x		
44		x	x				
47				x	x		
49		x	x	x	x		
50		x	x		x		
SUM	15	16	17	17	18	14	2
adjusted p	1 (0.3005)	1 (1)	1 (0.4813)	1 (0.2818)	1 (0.4688)	1 (0.2728)	-

Participants had the opportunity to describe specific countermeasures or strategies they have used against *A. lycopersici* in more detail. Ten participating farms (20 %) supplied a free-text answer. Answers usually consisted of specific combinations of countermeasures that had been explicitly asked about. One participant mentioned an additional (not previously asked about) measure - "herbal mixtures". Another farm mentioned, that the first and strongest symptoms usually occur in areas that are most exposed to sunlight. Detailed answers can be viewed in Tab. S2 in the Appendix Chapter 3.

Of the 15 farms that have applied acaricides, nine farms supplied specific detail about which acaricides they applied. Abamectin-based products were used by all nine farms that provided this detailed information. Spirodiclofen, potash soap and Azadirachtin were each applied on one farm.

Of the nine farms that applied predatory mites specifically against *A. lycopersici*, *A. swirskii* was chosen most often; five farms applied this mite. *Phytoseilulus persimilis*, *Amblyoseius cucumeris*, *Amblyoseius barkeri* and *Amblyoseius californicus* were each chosen by one farm.

Besides predatory mites specifically introduced against *A. lycopersici*, a total of 14 different Arthropods, and one nematode species were introduced on participating farms. Data on this is provided in Tab. S1 in the Appendix Chapter 3 for farms with and without *A. lycopersici* presence.

The intensification factors that *A. lycopersici* presence is shown to be dependent on are all more prevalent in integrated rather than organic farms (Tab. 11).

Tab. 11: Factors that *A. lycopersici* occurrence was statistically dependent on, shown separately for integrated and organic farms. In total there were 27 integrated farms (54 %) and 23 organic farms (46 %). Note that if the total for one of the factors is not 27 or 23 respectively, this means that not all participants answered this question.

	Integrated	Organic
Production area ≥ 6000 m²	17 of 27	0 of 23
Heating in cold months	23 of 26	12 of 23
Cultivation in artificial substrate	21 of 27	0 of 23
Cultivation break between tomato seasons ≤ 3 months	18 of 24	0 of 20
A. lycopersici present	23 of 27	10 of 23

3.5 Discussion

Survey metadata

The survey that was carried out focussed on pests and diseases in tomato production. At a later stage during questioning the questionnaire revealed that the main focus of the survey was the pests *A. lycopersici* and *Tuta absoluta*. In this study, pest-specific data was only presented for *A. lycopersici*.

Fifty participants took part in the survey between January and August 2019. With this limited sample size and due to a low response rate for some of the non-mandatory survey questions there was insufficient statistical power to run quantitative analyses - to model dependencies and derive reliable conclusions about several of the potential explanatory factors for *A. lycopersici* incidence and damage levels. This study does, however, provide several interesting findings from a qualitative angle.

The fact that only 3.5 % of the 1448 tomato producers in Germany participated and at the same time 34.2 % of the 385.63 ha German tomato production area is covered by those 3.5 % reveals, that mainly farms with considerably larger tomato production area than the average farm participated in the survey. This imbalance needs to be taken into account when interpreting the results. Thirty-eight of the 50 participants (76 %) included in the analysis were contacted via phone. The phone numbers of these tomato producing farms were obtained via google search engine. This means there is a selection bias for farms that maintain a web page with unknown effect on the results. Those factors in consequence limit the representativeness of the results. Having acknowledged this limitation, the displayed data is useful giving as it does, a unique insight into German commercial tomato production facing *A. lycopersici* infestation which, to date, is not available in a structured and published format.

It was possible to avoid regional clusters of participants in this survey. The 50 participants were spread over all ten postal code areas (first digit of the German postal code), although, display of participants by the two-digit postal code reveals several areas where there were no participating farms. These areas might not have any professional protected tomato cultivation, or it may simply be that no farms in this area were contacted. Despite the fact that there are some areas where no farms participated, the map visualises that *A. lycopersici* occurs

throughout Germany from south to north and west to east – its presence is not restricted to one specific region (Fig 1).

Relevance of *A. lycopersici* as a pest in German tomato cultivation

This study focusses on the farmers perception. For all results displayed it must be considered, that they are based on the assumption that the participants answers are valid. The number of participants that experienced first *A. lycopersici* occurrence on their farms culminates towards the end of the surveyed window from the year 2004 to 2018 (Fig. 3). It is possible that *A. lycopersici* may have gone unnoticed for some time due to there being less experience with this pest in practical cultivation in the past when symptoms appeared late and were of minor nature. However, in the more devastating *A. lycopersici* incidences in which sometimes whole crops have been lost, it is unlikely that farmers would not have identified that *A. lycopersici* was the cause. Twenty-four of the 33 farms (72.7 %) that experienced an *A. lycopersici* occurrence, experienced *A. lycopersici* presence and damage in every year following the year of first occurrence; initial infestation significantly increased the likelihood that *A. lycopersici* would continuously present in consecutive years. This shows, that within-farm eradication attempts are either not conducted at all, are conducted in an inefficient way, or they are simply not possible with the given circumstances in the farms that face continuous *A. lycopersici* presence. These results indicate that outbreaks in subsequent years could be a consequence of an initial infestation rather than a result of new independent migration events. However, a continued infestation through the use of fresh plants from external nurseries or other entrances with plant material or tools and packaging cannot be excluded as a potential yearly infestation source.

A. lycopersici reached the highest relative importance in the group of farms with *A. lycopersici* presence and also in the overall ranking of relative pest importance amongst all participating farms (Tab. 2). *A. lycopersici* was closely followed by whiteflies and by *Tuta absoluta*. "Importance" here refers to the plant protection effort exerted on the specific farm against the specifically named pest. The high plant protection effort demanded by *A. lycopersici* indicates a significant economic relevance for tomato producers and at the same time that there likely is room for improvement of *A. lycopersici* management in practice. In most cases the high plant protection effort seems to have prevented the farms from experiencing significant yield losses

caused by *A. lycopersici*. Only 12 of 33 farms (36.4 %) reported that yields were negatively affected by *A. lycopersici*. Unfortunately give that only nine participants reported a specific yield loss it is not possible to reliably quantify yield loss.

Due to the limited number of participants and/or answers, it was not possible to identify significant differences in the importance of specific pests (Tab. 2) or diseases (Tab. 3) with the exception of *P. infestans,* when the two groups of farms - with and without *A. lycopersici* presence - were compared. There were no mentions of *T. absoluta* as being important on farms without *A. lycopersici* presence in contrast to reported high relative importance on farms with *A. lycopersici* presence. Additionally, *P. infestans* was significantly more important on farms without *A. lycopersici,* compared to farms with *A. lycopersici*. This could mean that *A. lycopersici* favours some conditions similar to those favourable for *T. absoluta* and opposed to those favourable for *P. infestans*.

Intensification factors

Farms with large production areas and short cultivation breaks between tomato sets more often reported *A. lycopersici* presence (Fig. 4). Production area and cultivation break, among other factors included in this survey, can be categorised as intensification factors. As shown for production area and cultivation break, intensification factors tend to correlate or are mutually dependent upon one another. Mutual dependence for instance is the case for heating in cold months and short cultivation breaks as heating is usually only required when cultivation takes place in colder months, and cultivation in colder months usually only takes place when the cultivation break between tomato sets is short. Solely looking at large production area, it is questionable whether a large production area itself has an influence on *A. lycopersici* incidence. The described correlation likely is a result of the correlation between some of the intensification factors. *A. lycopersici* incidence was significantly higher in farms that heated during the colder months (Tab. 9). *A. lycopersici* requires temperatures of around 25 °C to reach its highest reproduction rate (Haque and Kawai 2003). This likely explains why first *A. lycopersici* symptoms are noticed earlier in the season on farms that heat in the cooler months (Tab. 9). Since only seven farms in the group without heating reported the months in which first symptoms were noticed, the conclusiveness of the presented data on heating in relation to *A. lycopersici* incidence is limited.

Growing in artificial substrate in special bags allows for precise water and fertiliser dosage to achieve optimal plant growth and high yields (IVA 2017). Since substrate bags, as opposed to natural soil, can easily be changed and renewed, problems with soilborne diseases are minimised. This allows farmers to disregard crop rotations and grow crops such as tomato over multiple seasons in a row in specialised greenhouses (IVA 2017). However, cultivation without crop rotation and with minimal breaks between tomato sets, as cultivation in substrates such as rock wool allows, comes at a cost. It might favour *A. lycopersici* survival by providing almost a year-round presence of the host crop. Organic growing associations usually only permit cultivation in natural soil (Bioland e.V. 2020). At the same time, due to soilborne diseases wider crop rotations are realised in farms that cultivate in natural soil that naturally result in a larger cultivation break / more time without a suitable host plant present. As mentioned, some of the factors overlap, and it is not possible to derive if and how severely the growing medium (Tab. 5) or substrate affect *A. lycopersici* incidence and persistence. To answer this question detailed studies on *A. lycopersici* survivability in soil, on plant residues, on greenhouse structures at varying temperature and humidity or on the effect of rotations with specific crops are needed. In summary, the data accumulated with this survey supports the assumption that *A. lycopersici* favours one or more of the often-correlating intensification factors investigated in this survey: i) short breaks between tomato seasons, ii) heating in cold months, iii) cultivation in non-natural soil, and iiii) large cultivation area. Since these factors were more prevalent in integrated farms that participated as compared to the organic farms (Tab. 11), an explanation is provided as to why *A. lycopersici* incidence is significantly higher in the participating integrated farms (Tab. 4).

Plant protection measures against *A. lycopersici*

The nine farms that reportedly achieved *A. lycopersici*-free seasons after previous seasons with *A. lycopersici* presence (Fig. 3) were thoroughly checked for similarities in production factors and plant protection measures but no factors could be identified. The absence of a key countermeasure on those farms could mean that successful on-farm eradication relies on the creation of unfavourable conditions for *A. lycopersici*, both during and between the growing seasons.

There were no significant differences in specific countermeasures taken against *A. lycopersici* between farms on which *A. lycopersici* negatively affected yields, compared to those on which it did not (Tab. 10). This means that a standard effective countermeasure could not be identified amongst the participating farms. It is possible that not only the type of measure, but also the early detection and fast reaction on the initial outbreak is of importance. This could be traced back to the lack of reliable early detection methods for this pest (Pfaff et al. 2020)

The chance for participants to describe custom strategies and countermeasures against *A. lycopersici* did not reveal any novel methods not already reported or published in the literature. Strategies mostly consisted of repeated treatment with acaricidal substances, use of predatory mites, the removal of infested plant material or a combination of all three approaches. One participant responded to this question by stating that the first and strongest symptoms occur in the areas that are most exposed to sunlight. Assuming that those are the tomato plants most likely experiencing drought stress, this confirms previous findings that there is stronger population growth on plants that are in a state of drought stress (Pfaff et al. 2020). Naturally, the greenhouse areas most exposed to sunlight are the warmest and this favours *A. lycopersici* which has shown to have its peak population growth at around 25°C (Haque and Kawai 2003).

During the survey window, the products Vertimec Pro and Agrimec Pro - both containing Abamectin, were the only products specifically authorised for use against *A. lycopersici* in Germany. This explains why they were chosen by nine of the 15 farms that applied acaricides. Efficacy of Abamectin against *A. lycopersici* has been shown in the past (Royalty and Perring 1987; Kashyap et al. 2015). The other three compounds applied against *A. lycopersici*, Spirodiclofen, potash salt and Azadirachtin each mentioned by a different single farm were authorised in tomato, but not specifically against *A. lycopersici*, although a side effect on *A. lycopersici* is possible and likely the reason why they were mentioned. Abamectin is harmful to several predatory mites (Alhewairini and Al-Azzazy 2021). The negative effects on beneficial arthropods likely explains why Abamectin treatments often are restricted by farmers to *A. lycopersici* infection nests, or why local Abamectin treatments are combined with broader Sulphur treatments e.g., described by three farms. A broad variety of beneficial arthropods were introduced on the participating farms. Even if there were further acaricidal compounds available against *A. lycopersici* they would need to be highly specific in targeting *A. lycopersici*

to not interfere with the established regime of beneficial arthropods in commercial tomato cultivation.

Among the participating farms, *A. swirskii* was the beneficial arthropod most often introduced specifically against *A. lycopersici*. *A. swirskii* was introduced on five of the participating farms. *A. swirskii* predates all life stages of *A. lycopersici* (Park et al. 2010). However, *A. lycopersici* has the ability to seek refuge from predators between trichomes on tomato plants (van Houten et al. 2013) and in doing so is likely able to limit the effectiveness of introduced predators to an uncertain extent.

3.6 Conclusion

This study provides a detailed picture of 50 tomato producing farms and how they are affected by *A. lycopersici*. Yearly, the number of farms where a first *A. lycopersici* occurrence is reported, has increased between 2005 and 2018 amongst participating farms and *A. lycopersici* incidence is not concentrated to certain regions in Germany. *A. lycopersici* was the pest with highest relative importance on the participating farms and 22 of 23 farms with a cultivation area of 4800 m² or more report the presence of *A. lycopersici*. Repetition of the survey to detect possible changes in relative importance and on the farms affected, would clarify whether these findings are consistent over time, or if the status of *A. lycopersici* in Germany is still subject to change. Several intensification factors (1. Heating in cold months, 2. Large cultivation area, 3. Short break between tomato seasons, and 4. Not cultivating in natural soil) statistically favoured *A. lycopersici* occurrence, but autocorrelation prohibited the identification of a causal link to specific factors. Initial infestation of *A. lycopersici* significantly increased the chance for continuous presence in consecutive years. Detailed trials on *A. lycopersici* survivability and population dynamics under varying environmental conditions could help provide causal links to some of the afore-mentioned factors. Plant protection strategies in different combinations often consisted of broad treatments of sulphur, local abamectin treatments, removal of infested plant material or introduction of a wide variety of beneficial arthropods. None of the countermeasures could be identified as providing better or lasting control of *A. lycopersici* with

the data gathered in this survey. Therefore, efficacy trials under practical conditions are advisable.

3.7 References

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General Discussion

Handling *Aculops lycopersici* in the present and future

In their review on interactions between eriophyoid mites and their host plants, de Lillo et al. (2018) stress the importance of identifying effective non-chemical methods for the control of *A. lycopersici* on tomato. This valid statement is supported by different developments of the past, and probable developments of the near future. Looking into the past the regulation (EC) No 1107/2009 needs to be mentioned. It was implemented into national law by the different member states of the EU in the early years of the 2010s. Parallel to this, a pesticide review program was being carried out. This pesticide review program took a hazard-based approach to assessing active substances, in contrast to the former approach, which was risk-based. This pesticide review program resulted in a considerable reduction in the availability of active substances for use within the EU (van Leeuwen et al., 2010). This also resulted in a reduction in the number of active substances that were available for the control of *A. lycopersici*. A table of active substances with acaricidal properties against eriophyoids authorised for plant protection in the EU is provided by Vervaet et al. (2021). Notably, the authorisation of active substances, does not necessarily mean that plant protection products containing the respective active substances are authorised at the member state level for use against *A. lycopersici*. This fact, in the case of Germany, narrows the number of chemical active substances available for use against *A. lycopersici* substantially (BVL 2022). Looking towards the future, the pesticide reduction goals of the "farm to fork" strategy of the European Union (European Commission 2020), makes an increase in the number of available active substances unlikely. This strategy will rather contribute to a further decline in the number of active substances which can be applied. One of the nine currently authorised active substances with efficacy against *A. lycopersici* listed by Vervaet et al.(2021), Cypermethrin, is already classified as candidate for substitution. Another consequence of this strategy is potentially a shift away from using active substances with proven high efficacy, to using active substances with lower efficacy.

The good results of the non-chemical method described in chapter 3 (use of glue-rings to restrict mite mobility on the plant), further support the statement by de Lillo et al. (2018), that non-chemical methods can pose as key factor in the control of *A. lycopersici*. Furthermore, it is a good example for a method that manipulates the crop and its environment towards

unfavourable conditions for *A. lycopersici*. There is the potential for this particular method – stem glue-ring application – to potentially be utilised in low- as well as high-tech cultivation environments, independently of whether it is organic or integrated cultivation, and this means that this method is a potential *A. lycopersici* control option for a broad spectrum of farms. The trial should be repeated and the method should be investigated in subsequent trials.

While the glue-ring method is an option, applying rings to every plant at a weekly interval comes with unavoidable additional costs. The magnitude of these costs will depend on whether partial or complete automation of the glue ring application will be possible in the future. An alternative way to reduce the cost of glue ring treatment would be to limit application to the areas with *A. lycopersici* presence, or to apply the treatment at a lower frequency should tests show that this still leads to adequate results. An alternative, non-sticky, substance that still creates a barrier would be an improvement of the glue-ring method, as it would mitigate the risk of contaminating tools, clothes or fruit with a sticky substance.

The earlier infestations are detected, the more leaf-surface can be protected with the glue ring method. Therefore, especially an early and efficient detection of infestations is of high value.

Non-imaging spectroscopic monitoring, a method for the early detection of *A. lycopersici* presence, was investigated and described in chapter 1. The non-imaging spectroscopic monitoring is a method that is in an experimental stage, but the results outlined in chapter 1 act as a first proof of concept for this method. In light of recent advances in sensor technology and their utilisation for plant protection purposes, a broader implementation of spectral sensors (Mahlein 2016; Roper et al. 2021) in commercial tomato cultivation seems likely in the near future. Due to sinking costs, mainly of RGB- but also of hyperspectral cameras, and increasing computing capacities in general, especially spectroscopic imaging technology is an ideal candidate for practical solutions (Zhao et al. 2020). This technology provides a chance for more precise plant protection in general, and more precise and efficient application of barrier methods as investigated in chapter 2, in particular in high technology greenhouses. For low technology greenhouses, where advanced and automated monitoring will not be available in the foreseeable future there is also room for innovation. Technological solutions for monitoring in low technology greenhouses should be time efficient and low cost, e.g. as shown with a proof of concept for different greenhouse pests and beneficials by Böckmann et al. (2021). Climate, irrigation, and growing substrate conditions typically show a higher variance

in low- as compared to high technology greenhouses. One approach for better monitoring without technological gadgets would be to preferably check those greenhouse areas that are most prone to drought stress for evidence of first symptoms. It was shown in chapter 1 that plant damage caused by *A. lycopersici* progresses faster on plants experiencing drought stress, or those areas most exposed to sunlight (an environment likely to coincide with drought stress). Similar observations were made on one farm in the research for chapter 3. Aside from replacing chemical plant protection with non-chemical alternatives where feasible, precise monitoring such as the approach developed in chapter 1, would improve the precision with which plant protection products are applied in commercial cultivation. This again, would allow for a reduction in the total amount of product applied, as healthy plants or healthy plant sections could be left out.

Beyond the research discussed in this work, there are further ways to make commercial tomato production less favourable for *A. lycopersici* in the future. By way of example, the potential employment of endophytic fungi in alleviating the severity of *A. lycopersici* infestations is as yet underexplored. This point is made by Vervaet et al. (2021). The non-pathogenic fungus *Fusarium solani* strain K has been shown to improve the direct tomato plant response to *T. urticae* by upregulating defence signalling pathways. It has also been shown to improve defence indirectly by changing the volatile signature of tomato plants in a way that makes them more attractive for a predator of *T. urticae*: *M. pygmaeus* (Pappas et al. 2018). Similar effects might be possible for *A. lycopersici* and this would certainly be worth investigating. As was pointed out in this work, the trichomes of currently cultivated tomato varieties can be considered a double-edged sword when it comes to *A. lycopersici*. Trichomes provide tomato plants with protection from different pests (Simmons and Gurr 2005), but they also provide shelter for *A. lycopersici* from natural enemies (van Houten et al. 2013). Modifying the trichomes of cultivated tomato through targeted breeding programs might provide a solution to this dilemma. There are indications for differences in susceptibility to *A. lycopersici* amongst different tomato varieties that has been able to be traced back to differences in trichome structure (Kitamura and Kawai 2006). The different trichome types of several wild varieties and cultivated tomato and the allelochemicals some of them secrete are well-described (Simmons and Gurr 2005; Zeist et al. 2019), and are considered to provide opportunity for

successful breeding programs, especially when accelerated by new biotechnological approaches for plant breeding (Zeist et al. 2019).

A. lycopersici and climate change

It is not justified to argue that *A. lycopersici* is on the rise in German tomato production solely due to the fact that climate change provides better conditions for it to establish and thrive. It can, however, certainly be argued that the increase in incidence of dry summers with high temperature that have been experienced in the recent past decades (statista 2022) and that might intensify further in the coming decades, are certainly a factor that *A. lycopersici* benefitted from, and will continue to benefit from. Throughout the trials conducted for this study between 2016 and 2020, the enhancing effect of drought stress on population development was observed and also reported with statistical significance (chapter 1). It was also observed in several greenhouse trials conducted at the trial station, that symptom expression was more severe in the greenhouses and greenhouse areas exposed to higher temperatures and more sunlight (own observation). Also, a grower reported to usually observe first symptoms in areas of the greenhouse most exposed to sunlight (described in chapter 3). Almería (southern Spain) has the largest concentration of greenhouses for tomato production in the EU. In Almería the average and peak temperatures, as well as number of sunlight hours, are higher when compared to the growing conditions in Germany. To shade plants from too much sunlight, and to reduce temperatures within the greenhouses, the top surfaces of greenhouses are chalked; a chalk slurry is sprayed on top of the greenhouses and a film cover of chalk forms when dried, and this serves to increase the amount of sunlight that is reflected (hortidaily 2016). In case temperatures and sunlight hours continue to increase in Germany, the profitability of shading methods or systems for summer months might increase and the use of such methods should be considered more widespread. Not only would plants be provided more evenly with their optimum growing temperatures and light intensities, but the development of pests that benefit from plants experiencing heat stress or drought, like *A. lycopersici*, would be impaired. An additional synergy could be achieved by establishing the shading with intelligent photovoltaic solutions on top of greenhouses, contributing to the "agri-photovoltaic" goals established in 2022 (BMEL 2022).

Intensive or extensive greenhouse tomato production?

In chapter 3, it was shown that *A. lycopersici* occurs more often, and causes more damage on farms with intensive tomato production (short cultivation cycles, production in substrate bags, heating in cold months), which tend to be integrated rather than organic farms.

Considering agriculture in general, and field crops in particular, there are several indications that organic farming as it is practiced today in central Europe cannot be considered a superior alternative to (true) integrated farming combined with "land-sparing" in terms of biodiversity conservation (Tscharntke et al. 2021; Collas et al. 2023) or climate protection once CO_2 opportunity costs are factored into the equation (Breunig and Mergenthaler 2022; Collas et al. 2023). However, the question needs to be raised whether this also applies to the greenhouse vegetable production. This is because greenhouse vegetable production covers much less space than field crops in agriculture and has additional CO_2 intensive inputs such as heating in colder months when located in Germany. As a result, a less intensive production (not necessarily after organic farming guidelines) of greenhouse crops in Germany could not only come with phytosanitary benefits and reduced impact of pests such as *A. lycopersici*, but it also could result in less CO_2 emissions. This creates room for further research to face the conflict of objectives when aiming for truly sustainable food production. Considering *A. lycopersici* in particular, it was not possible to quantify or identify the effect of particular intensification factors due to autocorrelation. Future studies can remedy this knowledge gap and identify the intensification factors that have the strongest effect on *A. lycopersici* incidence and infestation severity.

Conclusion

A. lycopersici and its unique relation to tomato crops in commercial cultivation make for a textbook example of how manifold the challenges posed by a single pest can be. Chemical plant protection products are either limited in number and efficacy or are not an option due to the widespread implementation of beneficial arthropods in today's tomato production. This means that ingenuity on the part of farmers and researchers, as well as companies active in developing monitoring techniques, distributing beneficial arthropods and working on tomato production systems, is required in a combined effort to make integrated pest management work. In different periods of time, solutions might be found in habitat manipulation such that

unfavorable conditions are created for *A. lycopersici* without impairing yield or aiding other pests. This includes the investigated barrier method, adaption of climatic conditions or optimisation of water supply and sun exposure, in the utilisation of endophytic fungi and in the long run, breeding of resistant or tolerant plants. Solutions might be found in the identification of suitable beneficial arthropods that prey on *A. lycopersici*, and in optimising their introduction specifically to combat *A. lycopersici* infestations. Last but not least, further work on early detection devices will substantially contribute to the success of any implemented *A. lycopersici* countermeasure.

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Appendix

Appendix Chapter 1

In order to investigate whether the sticky tape imprint method is suitable for quantifying *A. lycopersici* numbers on plant stems 30 plant stem pieces were cut from a tomato plant which was colonised by *A. lycopersici.* The total number of *A. lycopersici* individuals was counted on each stem piece. In the next step from each stem piece a sticky tape imprint was taken. Afterwards the number of *A lycopersici* individuals found on the imprints was counted. The results are displayed below in Figure S1.

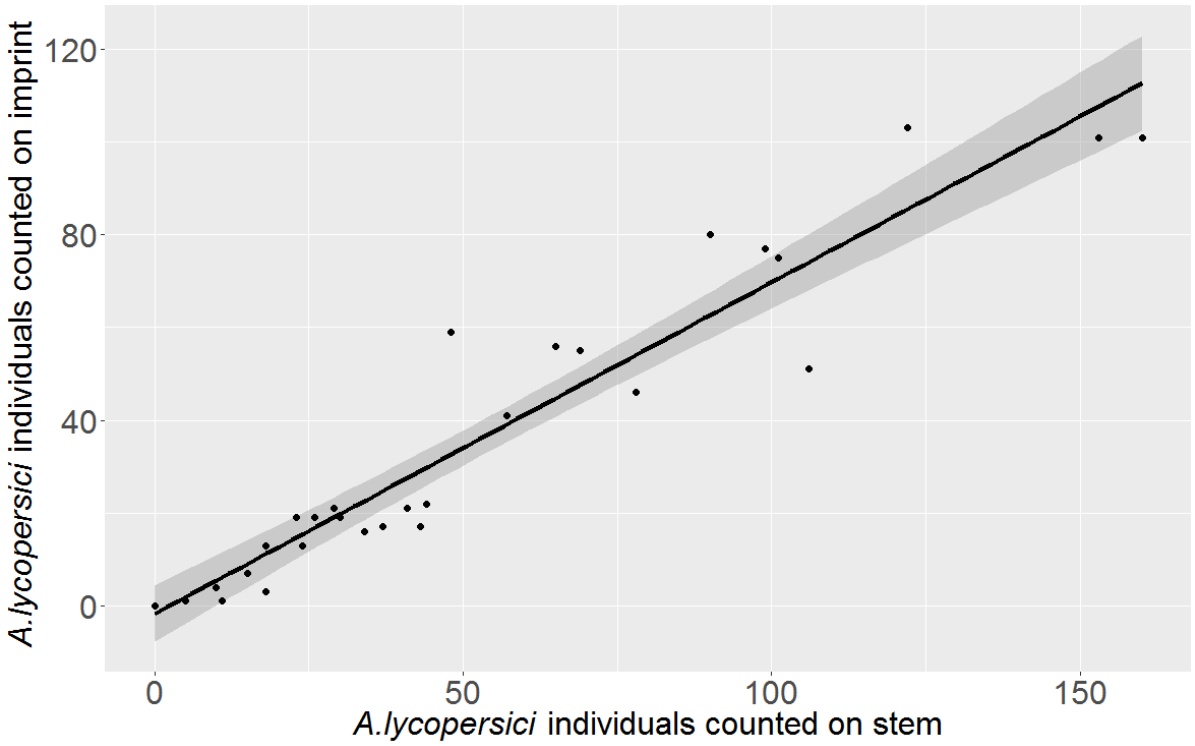

Fig. S1: The black dots display 30 different counts of *A. lycopersici* individuals each made on an individual plant stem piece in relation to counts made on sticky tape imprints after they were taken from the respective stem pieces. The black line displays a linear regression of imprint counts in dependence of stem counts. The grey band displays a 95% confidence interval for predictions based on the linear regression. The adjusted R^2 value for the model is 0.90.

Table S1: *Correct classification comparison separated by water supply* Displayed is the classification based on the fluorescence measurements separated for plants with sufficient and low water supply level and in both of these groups separated for inoculated and control plants. The grey "combined" columns state the percentage of correctly classified plants for each of the water supply groups.

	fluorescence classification in dependence on water supply					
	sufficient water supply level			low water supply level		
	inoculated (n=24)	control (n=24)	correct classification rate	control (n=24)	inoculated (n=24)	correct classification rate
dpi	class. correct:	class. correct:	% correct:	class. correct:	class. correct:	% correct:
4	13	10	48	12	14	54
6	12	8	42	12	15	56
8	7	16	47	9	12	44
10	23	4	56	24	5	60
12	9	12	44	8	18	54
14	10	19	60	10	20	63
16	21	12	69	24	8	67
18	13	24	77	18	23	85
20	20	22	88	24	21	94
22	21	21	88	24	23	98
24	21	24	94	23	23	96
26	24	24	100	24	23	98
28	23	21	92	23	23	96

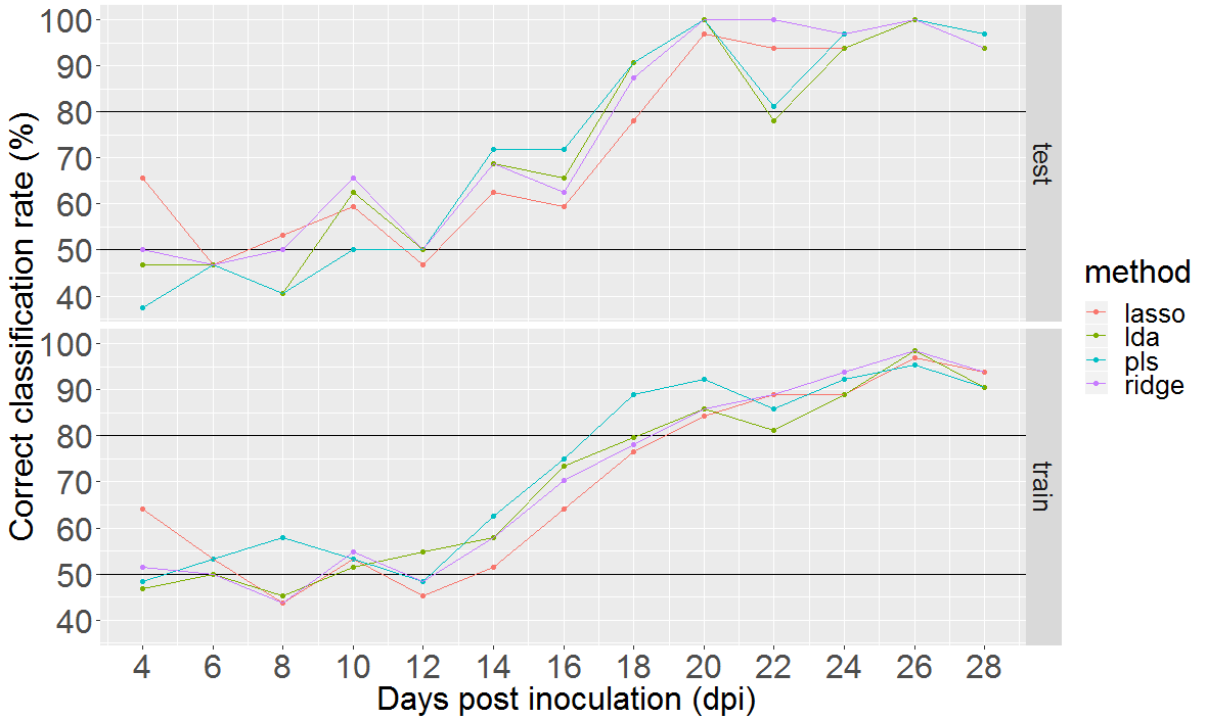

Fig. S2: Comparison of the four applied methods (1: ridge regression, 2: lasso regression, 3: PLS with six components, 4: LDA with scores of six principal components) Correct classification rate expressed as a percentage is shown on the Y-axis. The sampling dates are displayed on the X-axis. In the top part of the Figure the results for the test dataset are displayed. In the bottom part the results for the training dataset are displayed.

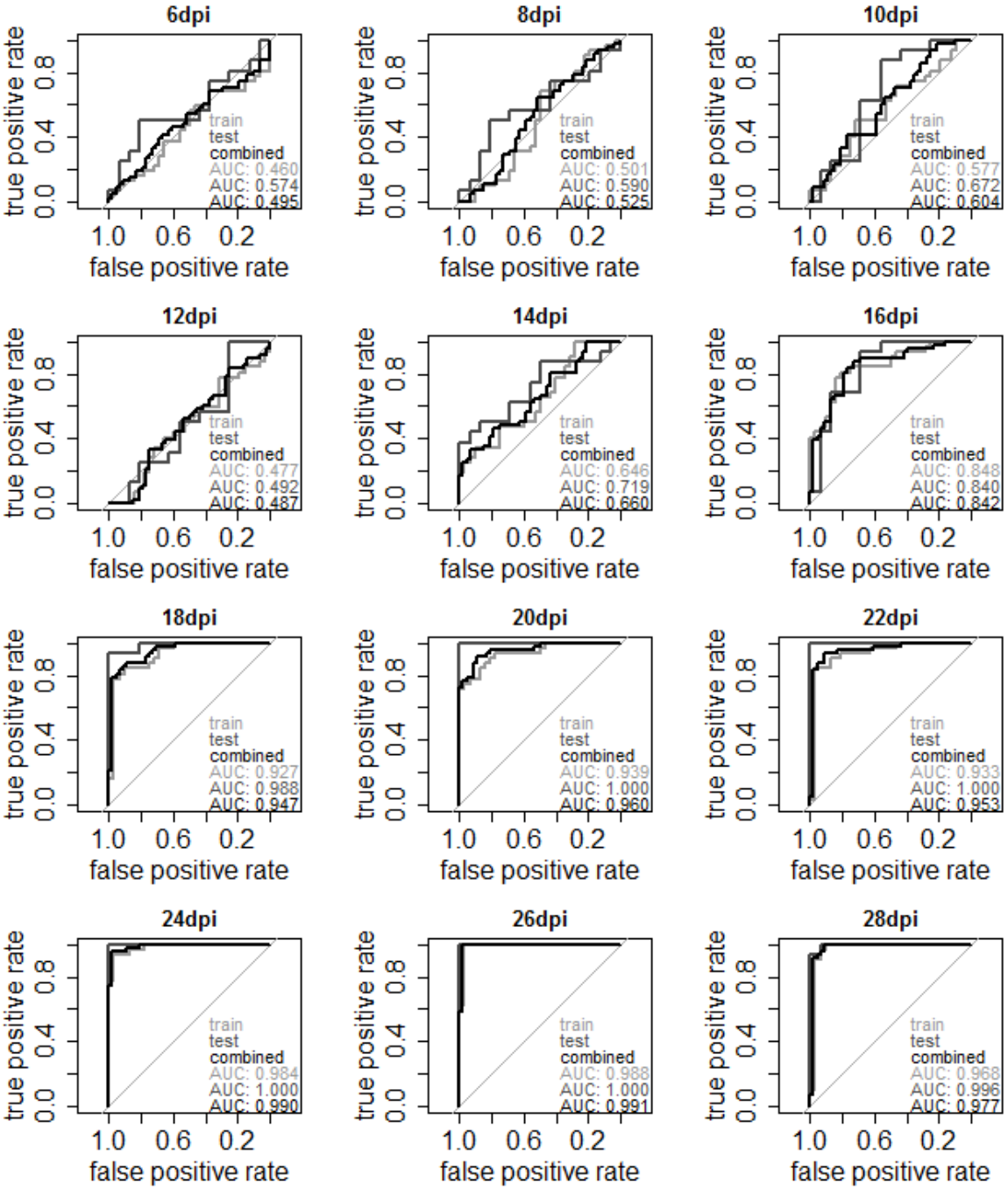

Fig. S3: Receiver Operator Characteristic (ROC) curves for calibration using the ridge regression at the different sampling dates from 6 dpi till 28 dpi. Separate curves for the combined dataset, the train dataset and the test dataset. AUC = Area under the Curve.

Appendix Chapter 2

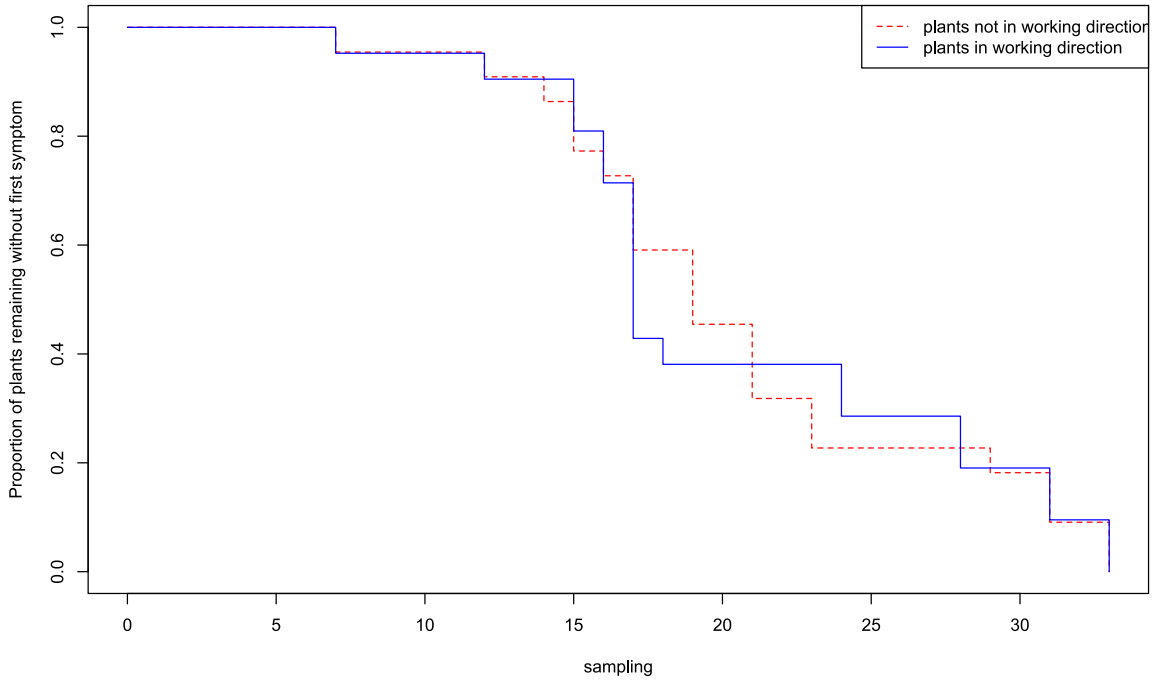

Fig. S1: Kaplan Meyer curve for first symptoms occurring on the plants in working direction (solid line, n = 24) compared with plants not in working direction (dashed line, n = 24). A survival analysis with the log rank test revealed that there was no significant difference in symptom development between the two groups (p=1.0).

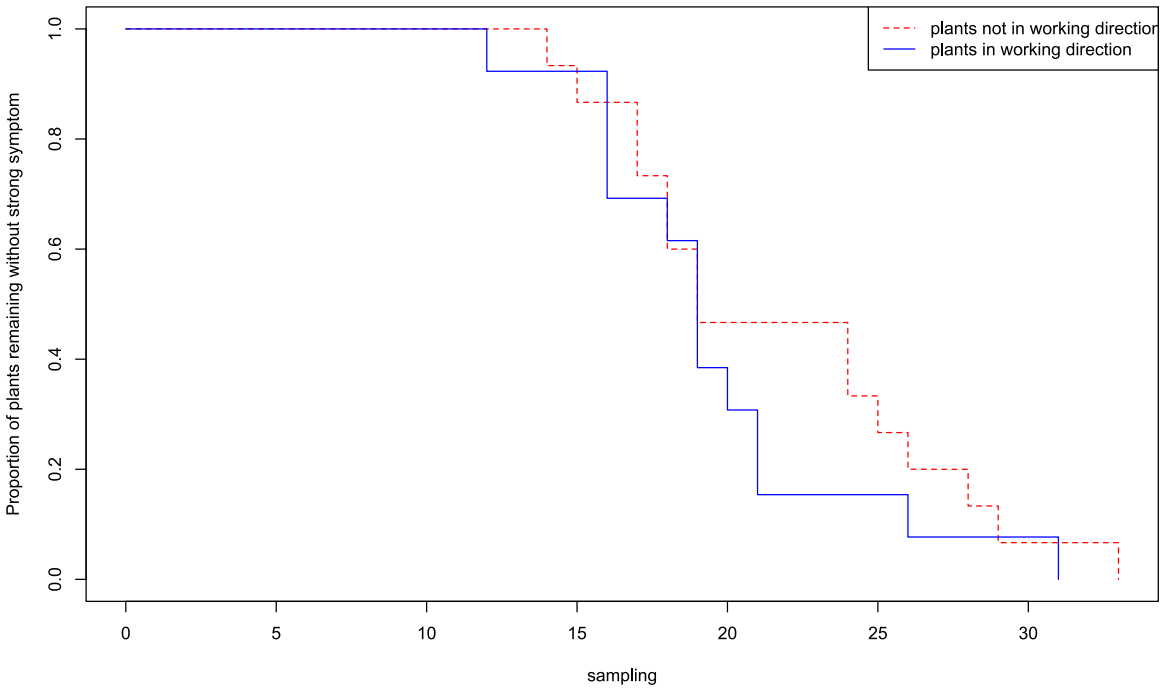

Fig. S2: Kaplan Meyer curve for first strong symptoms occurring on the plants in working direction (solid line, n = 24) compared with plants not in working direction (dashed line, n = 24). A survival analysis with the log rank test revealed that there was no significant difference in symptom development between the two groups (p = 0.4).

Appendix Chapter 3

Tab. S1: beneficial arthropods introduced on participating farms shown separately for farms with *A. lycopersici* presence and those without.

Beneficial organism	*A. lycopersici* present (28 of 33 farms reported beneficials)	*A. lycopersici* absent (10 of 17 farms reported beneficials)	total frequency
Encarsia formosa	23	9	32
Macrolophus pygmaeus	18	3	21
Eretmocerus sp.	8	1	9
Amblyseius swirskii	5		5
Phytoseiulus persimilis	3	1	4
Braconidae	1		1
Amblyseius cucumeris	1		1
Orius majusculus	1		1
Aphidius ervi		3	3
Amblyseius barkeri	1		1
Amblyseius californicus	2		2
Dacnusa sibirica	1		1
Steinernema feltiae	1		1
Aphidoletes aphidimyza	2		2
Aphidius colemani	2	2	2

Tab. S2: Farms that provided specific information on strategies against *A. lycopersici* infestation.

FarmID	Detailed description of countermeasure
2	Sulphur treatment with vaporizer
3	First and strongest symptoms in the areas most exposed to sunlight
4	preventive predatory mites, after *A. lycopersici* infestation Sulphur treatments
21	removal of symptomatic leaves followed by acaricide treatments
40	After *A. lycopersici* infestation local treatments with abamectin, broad treatments with sulphur
8	After *A. lycopersici* infestation local but spacious treatment around symptomatic areas with Sulphur repeated three times with three to four days time between single treatments
12	Treatment with herbal mixtures
13	So far only local and late *A. lycopersici* infestations which were contained with removal of symptomatic plants
36	abamectin with high water volume, two to three treatments per infested nest
39	Removal of whole plants as soon as symptoms occur

Acknowledgements

First, I would like to thank Elias Böckmann for his supervision, his ideas and his support during the three years of project work that made this dissertation possible. This also includes his support during my writing time that went far beyond my time in his research group at the JKI in Braunschweig. Thank you, Elias, I enjoyed working with you!

I would like to thank Stefan Vidal for his support and supervision in all scientific and organisational matters. Whenever there were papers to be proofread or advice needed on how to proceed, Stefan always made the time.

I would like to thank Michael Rostás, Rainer Meyhöfer, Elias Böckmann and Stefan Vidal for agreeing to grade my dissertation and/or be part of my examination committee.

The technical and gardening personnel at the JKI played a key role in making this dissertation a success. Without them, this work would not have been possible - I learned a lot from you, for which I am grateful. Also important to mention, are the other doctoral students who were around druing my time at the JKI; talking to you was always helpful and I'm grateful to you for giving me the push to move this dissertation forward, especially when thoughts of discontinuing became all too tempting.

I would like to thank my grandfather Wilhelm Richardt, for his unconditional support throughout all of my studies – I am sure he would have been proud to see me finish. I would also like to thank my parents and my sisters for their support.

Finally, I would like to thank Rowan Titchener. Rowan kept me motivated throughout this endeavor and without her at my side I would not have finished this dissertation. In addition, I would like to thank Rowan for proof-reading sections of this dissertation - her corrections improved this dissertation markedly and made it a far more enjoyable read!