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Research Project

The effects of basic substances on the population dynamics of *Tetranychus urticae* and *Phytoseiulus persimilis*

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Abstract - Pest species destroy large numbers of agricultural crops each year and to protect their crops, growers use chemical pesticides. However, chemical pesticides can lead to the death of essential non-target organisms like predators of the pest species. Alternative crop protection strategies like biological control agents and biological pesticides are needed and becoming more popular. The problem is finding new targeted biological pesticides that control pests without affecting their predators. Basic substances are substances that are allowed to be used in organic agriculture by the European Commission and can potentially help protect plants against pests. This study investigated how three basic substances (cow milk, chitosan hydrochloride and stinging nettle extract) affect the survival of the pest *Tetranychus urticae* and its natural enemy *Phytoseiulus persimilis* and assessed how this would affect their population dynamic. We found that chitosan hydrochloride (1% v/v) and stinging nettle extract (3% v/v) had no effect on *T. urticae* and *P. persimilis* in their tested concentrations. Directly spraying cow milk was found to lower the survival of both *T. urticae* and *P. persimilis*. However, in a population dynamic experiment cow milk was not able to reduce the number of *T. urticae*. In conclusion, when predators experience a double negative effect of an increased death rate and less prey, the use of biological pesticide will be ineffective, since the prey can increase in numbers.

Introduction

Chemical pesticides

Agricultural crops face daily challenges like pests, diseases, and other environmental stresses. As a consequence, large amounts of crops are destroyed each year due to these factors (Oerke, 2006; Oerke & Dehne, 2004). To be able to reduce the damage of agricultural pests on crops, it is important to understand the dynamics of their populations. It is possible to estimate the moment that action is required by looking at how pest populations grow and fluctuate. To control pests in agriculture, multiple techniques have been developed, some of which are more environmentally friendly than others.

One way to control pests in agriculture is by using chemical pesticides, this is often a quick, easy, and cheap option to protect crops against many pests and diseases. However, intensive use of pesticides can lead to intense ecological damage through their toxic characteristics (Guedes *et al.*, 2016). Chemical pesticides have been excessively sprayed and many species are developing resistance to pesticides (Bass & Jones, 2018). Moreover, due to this excessive spraying in the past and present, current toxin levels in the environment are dangerously high (Silva *et al.*, 2019). Additionally, there is the problem of pest resurgence. Here, the pest population density suddenly increases after being treated with pesticides and reaches a higher abundance than non-treated pest populations (Hardin *et al.*, 1995).

Chemical pesticides can lead to dangers for essential non-target organisms. Therefore, testing these products before widespread use is of vital importance. For every chemical pesticide that is used in agriculture, multiple toxicity tests were performed to examine the potential danger of the chemical (OECD, 2021). However, these tests focus mainly on how single substances affect mortality or growth of individual organisms (Topping *et al.*, 2020). Chemical pesticides can be very persistent and can accumulate in the environment, where they are dangerous, even in low concentrations. Moreover, these toxicity tests are focused on the effects on individuals and less on the dynamics of populations leading to possible pest resurgence events. The dynamics of a prey or predator population can significantly change when the population density of one of the species changes. The study by Janssen and Van Rijn (2021) shows that negative effects of the pesticide on predators are likely the cause of pest resurgence. They show that the pesticides often have no effect on pest densities when predators are also affected by the pesticide. Although chemical pesticides are often easy to use, intensive spraying can have substantial consequences on population dynamics and the environment.

Population dynamics

Besides reducing the density of pest organisms, it is also possible that the dynamics of other species (like natural enemies) are affected by (bio)pesticides, which can cause new problems. For example, if a pesticide is used to control the population of a certain species and it has a non-target effect on its natural enemy, the prey population can then increase in density and become a pest. If pesticides affect both the predator and prey, the predator will experience a double negative effect according to existing models. To give an example, predator-prey dynamics can be described by the Lotka-Volterra equations (Lotka, 1910), where one represents the prey $x(t)$ and the other the predator $y(t)$ densities (see equations 1 and 2). $\alpha x(t)$ and $-\gamma y(t)$ describe the natural growth of prey and death of predator respectively. Predation of the pest is dependent on the predation rate of the predators (β) as well as the density of both the predator and pest ($x(t) \cdot y(t)$), assuming a "random" chance that a predator and pest individual meet. Growth of the predator is depending on predation rate of the predators (δ) as well as the density of both the predators and pests ($x(t) \cdot y(t)$).

$$(1)\ \frac{dx(t)}{dt} = \alpha x(t) - \beta x(t) y(t)$$

$$(2)\ \frac{dy(t)}{dt} = -\gamma y(t) + \delta x(t) y(t)$$

The long-term average densities of predators and prey can be estimated by calculating equilibria of these differential equations, and the pest equilibrium density can be obtained from setting the predator equation equal to zero. This gives y^* = 0 or $x^* = \gamma/\delta$. When a pesticide is introduced that results in mortality of both predator and prey, the prey will die more (α decreases), but so will the predator (y). The higher mortality of the pest does not affect the pest equilibrium (x^*), but the higher predator mortality (γ) results in a higher pest equilibrium (x^* increases with γ). Because the density of the pest increases when the predator is sensitive to the pesticide, spraying with these compounds might not result in decreases in pest densities. This leads to the need for alternative pest control methods that focus on reducing pest populations while leaving the predator populations unharmed.

Alternative pest control methods

As apparent from the ecological disadvantages of pesticides, alternative protection strategies like biological control agents are needed and becoming more popular (Van Lenteren *et al.*, 2020). Van Lenteren *et al.* (2018) describes four types of biological control: classical, natural, augmentative and conservation biological control. Classical biological control was the first used biological control, which is why it is called classical. Classical biological control is that natural enemies are collected from other areas and released on the new invasive area of the pest, when a pest is invasive in a new area and there are no predators yet present. Here, natural enemies are released once and often result in permanent control of the pest (Hajek & Delalibera, 2010). With natural biological control, the pest organisms are controlled by enemies that are already occurring naturally (Abdel-Baky & Abdel-Salam, 2003). This control method takes place without human interference. Augmentative biological control is the release of large numbers of natural enemies and can be divided into two types, inoculative and inundative (Eilenberg *et al.*, 2001). Inoculative biological control agents have control over the pest for a long time but not forever, for example for species with a longer generation time (Pickett & Gilstrap, 1986). Inundative biological control agents are not expected to have a long or permanent control over the pest, as for species with a short generation time (Eilenberg *et al.*, 2000). Lastly, conservation biocontrol consists of changing the environment in a way that promotes crop protection by natural enemies. This is done by making the environment less appealing for pests and more attractive for natural enemies (Landis *et al.*, 2000).

Additionally to using biological control agents and chemical pesticides, more environmentally-safe pesticides are now being developed and used (Glare *et al.*, 2012; Gupta &

Dikshit, 2010). These biological pesticides (biopesticides) consist of substances that are extracted from organic material and are not heavily altered. Biopesticides are biodegradable, less toxic, and often more targeted on specific species, which means that they are less harmful than chemical pesticides. There are numerous biopesticides known that protect plants through different modes of action (Copping & Menn, 2000). Due to their targeted working, it is important to find and develop a large array of biopesticides. Even though a large amount of research is already taking place in the field of biopesticides, there is a missing link between scientific research and manufacturers (Pavela & Benelli, 2016). In order to develop new biopesticides, they need to be screened for safety and toxicity and they need to meet the EU regulations. It is important to keep in mind that, even though biopesticides are not synthetically created chemicals, substances can still form dangers at high concentrations.

The potential of basic substances as biopesticides

The EU regulation of 2009 may provide a solution to increase the use of biopesticides with the added category of compounds called "basic substances" (EC No 1107/2009, Article 23). Basic substances are substances selected by the European Commission that can be found in nature, such as extracts from plants and other organisms, and which are already used in the food (supplement) industry. These selected substances are already classified as not harmful for organisms and the environment at specified concentrations, so it is much easier to be able to use them as plant protection products. Around 20 basic substances (including cow milk, chitosan hydrochloride and stinging nettle extract) have already been approved by the EU, however, their potential for crop protection is largely unknown. That is why screening of the basic substances against different targets is vital. The basic substances form a new route to be able to produce and use more biological pesticides.

Cow milk

Cow milk is approved as basic substance and can be useful beyond consumption. For example, it can be used as medium to grow bacteria that are used against pests (Young, 1982). Moreover, milk itself has been found to also have some negative effects on pests. Cow milk can be an effective biopesticide against different fungi species, such as powdery mildews on pumpkins (Bettiol, 1999; Ferrandino & Smith, 2007). Milk can inhibit the growth of the fungi and is applied through foliar spraying of milk in concentrations up to 100%. The milk is sprayed on different plant stages and in different frequencies, depending on the plant species (EC No 1107/2009, Article 23). Milk has also been used as virucide by cleaning agricultural tools with milk that has a protein content of at least 3.5% (EC No 1107/2009, Article 23).

Ferrandino and Smith (2007) tested the effect of milk on fungi with both whole milk, skim milk and unprocessed milk. However, they found that the raw unprocessed milk caused problems while spraying and did not show a better effect against powdery mildew (*Podosphaera xanthii*) than pasteurized milk from a grocery store. Milk acts against fungi through different mechanisms. It can prevent fungi from germinating, and milk contains salts and amino acids that might help the plant to resist the fungi (Bettiol, 1999). Moreover, milk can alter the pH on the leaf surface. However, not only the buffering effect of milk is responsible for the control on powdery mildew, as substances with that same function show a lesser effect against fungi (Ferrandino & Smith, 2007). A lot of research on cow milk has so far been focused on fungi, and less research has been done on arthropods. Gupta *et al.* (2015) show that full milk caused a 94.4% mortality rate of spider mites on rose plants after 48 hours. They also found that cow milk had no significant effect on the mortality rate of predators, suggesting that cow milk is promising for pest control in combination with biological control.

Chitosan hydrochloride

Chitosan hydrochloride is a biodegradable substance that is frequently used in many different products such as cosmetics and packaging material and in processes such as paper production (Srinivasa & Tharanathan, 2007). Chitosan is also used as medicine and it is found to have antimicrobial properties, which are most likely due to the positive charge of the molecules (Goy *et al.*, 2009). Chitosan is derived from chitin and is chemically very similar to cellulose. Chitin, the precursor of chitosan, can be extracted from microorganisms (i.e. cell wall of fungi) and animals such as insects and crustaceans (i.e. chitin exoskeletons). To acquire chitosan, chitin has to be deacetylated so the $NHCOCH_3$ group turns into a NH_2 group (Pusztahelyi, 2018).

Chitosan can also be used as a plant defence elicitor. Elicitors are substances that trigger the defence response of plants by activating downstream defence signalling cascades (Malik *et al.*, 2020). When chitosan gets in contact with the plant, it activates an induced systemic resistance. Consequently, transcription factors and defence responsive genes of the jasmonic acid (JA) and ethylene (ET) pathways are activated (Peian *et al.*, 2021). Upon activation of these pathways, the plant defence will be primed, resulting in a stronger and faster defence response upon herbivore feeding and infection by necrotrophic microorganism (Yan & Xie, 2015).

Stinging nettle extract

Urtica dioica (stinging nettle) and *Urtica urens* (small nettle) are plants that are known to grow on nitrogen rich soils and are often seen as weeds. Nettles have trichomes that release compounds that cause irritation to the skin when touched (Whitney & Gibbs, 2006). Besides their nutritional value, stinging nettles can also be of value in agriculture and can be effective against fungi and aphids. Numerous fungal species seem to be inhibited in growth by stinging nettle extract (Tapwal *et al.*, 2011). Stinging nettle extract contains high concentrations of phenolics, which seem to be correlated with their antioxidant and antimicrobial functions (Maaroufi *et al.*, 2017). Besides fungi and insects, there has been some research on the effects of nettle extracts on mites. Dąbrowski and Seredyńska (2007) tested the effect of stinging nettle extract on *T. urticae* and found that stinging nettle concentrations of 40, 20 and 10 g/L had a significant effect on the mortality of spider mites after 3 days. Moreover, they found that the mortality after 6 days was almost 60% for all concentrations compared with a mortality of 10% for the control. Stinging nettle extract shows great potential as a biopesticide against various pests.

The well-studied acarine predator-prey system

One well-known pest-predator system is that of *Tetranychus urticae* (the two-spotted spider mite) and *Phytoseiulus persimilis* (a predatory mite). As the earlier mentioned Lotka-Volterra model shows, if a biopesticide results in increased, unintended mortality of *P. persimilis* (predator), the density of *T. urticae* (prey) may increase, even when the biopesticide increases mortality of *T. urticae* as well. *T. urticae* is known to attack over 1100 different plant species, including many agricultural crops and they are known to be able to quickly develop resistance to pesticides, making it a highly relevant system to investigate (Attia *et al.*, 2013; Van Leeuwen *et al.*, 2010). This study will focus on investigating the effects of the basic substances cow milk, chitosan hydrochloride and stinging nettle extract on the mortality and population dynamics of the predator-prey system of *T. urticae* and *P. persimilis*. This will be investigated by measuring the change in mortality of the predators and prey separately after treatment with basic substances, and by investigating how cow milk influences the population dynamics of *T. urticae* and *P. persimilis* on small plants. By comparing mortality rates and investigating the population dynamics, we hope to gain insight in the potential of these basic substances to be used as plant protection products.

Materials and methods

Rearing

Spider mites (*T. urticae* (Van Leeuwen *et al.*, 2004)) were reared on a group of bean plants (*Phaseolus vulgaris* 'Speedy'). Each week the oldest bean plants would be replaced by new clean bean plants to provide new food. The rearing unit was positioned in a climate chamber at 25°C and 60% humidity with 18h of light and 6h of dark. The rearing unit of the predatory mites (*P. persimilis* (Revynthi *et al.*, 2018)) was placed onto a plastic tray inside a climate chamber at 25°C and 60% humidity with 18h of light and 6h of dark. *P. persimilis* was fed three times a week with *T. urticae* on 2-3 bean leaves from the *T. urticae* rearing unit. To make sure the predators could not escape the rearing unit, the tray was placed in a bigger tray with water which was placed inside a small tent.

To reduce variation in susceptibility due to age differences of the mites during the experiments, the hatching of all mites was synchronized, this is called an egg wave. Egg waves of *T. urticae* were made by placing 75 adult females on top of three bean leaves that were placed on cotton wool in a tray with water. After 3 days these females were removed, and the eggs were left for 17 days to develop into adults. New bean leaves were added once per week. Egg waves of *P. persimilis* were obtained by placing invested bean leaves from the *T. urticae* colony in a 15cm diameter Petri dish on top of a layer of Daishin agar 1% (Duchefa Biochemie, The Netherlands, D1004). After cooling down, but before solidifying, the invested bean leaves were placed upside down in the agar. After the agar had solidified, 20 adult female *P. persimilis* were added to the leaves and were removed after 24 hours. The Petri dish was closed with parafilm and placed onto a platform in a tray with water which was positioned inside a small tent, to prevent the mites from escaping. To make sure all mites were adults during the experiments, the eggs were left for 1 week to develop. After around 4-5 days, a new bean leaf with spider mites from the *T. urticae* rearing unit was added to the Petri dish to provide some more food. All egg waves and further experiments were put in climate chambers with 25°C and 60% humidity, 18h of light and 6h of dark.

Basic substances preparation

The concentrations of the basic substances were based on the concentrations that are already used in practice or mentioned in other research papers and were approved for agricultural usage by the EC (EC 1107/2009). Pasteurized full milk (Melkan, full milk, The Netherlands) was used at a 100% concentration (Ferrandino & Smith, 2007; G. Gupta *et al.*, 2015). Chitosan hydrochloride (DB-CHITIS 3.0 - Charge®, ADAMA) was sprayed with a solution of 1% v/v, based on current greenhouse uses against various fungi (DeBroers, Woodchem, The Netherlands). Stinging nettle extract was prepared following the guidelines of the EC (EC 1107/2009), where 75g fresh material of *Urtica dioica* and/or *Urtica urens* (or 15g dried material) is used. The material is soaked into 1L water for 3-4 days at room temperature and stirred daily. The extract is then filtered, and 5 times diluted. This was done by a local farmer (John Huiberts, The Netherlands), who uses the extract to control aphids in flower bulb fields. Based on the concentrations that he utilizes; a stinging nettle extract solution of 3% v/v was used. When needed, the substances were diluted in water and the control consisted of water.

Spraying setup

To be able to test how both mite species react to different substances, the leaves had to be treated as they would under greenhouse conditions, which is by spraying. Because a Potter spray Tower was not available for usage, we built an alternative method to control the application of the substances and control treatment (Figure 1). For the spraying of the liquids, an airbrush was used (Revell Airbrush 39200) inside a fume hood, where liquid was sprayed with 2.8 bar N_2. The spraying setup was secured to an iron stand with clamps, the airbrush was clamped at a 45° angle and the nozzle of the airbrush was at a 20cm height above the surface of the fume hood (Figure 1). The placement for the leaf disks was marked on the surface of the fume hood at a 10cm distance from directly under the nozzle of the airbrush (Figure 1). Every replicate was sprayed for 6 seconds. To make sure there

was no contamination of the substances with each other, the airbrush was cleaned with acetone and then demi water before and after spraying each substance.

To determine the accuracy of the airbrush, the amount of liquid that was sprayed by the setup was measured. Filter paper (Whatman, 10311809, Grade 597) was cut into Ø 2.5cm discs that represent the size of the leaf discs that were used in the experiments. To make sure the filter paper discs would stay in the right place, a piece of tape was attached to the back. The weight of each filter paper was measured inside an 2mL Eppendorf tube before spraying. The filter paper was attached to a Petri dish and sprayed for 6 seconds with demi water. Next, the filter paper was quickly but carefully removed without touching any other water droplets, put back in the Eppendorf tube and the weight was measured again.

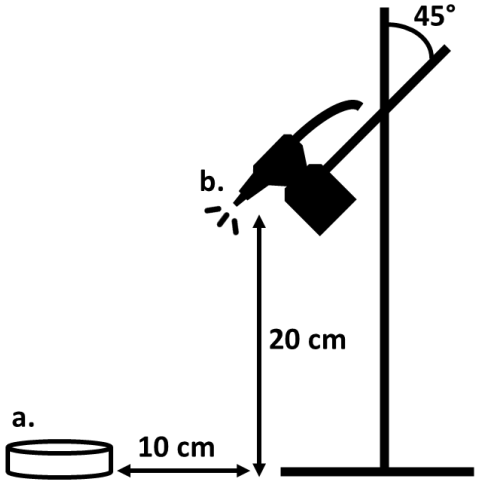


Figure 1. Spraying setup used to spray leaf disks in Petri dishes.
An airbrush is connected to an iron stand with clamps at a 45° angle. In the Petri dish (a.) leaf disk would be placed in the middle, at 10cm from the airbrush. The nozzle of the airbrush (b.) was at a 20cm height.

Survival experiments

To determine the effect of the different substances on *T. urticae* and *P. persimilis*, the effect was first determined for both species separately. For the survival and oviposition experiments with *T. urticae*, 17-day-old spider mites from an egg wave were used. Fresh Ø 2.5cm bean leaf disk were made and on each leaf disk one spider mite was placed. Per treatment 25 replicates were made and tested. Before every experiment, the variation of the airbrush was measured by spraying 3 filter papers before and after each treatment. After spraying, leaf disks were placed in a Petri dish with water to avoid leaf disk desiccation and escape of the individuals. A piece of cotton wool was placed underneath the leaf disk to keep the leaf from sinking or moving. To easily keep enough water inside the petri dishes, three small holes were made in the bottom of the Petri dishes, and they were placed in a big tray with water. After spraying, the leaves were left to air-dry, put in a climate chamber and the survival and number of eggs was assessed after 24, 48 and 72 hours. Water was added every day to make sure the water level was as high as possible so the mites would not be able to escape.

The same experimental setup was done for the survival experiments with *P. persimilis* with a few changes. The predators were synchronized in age and 7-day old predators were used since they develop quicker into adults. One predatory mite was tested per leaf disks. However, 4 days prior to the experiment the leaf disks had already been prepared and 10-15 *T. urticae* mites had been added to the leaf disks. This was done to make sure there would be an abundance of spider mite eggs for the predators to eat during the experiment. The leaf disks were put on cotton wool in a tray with water to keep them from desiccation. Besides these few changes, the experiment was carried out and the survival was assessed the same way as for the spider mites.

Population dynamics experiment

To measure the effect of milk on the population dynamics of spider mites and predatory mites for a longer period of time, an experiment was conducted using bean plants. For the experiment, 20 bean plants were grown in a clean climate chamber (25°C and 60% humidity) until they were 3 weeks old. The plants were divided into four treatments with each five replicates (plants). Two groups consisted of plants with only *T. urticae*, with one group sprayed with milk as the treatment and the group sprayed with water as the control. The other two treatments consisted of plants on which *T. urticae* and *P. persimilis* were both released in a 20:1 ratio, with one group sprayed with milk and the other with water.

At the start of the experiment, 100 spider mites that came from the same leaf from the rearing unit (to minimize variation in age and genetics) were placed on each plant. The spider mites were given time to settle and make web before adding predatory mites. After an initial three days settling period, 5 predatory mites were added to each plant of the two treatments that needed them. The predatory mites were then given half a day to settle as well before spraying took place. All the plants were sprayed in a fume hood using the airbrush with 2.8 bar N_2.

To ensure that all the leaves were sprayed equally, the airbrush was disconnected from the iron stand and held horizontally, and the plants were slowly turned around during spraying (Figure 2). The airbrush was continuously moved between the bottom of the plant to about 10cm above the plant. To make sure the nozzle was always kept at around 20cm distance from the middle of the plant, a piece of tape was placed on the bottom of the fume hood. Each plant was sprayed with 10mL water or cow milk.

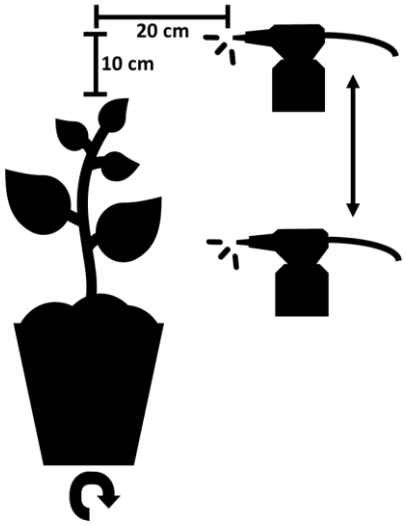


Figure 2. Spraying setup of population dynamic experiment
During spraying the plant was slowly turned around so each part of the leaf was equally sprayed. The airbrush was held at a 20cm distance from the plant which was marked on the ground. During spraying the airbrush was moved up and down the length of the plant till about 10cm above the plant.

After spraying, the plants were left to air-dry for about an hour and then moved to the climate chamber. Living adult mites from both species were observed twice a week on the intact plants by eye, using a bendable desk light and a magnifying glass, and they were counted while observing them for three weeks. It was later decided to also count the number of leaves of each plant. At the start of the fourth week, pictures were taken of the plants.

Data analysis

To determine if there was a significant difference in the survival probability between the controls and the treatments, R and R studio Version 1.1.463 (R Core Team, 2020) were used with the package survival (Therneau & Grambsch, 2000) to perform a Kaplan-Meier survivorship test on the data. The corresponding package survminer (Kassambara *et al.*, 2021) was used to visualize the data.

To determine if there was an effect of the treatment on oviposition by spider mites, a generalized linear mixed effect model (GLMER) analysis was performed. For the oviposition, all eggs were counted and included in the analysis. Egg counting was stopped the day after a mite had died. If the mites had laid eggs in the same 24 hours that they died, it was assumed that the mite died after 12 hours. Thus, hypothetically the mite could have laid twice as many eggs in 24 hours. Subsequently, the number of eggs were multiplied by 2 to correct for this. Before doing the analysis, the error distribution was visually checked for a normal distribution. A random factor of replicate was added in the model to correct for repeated measures. Separate models were made and compared where the variables of treatment and time were defined as interacting, non-interacting factors, or were left out of the model entirely. These GLMERs were subsequently compared using an ANOVA. Results were considered significant if a p-value of <0.05 was obtained by the ANOVA.

To compare the data of the population dynamics of the four treatments, a linear mixed effects model (lme of the package LMER) was used. The effect of the different treatments was investigated through stepwise model simplification by deletion. First, the model was simplified by grouping the two least significant treatment factors, whereafter, the simplified model was compared with the starting model by ANOVA. If a non-significant p-value was obtained ($p>0.05$), the model was successfully simplified without loss of explanatory power and the next two treatment factors were grouped. When a significant p-value was obtained ($p<0.05$), the model could not be simplified by

pooling the treatment factors, indicating a significant difference between these two treatment groups. This stepwise model simplification was done by pooling all treatment factors in a pairwise manner.

Results

The effects of the basic substances on the mortality of *T. urticae*

Spraying spider mites with cow milk induced significant mortality compared to the spider mites sprayed with water (Figure 3A., Kaplan-Meier survivorship test, $Chi^2 = 10.8$, d.f. = 1, $p = 0.001$). The cow milk seemed to glue the mites to the leaves so they often could not move or feed.
Spider mites sprayed with chitosan hydrochloride did not show a significantly different mortality than spider mites sprayed with water (Figure 3B., Kaplan-Meier survivorship test, $Chi^2 = 3.8$, d.f. = 1, $p = 0.051$). Lastly, spraying spider mites with stinging nettle extract showed no difference in mortality compared to spider mites sprayed with water (Figure 3C., Kaplan-Meier survivorship test, $Chi^2 = 0.2$, d.f. = 1, $p = 0.7$). Spider mites sprayed with stinging nettle extract were observed to get stuck to the leaf, often more than 24h after spraying. It was observed that some mites got stuck to the leaf via an egg that was partly inside their bodies. This meant that the mites could not move or oviposit. Therefore, the same survival analysis was done comparing alive and well mites with mites that were either stuck or dead (instead of comparing alive mites with only dead mites). It was found that the spider mites sprayed with stinging nettle extract did not significantly differ from the spider mites sprayed with water (Kaplan-Meier survivorship test, $Chi^2 = 1.2$, d.f. = 1, $p = 0.3$).

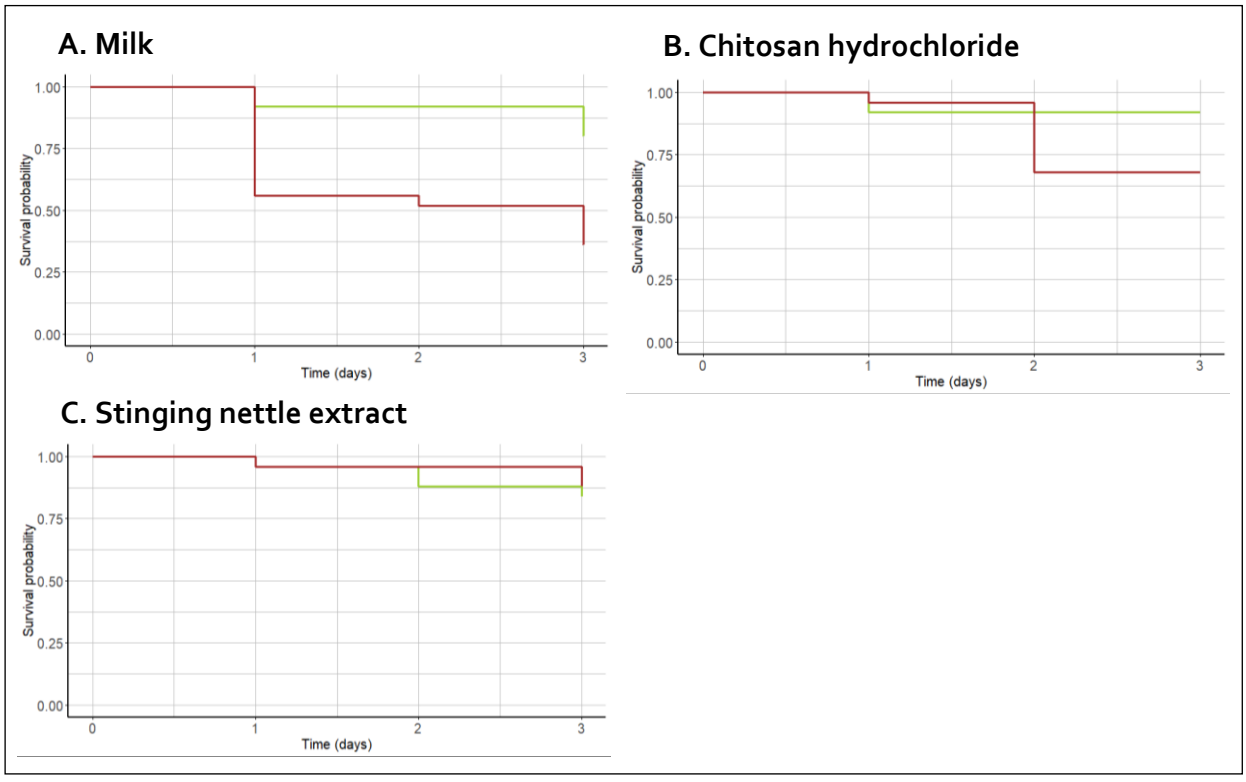


Figure 3. The effects of three basic substances on the survival probability of *T. urticae*.
The effects of cow milk (A), chitosan hydrochloride (B) and stinging nettle extract (C) on the survival probability of *T. urticae* over time. The control (spraying with water) is represented by the light green line and the different treatments are represented by the red line (n=25).

The effects of the basic substances on the oviposition of *T. urticae*

The oviposition of the spider mites during the survival experiments was also monitored. Treating spider mites with cow milk resulted in significantly lower oviposition by mites surviving the treatment than by mites that were sprayed with water (Figure 4A., GLMER, $Chi^2 = 53.38$, d.f. = 3, $p < 0.0001$). Spraying the mites with chitosan hydrochloride had no different effect on the average oviposition than spraying with water (Figure 4B., GLMER, $Chi^2 = 0.42$, d.f. = 1, $p = 0.517$). Lastly, the spider mites treated with stinging nettle extract did not differ in oviposition with spider mites sprayed with water (Figure 4C., GLMER, $Chi^2 = 0.48$, d.f. = 1, $p = 0.48$).

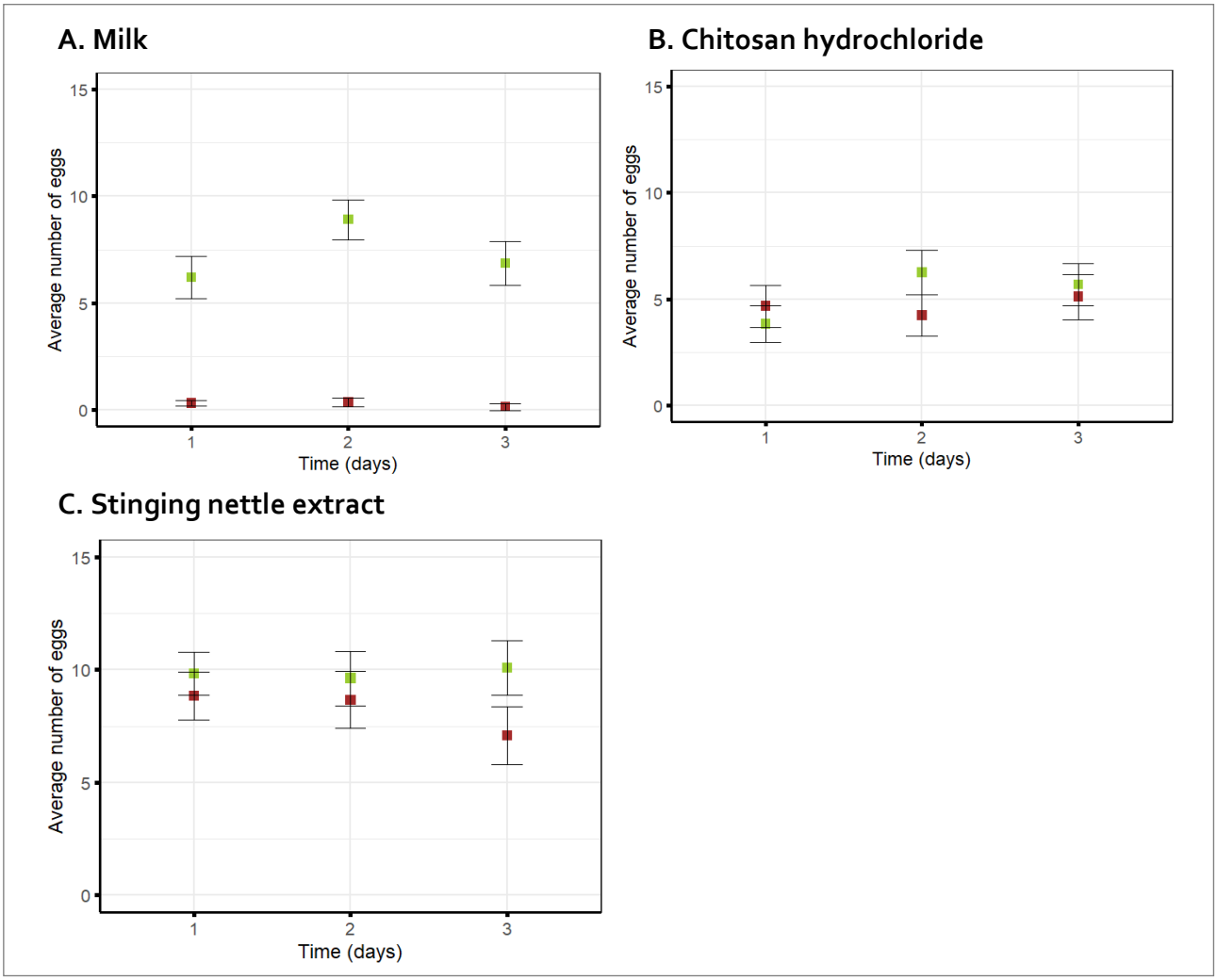


Figure 4. The effects of three basic substances on the oviposition of *T. urticae*.
The effects of cow milk (A), chitosan hydrochloride (B) and stinging nettle extract (C) on the oviposition of *T. urticae* over time. The oviposition is displayed as the average number of eggs that has been laid over time. The control (spraying with water) is represented by the light green squares and the different treatments are represented by the red squares. The whiskers show the standard error.

The effects of the basic substances on the mortality of *P. persimilis*

Spraying predatory mites with cow milk induced significant mortality compared to spraying with water (Figure 5A., Kaplan-Meier survivorship test, $Chi^2 = 5.4$, d.f. = 1, p = 0.02). The cow milk seemed to also glue some of the predatory mites to the leaves so they could not move or feed. After treating the predatory mites with chitosan hydrochloride or water, there was no difference between the two treatments since there was no mortality at all (Figure 5B.). Lastly, after spraying the predatory mites with stinging nettle extract or water, there was no mortality at all of the predatory mites (Figure 5C.).

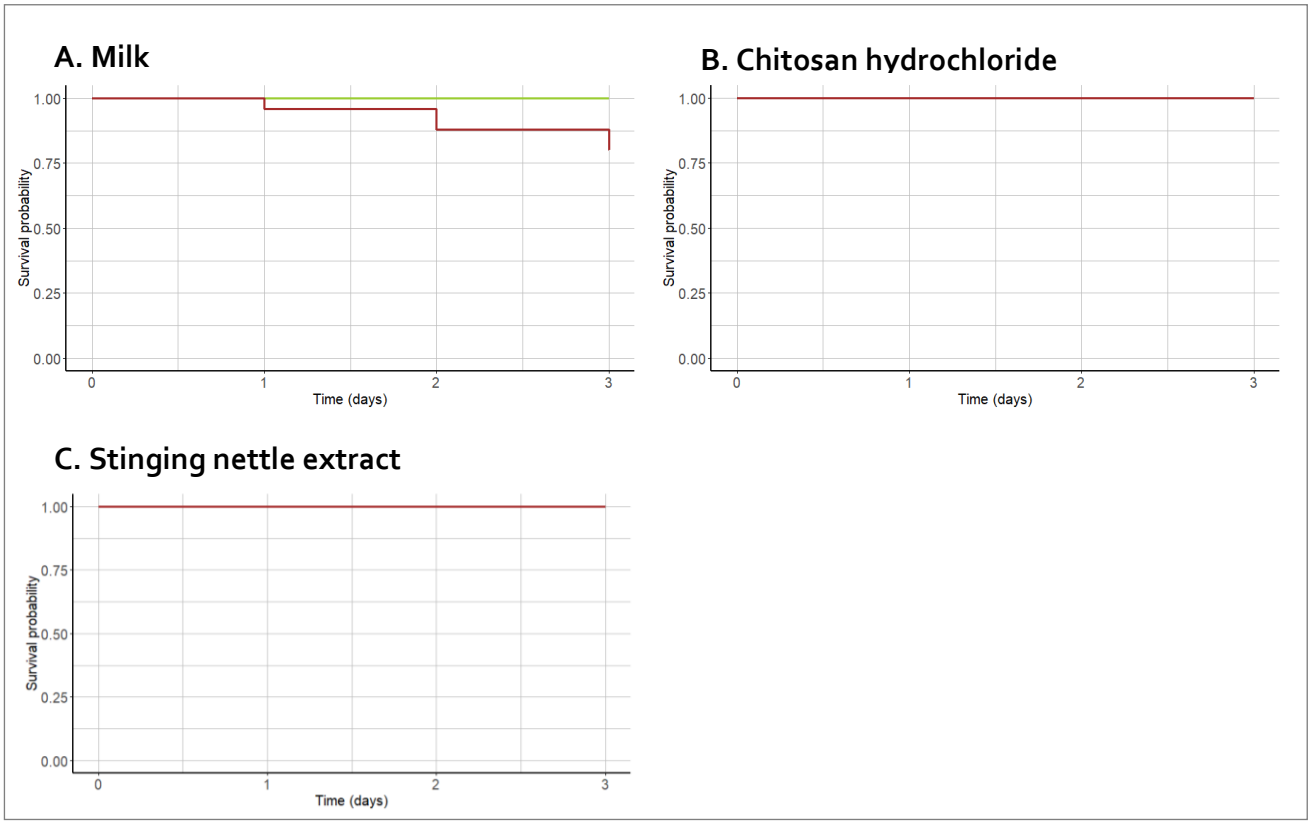


Figure 5. The effects of three basic substances on the survival probability of *P. persimilis*.
The effects of cow milk (A), chitosan hydrochloride (B) and stinging nettle extract (C) on the survival probability of *P. persimilis* over time. The control (spraying with water) is represented by the light green line and the different treatments are represented by the red line (n=25).

Effect of cow milk on population dynamics of *T. urticae* and *P. persimilis*

There was no significant difference in the number of spider mites found between the two treatments that were sprayed with water, where one treatment also contained predators and the other one did not (Figure 6A., both dark green lines, LMER, Likelihood ratio = 5.31, d.f. = 14; 11, p = 0.15). There was a significant difference found in the number of spider mites between the two treatments that were sprayed with cow milk, where one treatment also contained predators and the other one did not (Figure 6A., both light pink lines, LMER, Likelihood ratio = 17.8, d.f. = 11; 8, p = 0.0005). There was a significant difference found in the number of spider mites between the water spray treatments and the milk treatment that also contained predators (Figure 6A., the dark green lines compared to the light pink line with dots, LMER, Likelihood ratio = 11.2, d.f. = 11; 8, p = 0.0106).

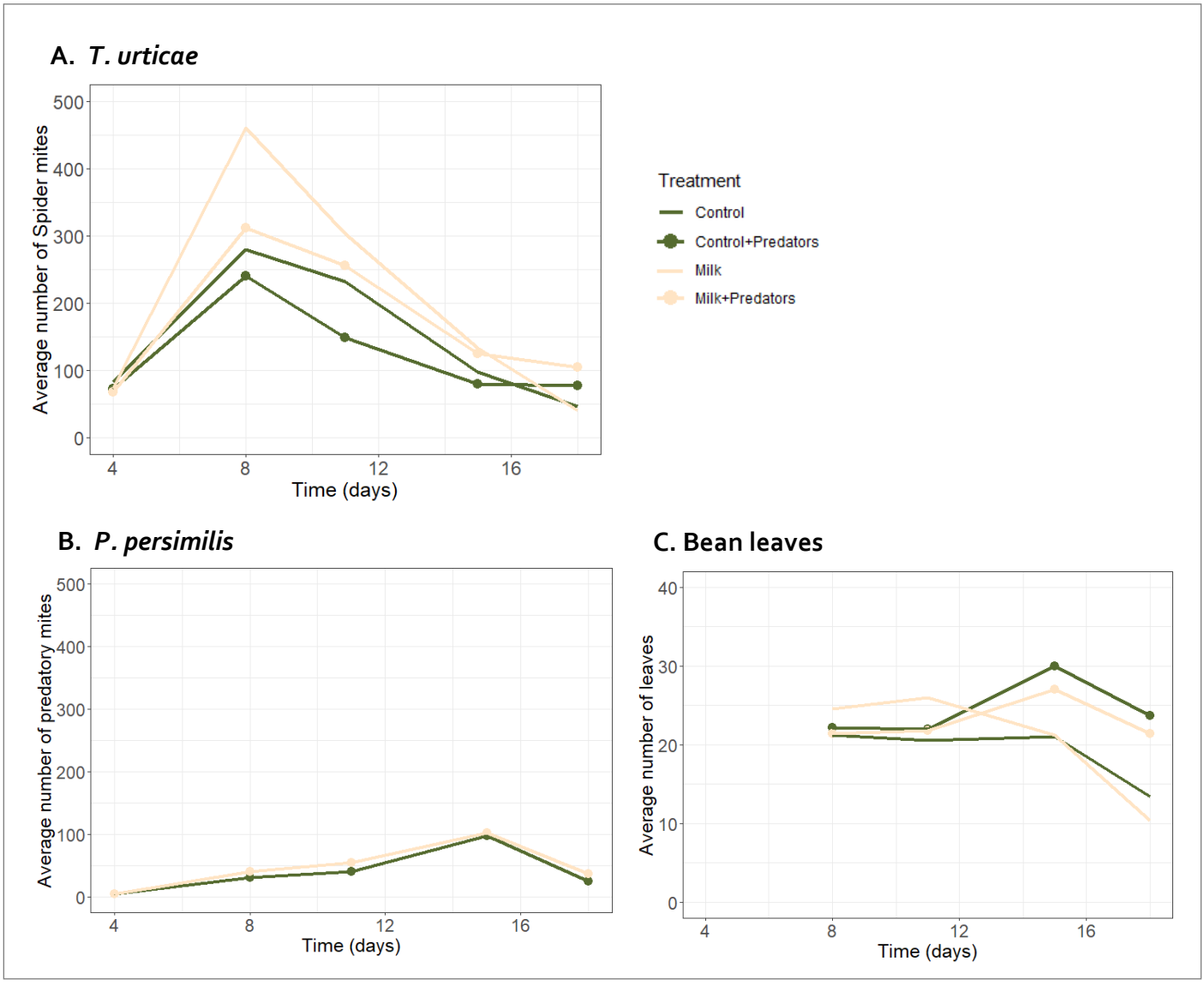


Figure 6. The population dynamics of *T. urticae* and *P. persimilis* over time treated with milk.
The effects of predators and milk or water on the dynamics of spider mites on intact bean plants (A), the effect of cow milk on predators (B) and on the size of the plants (numbers of leaves C). The dark green colours represent the control (spraying with water) and the light pink represents the cow milk treatment. The dots in the lines are the groups that include predators. SD and SE can be found in the Supplementary Materials. Pictures of the plants at day 22 can also be found in the Supplementary Materials.

There was also a significant difference in the number of spider mites between the water spray treatments and the milk treatment without predators (Figure 6A., the dark green lines compared to the light pink line, LMER, Likelihood ratio = 15.1, d.f. = 11; 8, p = 0.0018).

There was no significant difference in the number of predators between the treatments sprayed with water and the treatments sprayed with cow milk (Figure 6B., LMER, Likelihood ratio = 0.46, d.f. = 8; 7, p = 0.50).

There was no significant difference in the average number of leaves per plant between both treatments that included predatory mites (Figure 6C., two lines with dot, LMER, Likelihood ratio = 1.27, d.f. = 10; 8, p =0.530). There was also no significant difference in the average number of leaves per plant between both treatments that had no predatory mites (Figure 6C., two lines without dot, LMER, Likelihood ratio = 4.26, d.f. = 8; 6, p = 0.119). All the other treatments were significantly different from each other (Table 1., LMER)

Table 1. P-value table of pairwise model comparison for average number of leaves per treatment. P-values indicate differences between models where factor 1 and factor 2 were pooled and compared with a null model using LMER.

Factor 1	Factor 2	Likelihood ratio	d.f.	P-value
Control	Milk	4.26	8, 6	0.119
Control with predators	Milk with predators	1.27	10, 8	0.530
Control	Control with predators	11.11	10, 8	0.0039
Control	Milk with predators	6.80	10, 8	0.033
Milk	Milk with predators	13.58	10, 8	0.0011
Milk	Control with predators	19.28	8, 6	0.0001

Discussion

I aimed to investigate the effects of cow milk, chitosan hydrochloride and stinging nettle extract on the survival of *T. urticae* and *P. persimilis*, and on the dynamics of populations of this pest and predator. Full cow milk was found to negatively affect survival of *T. urticae* and *P. persimilis*. In contrast, the concentrations of chitosan hydrochloride and stinging nettle extract used had no significant effect on both species and were therefore not tested further. An experiment on population dynamics of *T. urticae* and *P. persimilis* on small bean plants showed that plants treated with cow milk had a significantly higher density of spider mites, but that milk had no effect on the density of predatory mites. Plant performance, measured as the number of leaves per plant was also not affected by the milk treatment, however, plants with predators kept significantly more of their leaves than the plants without predators.

Stinging nettle extract and chitosan hydrochloride had no effect in the tested concentrations

The fact that stinging nettle extract and chitosan hydrochloride had no effect on the mortality of *T. urticae* or *P. persimilis* suggests that the population dynamics of these two species would not be influenced by the use of these basic substances in the tested concentrations. Looking at Equation 1 and 2, this means that the natural growth of prey ($\alpha x(t)$) and the death rate of the predator ($-\gamma y(t)$) are not changed by the treatment. The predation rate of the predators (β) and their growth (δ) would probably remain unchanged as well, meaning that the pest density of the prey as well as the density of the predator will stay the same.

Contrary to our findings, Dąbrowski and Seredyńska (2007) showed strong effects of stinging nettle extract against spider mites, but they used higher concentrations. The concentrations used in our study were based on concentrations that are currently used by growers to control aphids. It is most likely that these concentrations were too low to directly affect the

mortality of the mites. This means that current uses of stinging nettle extract against fungi or aphids does not unintentionally affect spider mites or *P. persimilis* populations. However, some spider mites were found attached to the leaf through an egg, unable to move or lay more eggs. This suggests that higher concentrations of stinging nettle extract could affect more mites in this way and make them unable to lay more eggs.

Chitosan hydrochloride was found to act as elicitor and prime plants by activating for example the jasmonic acid pathway (Doares *et al.*, 1995). This is found to be highly effective against various fungi species such as *Botrytis cinerea* (Peian *et al.*, 2021). Surprisingly, we found that the same concentrations that prime the plants against fungal growth seem to have no effect on the mortality or oviposition of *T. urticae* and *P. persimilis*. However, with our experimental setup, the plants were not necessarily primed by chitosan since the treatment was applied after the introduction of the mites. This would result in less time for the plant to properly prepare for effective defence against the spider mites. Although chitosan hydrochloride could negatively affect mites indirectly through the activation of plant defence mechanisms, it did not have a direct effect on the mites.

Direct milk spraying acts like glue but affects both mite species

Cow milk, and especially full milk, is high in sugars and fat, both of which cause stickiness. This property of milk caused spider mites and predatory mites that were directly sprayed with milk to be glued to plant leaves. This caused a discernible increase in mortality rates of both the prey and predatory mites. The fact that both mite species were affected by the milk treatment suggests that it might not result in overall decreases in pest densities. Looking at Equation 1 and 2, in this scenario prey density (α) will decrease, which will not affect the pest equilibrium ($x^* = \gamma/\delta$). Additionally, the predator mortality (γ) increases with the use of milk, causing x^* to increase meaning that pest density will increase as well. This means that, in the end, milk will eventually have a negative effect on crop protection and the number of spider mites will increase. The study by Janssen and Van Rijn (2021) supports this by showing that the use of pesticides in the presence of natural enemies is ineffective. Even though milk is not a pesticide it will have the same outcomes on the prey population since it does not leave the predator unharmed.

Similar to our study, Gupta *et al.* (2015) also found that direct treatment with cow milk increased the mortality of spider mites. However, they only tested the direct effect of milk on spider mites. Mites are known to occur at the underside of leaves or in different plant structures to be able to protect themselves. Thus, the mites will not be glued to the leaf and can continue reproducing. In practice it is not possible to directly spray every single mite with cow milk. This shows that additionally to direct survival experiments, population dynamic experiments should be done.

Cow milk has a negative effect on spider mite control

The population dynamics experiment on small plants indicates that indeed larger-scale spraying does not work. In fact, spider mites were even found to do better when treated with milk. The addition of milk caused the natural growth of the prey ($\alpha x(t)$) to increase. This might be due to a stress response when the spider mites are treated with milk, where they suddenly lay more eggs than the control. This is also known as hormesis and occurs when an organism experiences low amounts of stress due to treatment and can initiate compensatory behaviour, like laying more eggs (Calabrese, 2008; Calabrese & Baldwin, 2002). In the survival experiments, milk was sprayed directly on the spider mites, however, in the population dynamics experiment milk was sprayed on top of the leaves and spider mites have mostly hidden at the underside of the leaves. The spider mites might still have experienced stress from the milk treatment, but they may not have died, like in the survival experiment. Therefore, the spider mites could have laid more eggs due to this small amount of stress when treated with milk compared to the control group. Moreover, with the treatment of milk the natural growth of the prey population in the presence of predatory mites also increased compared to the control treatment with and without predators. This shows that the prey population

will increase in the presence of full cow milk. The death rate of the predator ($-\gamma y(t)$) did not seem to change after treatment with milk in the population dynamic experiment, this might also be due to the fleeing of the predators to the bottom side of the leaves. Contrastingly, the population experiments also showed the positive effect predators can have on the survival of the plants. This is a great example of biological pest control and that it is also a very effective and reliable alternative to spraying (bio)pesticides (Van Lenteren *et al.*, 2020).

Cow milk does not control spider mites, and the experiments showed that it does influence the spider mite population growth. Since milk is also effective against different fungi (Bettiol, 1999; Ferrandino & Smith, 2007), the effect on spider mites populations should be kept in mind when treating crops with milk to inhibit fungal growth. When spider mites and natural enemies are present, treating the plants with milk could lead to population growth of the spider mites, which, in turn, will affect plant survival.

Conclusion

Even though the direct spraying of cow milk did negatively affect the survival and oviposition of individual spider mites, this effect was unable to be replicated on spider mite populations on intact bean plants. Predatory mites were also negatively affected by direct milk treatment and consequently, treatments with milk can result in higher spider mite densities. Cow milk, chitosan hydrochloride and stinging nettle extract were not able to control spider mites, but there are still other basic substances that can potentially be useful in crop protection. More research can be done to explore the potential of other basic substances to protect crops, but it should always consider the effects of these substances on natural enemies and on population dynamics.

References

Abdel-Baky, N. F., & Abdel-Salam, A. H. (2003). Natural incidence of Cladosporium spp. as a bio-control agent against whiteflies and aphids in Egypt. *Journal of Applied Entomology*, *127*(4), 228–235. https://doi.org/10.1046/j.1439-0418.2003.00662.x

Attia, S., Grissa, K. L., Lognay, G., Bitume, E., Hance, T., & Mailleux, A. C. (2013). A review of the major biological approaches to control the worldwide pest Tetranychus urticae (Acari: Tetranychidae) with special reference to natural pesticides: Biological approaches to control Tetranychus urticae. *Journal of Pest Science*, *86*(3), 361–386. https://doi.org/10.1007/s10340-013-0503-0

Bass, C., & Jones, C. M. (2018). Editorial overview: Pests and resistance: Resistance to pesticides in arthropod crop pests and disease vectors: mechanisms, models and tools. *Current Opinion in Insect Science*, *27*, iv–vii. https://doi.org/10.1016/j.cois.2018.04.009

Bettiol, W. (1999). Effectiveness of cow's milk against zucchini squash powdery mildew (Sphaerotheca fuliginea) in greenhouse conditions. *Crop Protection*, *18*(8), 489–492. https://doi.org/10.1016/S0261-2194(99)00046-0

Calabrese, E. J. (2008). Hormesis: Why it is important to toxicology and toxicologists. *Environmental Toxicology and Chemistry*, *27*(7), 1451–1474.

Calabrese, E. J., & Baldwin, L. A. (2002). Defining hormesis. *Human and Experimental Toxicology*, *21*(2), 91–97. https://doi.org/10.1191/0960327102ht217oa

Copping, L. G., & Menn, J. J. (2000). Biopesticides: A review of their action, applications and efficacy. *Pest Management Science*, *56*(8), 651–676. https://doi.org/10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U

Dąbrowski, Z. T., & Seredyńska, U. (2007). Characterisation of the two-spotted spider mite (Tetranychus urticae KOCH, Acari: Tetranychidae) response to aqueous extracts from selected plant species. *Journal of Plant Protection Research*, *47*(2), 113–124.

Doares, S. H., Syrovets, T., Weiler, E. W., & Ryan, C. A. (1995). Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(10), 4095–4098. https://doi.org/10.1073/pnas.92.10.4095

Eilenberg, J., Enkegaard, A., Vestergaard, S., & Jensen, B. (2000). Biocontrol of pests on plant crops in Denmark: Present status and future potential. *Biocontrol Science and Technology*, *10*(6), 703–716. https://doi.org/10.1080/09583150020011681

Eilenberg, J., Hajek, A., & Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl*, *46*(4), 387–400. https://doi.org/10.1023/A:1014193329979

Ferrandino, F. J., & Smith, V. L. (2007). The effect of milk-based foliar sprays on yield components of field pumpkins with powdery mildew. *Crop Protection*, *26*(4), 657–663. https://doi.org/10.1016/j.cropro.2006.06.003

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Köhl, J., Marrone, P., Morin, L., & Stewart, A. (2012). Have biopesticides come of age? *Trends in Biotechnology*, *30*(5), 250–258. https://doi.org/10.1016/j.tibtech.2012.01.003

Goy, R. C., De Britto, D., & Assis, O. B. G. (2009). A review of the antimicrobial activity of chitosan. *Polimeros*, *19*(3), 241–247. https://doi.org/10.1590/S0104-14282009000300013

Guedes, R. N. C., Smagghe, G., Stark, J. D., & Desneux, N. (2016). Pesticide-Induced Stress in Arthropod Pests for Optimized Integrated Pest Management Programs. *Annual Review of Entomology*, *61*, 43–62. https://doi.org/10.1146/annurev-ento-010715-023646

Gupta, G., Kaur, G., & Kumar, N. R. (2015). Effect of cow milk on sucking pests and insect predators on rose. *Journal of Eco-Friendly Agriculture*, *10*(2), 145–149.

Gupta, S., & Dikshit, A. K. (2010). Biopesticides: An ecofriendly approach for pest control. *Journal of Biopesticides*, *3*(1 SPEC.ISSUE), 186–188.

Hajek, A. E., & Delalibera, I. (2010). Fungal pathogens as classical biological control agents against arthropods. *BioControl*, *55*(1), 147–158. https://doi.org/10.1007/s10526-009-9253-6

Hardin, M. R., Benrey, B., Coll, M., Lamp, W. O., Roderick, G. K., & Barbosa, P. (1995). Arthropod pest resurgence: an overview of potential mechanisms. *Crop Protection*, *14*(1), 3–18. https://doi.org/10.1016/0261-2194(95)91106-P

Janssen, A., & van Rijn, P. C. J. (2021). Pesticides do not significantly reduce arthropod pest densities in the presence of natural enemies. *Ecology Letters*, *00*, 1–15. https://doi.org/10.1111/ele.13819

Kassambara, A., Kosinski, M., & Biecek, P. (2021). *Survminer: Drawing Survival Curves using "ggplot2."* R Package Version 0.4.9. https://cran.r-project.org/package=survminer

Landis, D. A., Wratten, S. D., & Gurr, G. M. (2000). Habitat Management to Conserve Natural Enemies of Arthropod Pests in Agriculture. *Annual Review of Entomology*, *45*(1), 175–201. https://doi.org/10.1146/annurev.ento.45.1.175

Lotka, A. J. (1910). Contribution to the Theory of Periodic Reactions. *The Journal of Physical Chemistry*, *14*(3), 271–274. https://doi.org/10.1021/j150111a004

Maaroufi, L., Sazzad Hossain, M., Tahri, W., & Landoulsi, A. (2017). *Journal of Medicinal Plants Research New insights of Nettle (Urtica urens): Antioxidant and antimicrobial activities*. *11*(4), 73–86. https://doi.org/10.5897/JMPR2016.6278

Malik, N. A. A., Kumar, I. S., & Nadarajah, K. (2020). Elicitor and receptor molecules: Orchestrators of plant defense and immunity. *International Journal of Molecular Sciences*, *21*(3). https://doi.org/10.3390/ijms21030963

OECD. (2021). *OECD Guidelines for the Testing of Chemicals*. Accessed 26 Jan 2021. https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals_72d77764-en

Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Science*, *144*(1), 31–43. https://doi.org/10.1017/S0021859605005708

Oerke, E. C., & Dehne, H. W. (2004). Safeguarding production - Losses in major crops and the role of

crop protection. *Crop Protection*, *23*(4), 275–285. https://doi.org/10.1016/j.cropro.2003.10.001

Pavela, R., & Benelli, G. (2016). Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. *Trends in Plant Science*, *21*(12), 1000–1007. https://doi.org/10.1016/j.tplants.2016.10.005

Peian, Z., Haifeng, J., Peijie, G., Sadeghnezhad, E., Qianqian, P., Tianyu, D., Teng, L., Huanchun, J., & Jinggui, F. (2021). Chitosan induces jasmonic acid production leading to resistance of ripened fruit against Botrytis cinerea infection. *Food Chemistry*, *337*(August 2020), 127772. https://doi.org/10.1016/j.foodchem.2020.127772

Pickett, C. H., & Gilstrap, F. E. (1986). Inoculative Releases of Phytoseiids (Acari) for the Biological Control of Spider Mites (Acari: Tetranychidae) in Corn. *Environmental Entomology*, *15*(4), 790–794. https://doi.org/10.1093/ee/15.4.790

Pusztahelyi, T. (2018). Chitin and chitin-related compounds in plant–fungal interactions. *Mycology*, *9*(3), 189–201. https://doi.org/10.1080/21501203.2018.1473299

R Core Team. (2020). R: A language and environment for statistical computing. In *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*.

Revynthi, A. M., Egas, M., Janssen, A., & Sabelis, M. W. (2018). Prey exploitation and dispersal strategies vary among natural populations of a predatory mite. *Ecology and Evolution*, *8*(21), 10384–10394. https://doi.org/10.1002/ece3.4446

Silva, V., Mol, H. G. J., Zomer, P., Tienstra, M., Ritsema, C. J., & Geissen, V. (2019). Pesticide residues in European agricultural soils – A hidden reality unfolded. *Science of the Total Environment*, *653*, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441

Srinivasa, P. C., & Tharanathan, R. N. (2007). Chitin/chitosan - Safe, ecofriendly packaging materials with multiple potential uses. *Food Reviews International*, *23*(1), 53–72. https://doi.org/10.1080/87559120600998163

Tapwal, A., Nisha, Garg, S., Gautam, N., & Kumar, R. (2011). In Vitro antifungal potency of plant extracts against five phytopathogens. *Brazilian Archives of Biology and Technology*, *54*(6), 1093–1098. https://doi.org/10.1590/S1516-89132011000600003

Therneau, T. M., & Grambsch, P. M. (2000). *Modeling Survival Data: Extending the Cox Model*. Springer.

Topping, C. J., Aldrich, A., & Berny, P. (2020). Overhaul environmental risk assessment for pesticides. *Science*, *367*(6476), 360–363. https://doi.org/10.1126/science.aay1144

Van Leeuwen, T., Stillatus, V., & Tirry, L. (2004). Genetic analysis and cross-resistance spectrum of a laboratory-selected chlorfenapyr resistant strain of two-spotted spider mite (Acari: Tetranychidae). *Experimental and Applied Acarology*, *32*(4), 249–261. https://doi.org/10.1023/B:APPA.0000023240.01937.6d

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., & Tirry, L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite Tetranychus urticae and other important Acari: A review. *Insect Biochemistry and Molecular Biology*, *40*(8), 563–572. https://doi.org/10.1016/j.ibmb.2010.05.008

Van Lenteren, J. C., Alomar, O., Ravensberg, W. J., & Urbaneja, A. (2020). Integrated Pest and Disease Management in Greenhouse Crops. In *Integrated Pest and Disease Management in Greenhouse Crops*. https://doi.org/10.1007/978-3-030-22304-5

van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J., & Urbaneja, A. (2018). Biological control using invertebrates and microorganisms: plenty of new opportunities. *BioControl*, *63*(1), 39–59. https://doi.org/10.1007/s10526-017-9801-4

Whitney, P. J., & Gibbs, G. (2006). The common stinging nettle: Resource or risk? *Biologist*, *53*(4), 178–182.

Yan, C., & Xie, D. (2015). Jasmonate in plant defence: Sentinel or double agent? *Plant Biotechnology Journal*, *13*(9), 1233–1240. https://doi.org/10.1111/pbi.12417

Supplementary materials

Material 1. Standard deviation (SD) and standard error (SE) from the population dynamics experiment (Figure 6).

A. Spider mites

Treatment	Time	SD	SE
Control	4	8.792042	3.931921
Control	8	109.8704	49.13553
Control	11	86.37303	38.62719
Control	15	28.20106	12.6119
Control	18	22.36515	10.002
Control+Pred	4	8.258329	3.693237
Control+Pred	8	39.4487	17.642
Control+Pred	11	22.15664	11.07832
Control+Pred	15	23.43786	13.53186
Control+Pred	18	16.64332	9.609024
Milk	4	11.25611	5.033885
Milk	8	128.8379	57.61805
Milk	11	88.63521	39.63887
Milk	15	48.1643	21.53973
Milk	18	23.04778	10.30728
Milk+Pred	4	1.949359	0.87178
Milk+Pred	8	52.23696	37.40642
Milk+Pred	11	142.838	13.53622
Milk+Pred	15	25.64761	11.56623
Milk+Pred	18	40.86808	13.05118

B. Predators

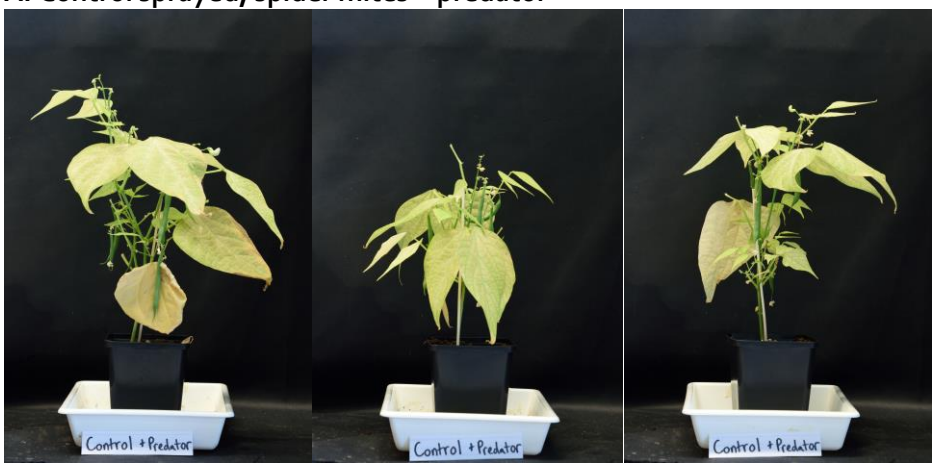
Treatment	Time	SD	SE
Control	4	0.547723	0.244949
Control	8	6.534524	2.922328
Control	11	10.87428	5.437141
Control	15	14.57166	8.412953
Control	18	10.14889	5.859465
Milk	4	0.547723	0.244949
Milk	8	7.648529	3.652396
Milk	11	10.82589	7.416198
Milk	15	22.11334	7.125853
Milk	18	27.51908	3.844188

C. Leaves

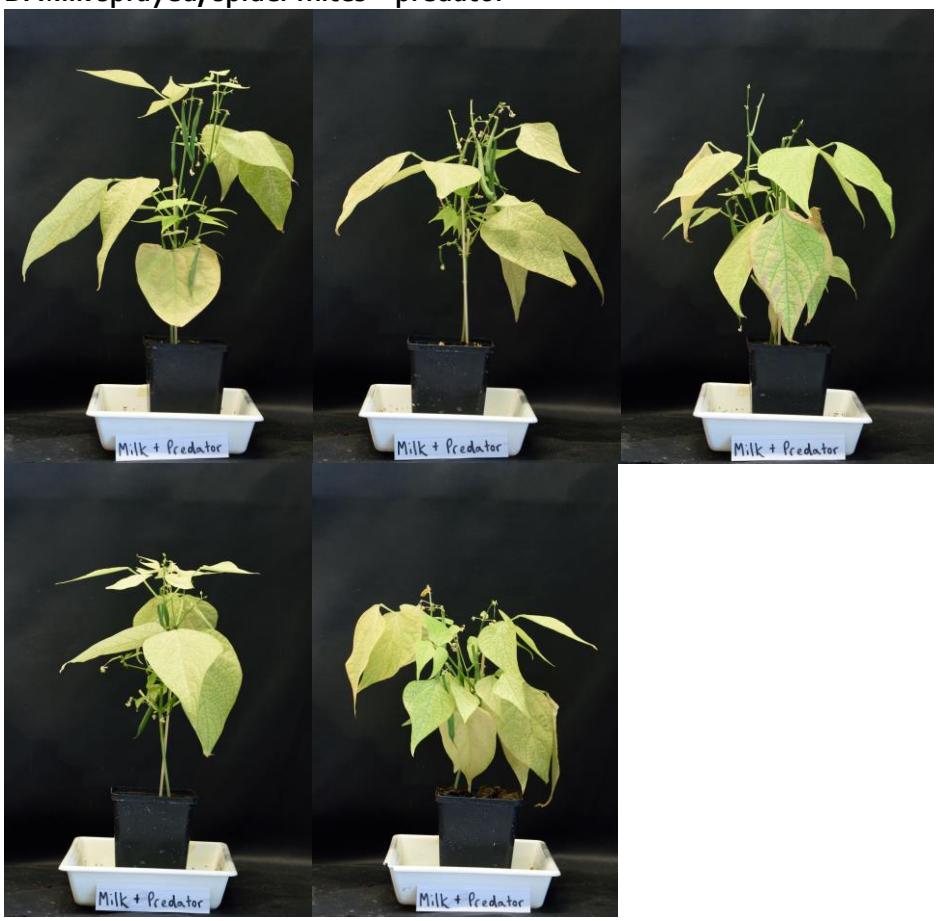
Treatment	Time	SD	SE
Control	4	NA	NA
Control	8	3.898718	1.74356
Control	11	3.286335	1.469694
Control	15	8.3666	3.741657
Control	18	4.615192	2.063977
Control+Pred	4	NA	NA
Control+Pred	8	3.49285	1.56205
Control+Pred	11	1.414214	0.707107
Control+Pred	15	4.582576	2.645751
Control+Pred	18	5.507571	3.179797
Milk	4	NA	NA
Milk	8	4.615192	2.063977
Milk	11	2.12132	0.948683
Milk	15	6.83374	3.056141
Milk	18	3.04959	1.363818
Milk+Pred	4	NA	NA
Milk+Pred	8	4.393177	1.74356
Milk+Pred	11	2.683282	0.866025
Milk+Pred	15	4.123106	3.605551
Milk+Pred	18	5.899152	3.666667

Material 2. At the end of the population dynamic experiment at day 22, pictures were taken of all the plants. Treatment A misses 2 replicates due to contamination with thrips.

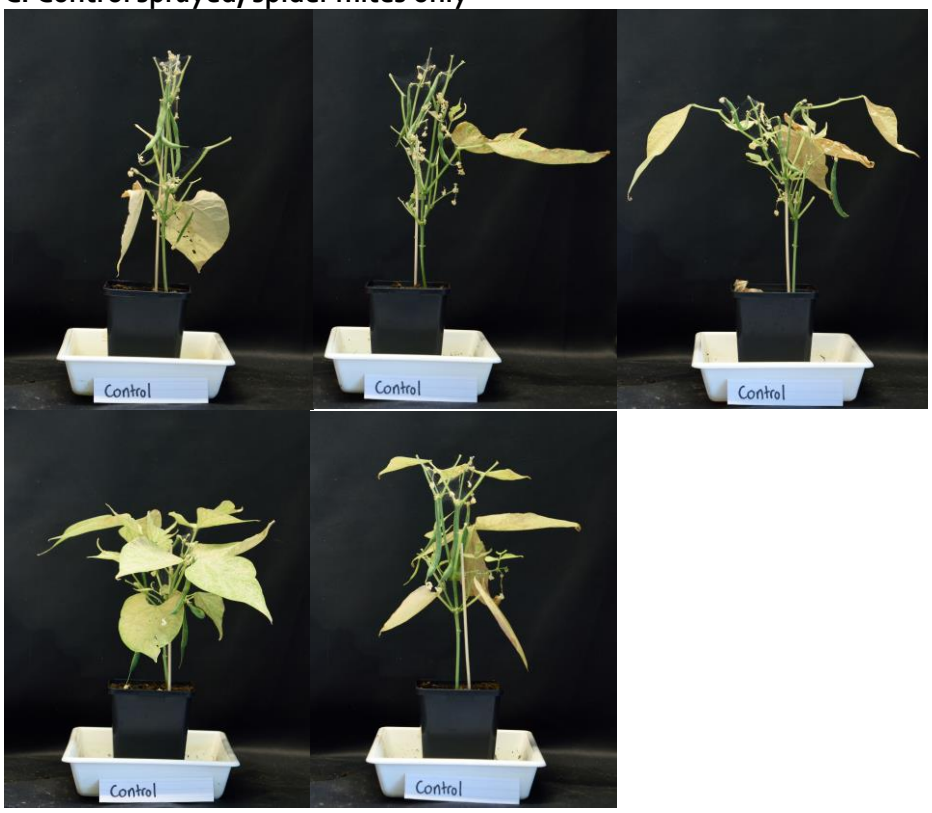
A. Control sprayed, spider mites + predator



B. Milk sprayed, spider mites + predator



C. Control sprayed, spider mites only



D. Milk sprayed, spider mites only