University of Veterinary Medicine
Department of Food Hygiene

Cocktails or not:

Dissipation of some pesticides from greenhouse tomatoes applied alone or in combination treatment

By

Cecilie Marie Andvord

Supervisor:
Lányi Katalin, PhD
Senior research fellow

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List of Abbreviations and Formulae

7^{th} EAP	- Seventh Environment Action Programme
DDT	- Dichlorodiphenyltrichloroethane
EC	- European Commission
EEA	- European Environmental Agency
EFSA	- European Food Safety Authority
EU	- European Union
FAO	- Food & Agriculture Organisation of the United Nations
LBI	- Lipid Biosynthesis Inhibitor
MS	- Member States
MRL	- Maximum residue levels (of pesticides in or on food and feed of plant and animal origin)
PPP	- Plant Protection Product
QuEChERS	- Quick, Easy, Cheap, Effective, Rugged and Safe
IPPC	- International Plant Protection Convention (commission on Phytosanitary measures – CPM)

Figures and Tables

1. Introduction

With a continuous increase in population, food productivity has never been as vital as it is today. The United Nations Population Fund (UNPF) stated that the world population would reach 10 billion by the year 2050. It is safe to say to meet this growing population; there is a need for streamlining in the production of food and feed. Graziano da Silva, Director-General of Food and Agriculture Organisation (FAO) of the United Nations, wrote that to meet this amount of people, global food production has to be increased by 60 percent by the year 2050 (Graziano da Silva, 2011). However, recent discoveries have shown that there is an increase in stagnating farms, thereby decreasing the window of error regarding food production (FAO, 2006).

There is a race against the clock; for every second, a few thousand people are born, and more food produced is needed. New strategies are continuously being developed to increase yield, but only time will show if it is enough.

An increased population is not the only reason for the scarcity of food. A large number of pests and diseases caused by fungi, insects, and animals frequently threaten crop yield. More than ``70,000 species of pests are known to damage crops´´ grown for agricultural purpose divided into; ``9,000 different insects and mites, 50,000 species of plant pathogens, and 8,000 species of weeds´´ (Pimentel, 2009). Regardless, the question remains; how can we fight the production loss? Some believe the answer is pesticides.

Pesticides are used frequently in the agricultural world, applied to optimize crop production and to protect stored crops. In previous years, the amount of chemicals used for agriculture has increased, causing a new growing problem, pesticide residues.

While agrochemicals are mostly selective of their target species, some portray a health risk. Previous pesticides linked as carcinogenic and toxic towards humans, animals, and environment such as Dichlorodiphenyltrichloroethane (DDT), was removed from the market (NPIC, 1999). Congenital disabilities, cancer, and even death have been associated with certain chemicals, leading to an increase in monitoring programs (See Appendices II).

In this regard, Maximum Residue Levels (MRL) have been applied to all food and feed, attended for animal and human consumption, and are set by law to ensure safety. MRL is the detection of any amount of pesticides. Example: the detection of spirotetramat, the ``parent compound or any of its four metabolites are residues´´ (FAO, 2016). While the MRL is set forth by the European Union, they do not include research on what happens when combining pesticides, which is why this experiment is crucial. Crops are frequently attached simultaneously by different types of pests, which is why a cocktail of pesticides are applied. Cocktails are beneficial financially, time effective, cause less damage to the crops, and have broad-spectrum protection. However, addressing any possible cross effects of active ingredients is essential.

This study aimed to measure the residue concentration of spirotetramat, the active ingredient in Movento insecticide, and metalaxyl-M found in Ridomil Gold MZ 68 WG (from now referred to as Ridomil) fungicide on greenhouse tomatoes. We performed an interaction study to see whether the measured concentration would change when the chemicals were applied in combination versus alone. This experiment was carried out by measuring the concentration right after treatment, 2 days, 4 days, and 8 days post-treatment.

A literature review of the agrochemicals Movento and Ridomil, regarding the history, mode of action, target species, and more; see [*Chapter 2.1*] and onwards, are followed by the experiment.

Residue levels of Movento and Ridomil were measured using greenhouse tomatoes at different days post-treatment. An additional experiment was carried out in terms of residue levels of tomato juice when chemicals were applied alone or combined post heat treatment.

1.1. Objectives

The increased human population occurs at an alarming speed allows for little error when it comes to food production. Pesticides are used to ensure a higher yield by killing target pests. Unfortunately, have many of these chemicals been linked to killing important pollinators and been linked to being toxic for the environment, animals, and humans.

Bees are essential in the pollination of greenhouse tomatoes, which is one of the many reasons this experiment was necessary. Evaluating the residue levels for unspecified interaction between the two chemicals is one of the aims. Done by comparing the different data obtained in the experiment for the chemicals when applied alone versus in combination made this possible.

Goals involved analysing the;

(1) residue levels of greenhouse tomatoes sprayed with Movento (spirotetramat) alone,

(2) residue levels of greenhouse tomatoes sprayed with Ridomil (metalaxyl-M and mancozeb) alone,

(3) residue levels measured when both chemicals were applied simultaneously, observing any change in concentration measured, and

(4) to compare the measured concentrations for residue post-pasteurization of tomato juice, when Movento and Ridomil when applied alone, or in combination.

This experiment was carried out by analysing the detected levels over time, starting the measurements right after application (once the pesticide was dried), on days 2, 4, and 8 days after the first application.

The EU has composed a list over Maximum Residue Levels (MRL) for all chemicals and a withdrawal period (WP) to ensure safe levels found in food and feed. The EU regulations do not incorporate research on pesticides regarding possible dissipation if applied in combination, which is why this interactive study is crucial. Today, applied pesticides are used as a cocktail, rather than individually to save time, despite little is known about inter-chemical dissipation.

Movento has a WP of 4 days, while Ridomil Gold has 7 days after the first application. Nonetheless, measuring of Ridomil occurred on day 8.

The second part of the experiment involves the measured levels, post- heat treatment, of the same chemicals of tomato juice. Spirotetramat and metalaxyl-M were applied alone, and in combination, post pasteurization before measuring the concentration.

Evaluation of application rates, success rates of pest control, and hazards are included, together with hazards towards bees as primary pollinators used in greenhouse tomatoes.

1.2. Hypotheses

1. Agrochemicals are often applied together as a cocktail. It is hypothesised that residue levels measured from the pesticides Movento, and Ridomil when applied alone versus together, changes the concentration measured. The belief is when applied in combination, the measured values of spirotetramat and metalaxyl-M should be lower, due to dissipation, then when applied alone.
2. Tomato juice is consumed after pasteurization. The prediction is that heat treatment influences the levels of chemicals measured, and we will see a decrease in concentration. More so when applied together, versus when applied alone.

2. Literature review

Agrochemicals have become one of the most favourable strategies used to ensure higher crop yield. However, more primitive strategies are traced back to before of the agriculturalization, roughly 10, 000 years ago (Pimentel, 2009). An example is the Pyrethrum daisies, *Chrysanthemum cinerariaefolium,* giving raise to pyrethrum and used as an insecticide (Unsworth, 2010).

Today, there are more than 100 different types of insecticide categories registered. Though this number is continuously increasing as new groups are discovered (Ware & Whitacre, 2004). Despite this, the consideration is pesticides fall into one of 4 main categories.

Pesticides are today divided into one of the four main categories as followed:

1) **Fungicides** – as the name implies, used to kill undesirable fungi on crops,
2) **Insecticides** – used to control unwanted insects. Either to decrease possible diseases insects might spread or to kill insects that would otherwise eat and destroy the crop.
3) **Herbicides** – often referred to as a weed killer, are used to destroy unwanted plants, and the last group,
4) **other** pesticides, include Rodenticides, used to kill unwanted rodents (WHO, 2008)

Today the majority of crops are lost due to weeds counting for 43 percent of a total loss, while animal pests account for 18 percent (Moore, Robson, & Trinci, 2019). Microbial diseases count for 16 percent of the loss, whereas fungi cause 80 percent of the microbial diseases. Combined, it makes a total of 68 percent production loss regarding different crops worldwide. ``More than 40 billion US Dollars was spent for the 3 million metric tons of pesticides worldwide´´, Yet between ``35 and 42 % of potential crops´´ are lost yearly (Pimentel, 2009). Could this be due to a change in the dissipation of chemicals when applied together?

2.1. Insecticide: Movento (Bayer)

Bayer has been and still is one of the largest pharmaceutical companies. In 2002 Bayer CropScience was formed. Now also exceeding in agrochemical inventions, Bayer has produced multiple chemicals for this sole purpose.

Movento, with spirotetramat as the parent compound, is a keto-enol insecticide produced first in 1990, by Bayer.
Spirotetramat has the molecular formula of $C_{21}H_{27}NO_3$.

The color of the powder is either light beige when pure, or white when manufactured.

It has a half-life of 9 days and 8 hours at a temperature of 25 degrees Celsius, forming spirotetramat-enol as a metabolite when in water (FAO, 2011).

Melting Point (°C)	142
pH	6.3
Density D4 20	1.22
Solubility in water at 20^{o} C pH 4	33.5 mg/L
pH 7	29.9 mg/L
pH 9	19.1 mg/L

(EPA, 2008)

The most common metabolites of spirotetramat are; BYI08330-ketohydroxy, BYI08330-monohydroxy, and BYI08330 enol-glucoside (see Figure 3) (Euopean Commission, 2016).

The active ingredient spirotetramat was 100g/L; CAS No. 203313-25-1. As previously mentioned, the MRL for Movento is 2 mg/kg (2000 µg/kg).

Movento is an insecticide used for the prevention of aphids, glasshouse whiteflies, spider mites, and thrips.

Plants should not be treated with Movento more than two times, and a minimum of seven days between the applications.

The recommended dose for Movento is 0.75 L/ha, or 800 to 1500 L/ha when sprayed.

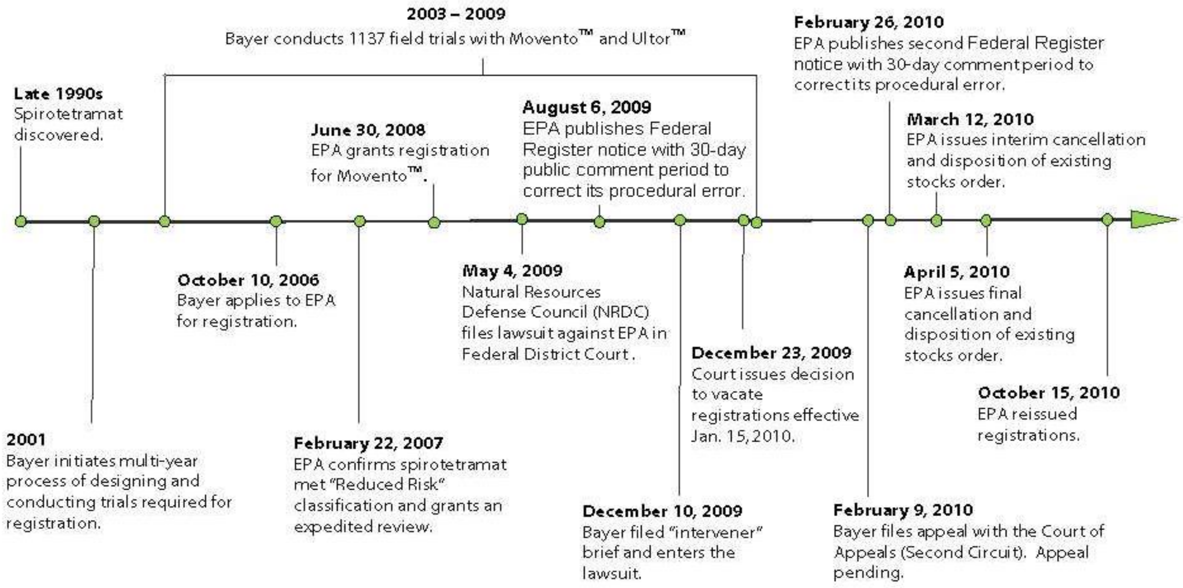
The International Organization for Standardization (ISO) approved spirotetramat as the common name, but the road to the stores has been long.

First discovered in the late 1990s, Movento took many decades before it's active substance was finally approved on May 1st 2014, by the European Union (Euopean Commission, 2016).

Approval happened following Regulation (EC) No 1107/2009 by the standing committee, including FCAH and more.

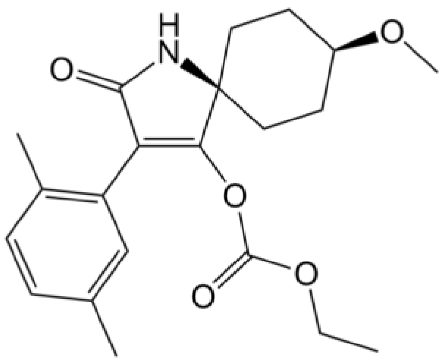
The first-time spirotetramat was approved, a lawsuit followed on May 9th 2009 by the Natural Resources Defence Council (NRDC) and Xerces Society (see Figure 1) (Mogerman, 2009).

Then December 10th 2009, Bayer entered the lawsuit by filing an intervener. Figure 1 below shows a timeline for Movento, from discovery until October 15th 2010, until the EPA reissued Movento.



1. Figure. The timeline of Movento (Eddy, 2010)

Movento effects many different insects, especially those considered as suckling insects, including aphids, whiteflies, mites, and thrips (Funk, 2011), and used on a variety of plants such as fruits, vegetables and nuts. Most frequently used on brussels sprouts, broccoli, cabbage, cauliflower, collards, kale, lettuce, potatoes, onions, carrots, swede, turnip, and parsnip (Bayer CropScience, 2016).



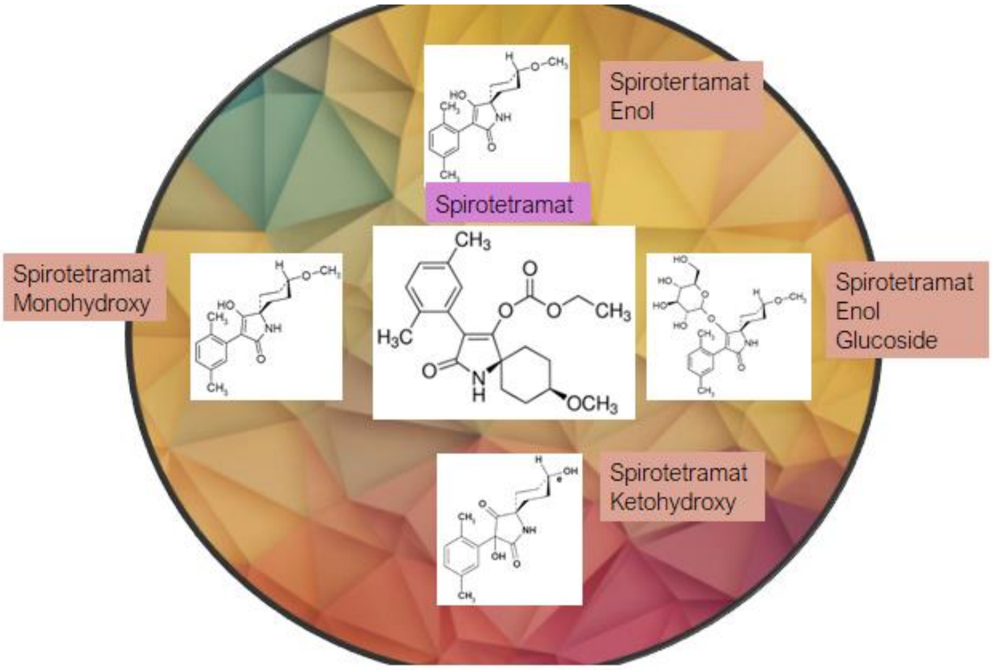
2. Figure. The structural formula of spirotetramat, Movento's active ingredient

2.1.1. Mode of action of Movento

Approved in 2014, Movento was considered a different pesticide with duel action (Bayer CropScience, 2016). Classified into group 23 insecticide.

The first mode of action being the mobility of the 2-SYS two-way systematicity, giving protection to the plant as a whole, and the other inhibiting the lipid biosynthesis (LBI).

Spirotetramat cis-enol is the active and mobile form of spirotetramat (Bayer CropScience, 2019).
Spirotetramat can move up and down the phloem and xylem of plants. Previous systemic insecticides were only one-way systems moving upwards in the xylem. This new function affects all parts of the plants, even those not directly sprayed (Bayer CropScience, 2016). Therefore, independently from where a pest invades, a lethal dose is ingested, giving optimal protection.
The second mode of action is the chemicals' ability to inhibit lipid synthesis, a process known as lipogenesis. Spirotetramat inhibits the lipid biosynthesis of certain insects, thereby inhibiting their immature stages of development.
Blocking lipid metabolism hinders the survival of insects (Bayer CropScience, 2019). It plays a crucial role in how insects can reproduce, develop and grow of insects embryos, and insects metamorphosis, which is needed to enter the next stage.
The insects' cell membrane is composed of lipids, assisting the structural integrity of their cells. Acetyl CoA carboxylase is inhibited by spirotetramat, leading to lipid biosynthesis inhibitor (LBI). When this process is inhibited (during the LBI), the immature stages of the development are blocked.
Eggs can not hatch, larvae maturing process is blocked, while the ability of young insects to grow is hindered. Eventually leading to their death. Adult insects have reduced fertility or none at all (Nauen, Reckmann, Thomzik, & Thielert, 2007).
Regarding bees mortality and pupae the toxic dose of spirotetramat is considered safe without effecting their "behaviour and flight activity" (EFSA, 2013). However, two out of three colonies showed high rates in flight termination, despite Bayer's semi-field study, 1 x 96 g a.s./ha had no adverse effect.
While other research showed increase adult mortality or termination of bees brooding, there has been documented recovery after spirotetramat was removed (EFSA, 2013).



3. Figure. Spitotetramat as the parent compound and the most common metabolites **(FAO, n.d.)**

2.1.2. Target species of Movento

This pesticide is most efficient on Whiteflies, and bugs belonging to the aphid species such as blackfly and greenfly (Bayer CropScience, 2016). Spirotetramat showed effects on scales, mealybugs, psylla, mites, phylloxera, thrips, and aphids insects (EPA, 2008) while still considered safe for beneficial insects such as bees (Bayer CropScience, 2016).

Overall, spirotetramat is considered safe by Bayer CropScience industries for non-target species when applied in accordance with the instructions (Nauen, Reckmann, Thomzik, & Thielert, 2007). It is, therefore, important when Movento is applied, there should be a minimum of five meters buffer zone, to prevent non-target species (Bayer CropScience, 2016).

2.2. Fungicide: Ridomil Gold (Syngenta)

Ridomil Gold MZ 68 WG (will now be addressed as Ridomil), developed by the Canadian company Syngenta AG inc (from now on as Syngenta), is used as a fungicide solution in the agriculture world. It protects against fungal foliar and tuber blight diseases caused by Oomycete fungi, the *Phytophthora infestans*. This fungus is known to cause severe diseases on potato plants and tomatoes (Seidl Johnson, Jordan, & Gevens, 2015).

This fungicide granule contains a mixture of 4 percent metalaxyl-M, and 64 percent mancozeb (Syngenta AG, 2015). When added to water, the water-dispersible granules (WDG) get dissolved.

Ridomil is a fungicide manufactured by the Canadian company Syngenta and used in this experiment.

As previously mentioned, it is composed of two active ingredients; mancozeb, 640 g/kg CAS No. 8018-01-7, and metalaxyl-M 38.8 g/kg, CAS No. 70630-17-0 [Figure 4] (shows the chemical structure of both compounds).

The MRL for metalaxyl-M is 0.2 mg/kg (200 µg/kg), and 3 mg/kg for mancozeb (3000 µg/kg).

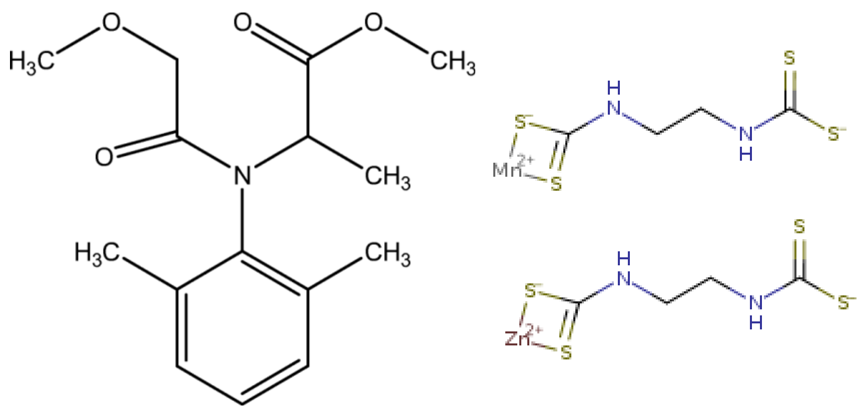
Ridomil has a withdrawal period, the minimum period of time that needs to pass since the administration (until the chemical residue is below MRL and considered safe), of 7 days when used on tomatoes; consequently, the final measurement was executed on day 8 post-treatment.

Ridomil is used to control late blight, caused by *Phytophthora infestans*, and other diseases caused by *Alternaria* and *Septoria*.

Plants should not be treated with Ridomil more than three times, our of fear for resistance, and a minimum of seven to twelve days in between.

The recommended dose for Ridomil is 2.5 kg/ha, in a minimum of 200 litres of water, or 400 to 800 L/ha when sprayed.

An interval between 10 and 14 days is used when metalaxyl-M is applied. Ridomil Gold should be discontinued if infested with blight, and a contact fungicide should be used instead (Syngenta AG, 2015).



4. Figure. The chemical structure of metalaxyl-M (left side) and mancozeb (right). ***Note: Mancozeb is composed of the two dithiocarbamates: maneb and zineb.***

2.2.1. Mode of action of Ridomil Gold

Once the roots absorb Ridomil Gold, it has two modes of function. Systemically, metalaxyl-M is absorbed and distributed evenly throughout the plant by the sap transport in xylem and phloem. It is allowing new growth to be protected, even after the chemicals have been applied (Syngenta AG, 2015). Mancozeb forms a protective film covering the outer layer of the plants (Syngenta AG, 2015). This means that once applied, fungi cannot enter the plant due to the mancozeb protective layer. Mancozeb inhibits the germination of pest fungi (Syngenta, 2017).

On potatoes, the withdrawal period, the minimum time after the application that has to pass before harvesting, is 7 days, and should not be applied a minimum of two till three hours before rainfall.

Mancozeb has a degradation half-life between 5.8 and 55 hours and is not persistent when in water, the same goes for Matalaxyl-M, which has a half-life between 22.4 and 47.5 days (Nauen, Reckmann, Thomzik, & Thielert, 2007).

2.2.2. Target species of Ridomil Gold

Ridomil is effective against *Penicillium expansum*, a Blue mould in tobacco plants. *P. expansum* is a fungus of economic importance which could have a high impact on stored vegetables and fruits postharvest. Certain strains of these fungi are able to produce the unfavourable mycotoxin Patulin (Syngenta AG, 2019). Mycotoxin Patulin inhibit normal barrier function on the digestive system (Mahfoud, Maresca, Garmy, & Fantini, 2002).

When using Ridomil Gold 480SL, it states that this chemical should not be used together with other chemicals, including; insecticides, nematicide, herbicides, other agrochemicals used against fungi, and no fertilizer.

Regarding ginseng, Ridomil gold is used to combat Phytophthora, a fungus that caused decay of seeds and the seedlings. Pythium, a soil fungus, is also combatted by using Ridomil Gold. Table 1 below shows different plants Ridomil Gold can be used, and what fungi it battels.

At worst, Ridomil Gold might be slightly toxic to bees, as well as fish, avian, and aquatic invertebrates (Syngenta Canada Inc., 2014). Considering that the LC50/EC50 for bees in contact with Ridomil is more than 100 µg. However, growers planning to use bees for pollination should always wait the 8 days post-treatment, as stated in the instruction.

1. Table. Fungi effected by Ridomil (Syngenta Canada Inc., 2014).

Plant affected	Name of Fungi
Field cucumber, greenhouse cucumber, Greenhouse tobacco	Pythium
Tobacco	Blue mould
Ginseng and Snap Beans	Pythium and Phytophora (food rot)
Hops	Downy Mildew
Raspberries	Pythiumand Phytophthora
Blueberries	Phytophthora
Strawberries	Red stele
Spinach	Downy mildew
Radish	Suppression of Downey mildew
Potatoes	Pink rot is suppressed and Phythium leak, Foliar Late blight, Early blight, and Botrytis vine rot

3. Materials and methods

3.1. Growing greenhouse tomatoes

Soliance F1 interminate tomatoes were grown inside greenhouses at John von Neumann University, at the Faculty of Horticulture and Rural Development, located in Kecskemet, Hungary. These tomatoes were grown by hydroponic farming, without the usage of soil. Grodan Delta rocks wool cubes were used, as one tomato seedling was placed into one cube before adding a rock wool quilt acting as the growing medium — planting 72 seedlings into the following; 2x 3-rows, with 12 seedlings in each row. There were separable and detachable boxes. The tomatoes had deep red colour and were slightly flattened, weighing between 120 – 140 grams. Optimal to be grown with lower wire heights, due to their joint, and other places with higher temperature.

3.2. The experimental method

During our experiment, tomato plants were divided into different groups (see Table 2). Movento was prepared and applied to group 1 of the truss zone 1 at a concentration of 0.9 ml/L with a totally of 4 L of spray by row. The tomatoes in group II, also belonging to truss 1, were sprayed with Ridomil Gold MZ 68 WG at a concentration of 3 g/L. 4 litres from this spray where used on group II. Movento and Ridomil were used in the same quantity and dose, both when applied alone or as a cocktail, which was of importance, regarding the interaction study. Once the solution was prepared, each row was sprayed with a total of 4 litres. The different groupsets were not separated. [Table 2] below shows the experimental setting. The groups were not separated but noticed the variety of the tomato truss zone 2 in group I. They were treated with both chemicals after the statutory withdrawal period had expired regarding metalaxyl-M, 8 days post-application. Treatment was performed in the morning on day 0 and is showed in [Table 2].

2. Table. The experimental setup of treatments

Pesticide		**Dose**	**Concentration**	**Group**			
				I		**II**	
				Truss zone 1	**Truss zone 2**	**Truss zone 1**	**Truss zone 2**
Movento		0.75 (L/ha)	0.9 (ml/L)	+	+	-	-
Ridomil Gold MZ 68 WG		2.5 (kg/ha)	3 (g/L)	-	+	+	-

3.3. Tomato sampling

Sample quantity:

500 g per sampling time, tomatoes were picked from different points within the truss zone.

Sampling times:

Harvesting of the **control** group was done previous to chemical treatment. Tomatoes were taken from truss zone I of group I and II, and truss zone 2 when both chemicals were applied. The tomatoes sampled **right after application**, was performed after the pesticide had dried up from truss zone 1 of both groups, together with truss zone 2, when spirotetramat and metalaxyl-M were applied in combination. The next sampling day was done **2 days** post-treatment. Tomatoes from the group I and II, belonging to truss zone 1 was picked, and tomatoes in truss zone 2 when the chemicals were applied in treatment combination. At **4 days** post-treatment, tomatoes from truss zone 1, group I and group II was used. Together with truss zone 2, from when the chemicals were used in combination treatment. At **8 days** post initial treatment, tomatoes were picked from truss zone 1, of both groups and from truss zone 2 in regards to when the pesticides were used in combination. Below in [Table 3], you can see a summary of the sampling schedule used.

3. Table. Schedule of tomato sampling

TREATMENT			**SAMPLING**				
Truss zone	**Insecticide**	**Fungicide**	**Before treatment**	**Treatment day***	**After treatment**		
					Day 2	**Day 4**	**Day 8**
1	Movento	-	x	x	x	x	
	-	Ridomil	x	x	x	x	x
2	Movento	Ridomil	x	x	x	x	x

* *After the applied chemicals had dried up.*

3.4. The preparation of tomato juice

Tomatoes harvested and juiced were prepared from the sampling before treatment and other, the last day of the experiment. Regarding spirotetramat, Movento's active ingredient, this was on the 4^{th}-day post-treatment, while metalaxyl-M, the active ingredient in Ridomil, at day 8 post-treatment. Tomatoes were prepared raw and heat-treated at 70 °C for 45 minutes.

3.5. Laboratory analysis

3.5.1. Preparing the samples

Reference standards for both Moventos spirotetramat, and Ridomils' macozab and metalaxy-M was obtained from Sigma-Aldrich. Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) kits I-II intended for preparation of the samples were obtained from Phenomenex, bought through GenLab Ltd.

At VWR international, acetonitrile, formic acid, acetic acid, and ammonium acetate were obtained.

Mancozeb was observed to be partially insoluble in the solvents while being polar enough for the chromatography. Therefore it was decided to exclude this from the measuring, and the focus is on metalaxyl-M, the other active substance found in the fungicide Ridomil Gold.

QuECHERS extraction method was adopted to remove the target active ingredient from the tomatoes and tomato juice. [Table 3] above shows how the tomatoes were sampled before they were cut into small cubes on a wooden cutting board, using a stainless steel knife. The juice was then homogenized by using an IKA T-25 Ultra-Turrax® Labrotory homogenizer. This was obtained by using a stainless steel dispenser at a speed of 5000 rpm. Once fully homogenized, 3 samples of 10 g were obtained at an analytical balance. This amount was added into a 50 mL PTFE centrifuge tube, already containing 6 g MgSO4, 1g NaCL, 1 g trisodium-citrate, and 0.5 g disodium-hydrogen-citrate (QuECHERS kit I). Before the extraction, 100 μL of caffeine solution (120 μg/mL) was added to the sample as an International Standard (ISTD). Followed by 10 mL acetonitrile added to the sample. Then hand-shaken intensively for 1 minute, before being centrifuged for 5 minutes at 6000 rpm at room temperature.

The supernatant of 6 mL was first removed from the samples, to be used for the second extraction. This was pipetted into a new centrifuge tube containing 900 mg $MgSO_4$ and 150 mg primary-secondary Amine (PSA) (QuEChERS kit II). The tube was shaken intensely for 30 seconds before being centrifuged again for 5 minutes at 6000 rpm at room temperature. After 4 mL supernatant was removed, 40 μL of formic acid solution (5 % volume per volume) was added. Nitrogen steam was used to dry the sample before it was reconstituted with 1 mL of acetonitrile containing 0.1 v/v% (volume per volume) formic acid.

The tomato juice used in this experiment was extracted as described above. Before 10 mL sample was measured by using a calibrated automatic pipette. 10 mL were pipetted into centrifuge tubes. Compared with the tomatoes above, the Ultra-Turrax homogenization step was skipped.

The same extraction procedures were carried out for the samples used for matrix-matched calibration. This was prepared by adding a known amount of the target compounds; spirotetramat, metalaxyl-M, and ISTD to the tomato juice before being tested for any absence of the previously mention pesticides. A blank sample from the same bio tomato juice was prepared against each sample batch, before being analysed.

3.5.2. Instrumental analysis

Shimaduzu LCMS-8030Pluss system was used to measure the pesticide content of each sample. Followed by a Phenomenex Kinetex C18, 100 x 4,6 mm ID (2,6 μm particle size) LC column equipped with a 4 x 2 mm C18 guard column.

By gradually changing the ration of eluent ‘A’ (50 mM ammonium acetate in water, the pH set to 5,0 with acetic acid) was used on the gradient elution. Regarding eluent ‘B’, 0.1 volume per volume formic acid in acetonitrile. A 0,3 mL/min flow rate was used. One chromatographic run lasted 8 minutes.

The column oven was set to 30 °C, and sampled were kept at 5 °C in the autoinjector. A volume of 10 μL was injected.

Electrospray (ESI) ionization was used in the mass spectrometer as a positive multiple reaction monitoring (MRM) mode. As an interface 4.5 kV and 250 °C was used, and the desolations line was set to 300 °C, with a heat block at 350 °C.

Respectively, 1.78 kV was used as detector voltage, with a nebulization gas (N_2) and drying gas (also N_2) flow set to 3 L/min and 15 L/min. Argon was used as the collision gas and set to 230 kPa.

International standard (ISTD) regarding quantitation of the matric matched calibration; using caffeine. Caffeine, at a fixed amount, was added to the calibrators, before the samples reached a final concentration of 300 ng/mL.

Area rations of the target compounds were plotted against the ISTD concentration ratio to obtain the calibration curve. The acceptable calibration point was dependent only if the measured concentration fell within ±15 to its nominal one. Any calibration points, failing this requirement, were discharged. Out of the 13 calibration points, minimum 9 was to be acceptable, together with the linear's r^2 value calibration of minimal 0.99, for the validation of calibration batch. The calibration points, together with the samples were injected three times. The lowest level of quantitation (LO Q) was 0.2 ng/mL (=µg/kg), the lowest level of detection (LOD) was 0.02 ng/mL.

3.5.3. Processing the data

Shimadzu LabSolutions ® software was needed to process the raw chromatographic data. While the secondary analysis, together with the statistical calculations, were performed using MS Excel software.

4. Results and discussion

Movento and Ridomil were applied to greenhouse tomatoes, alone or combined before the residue levels of the chemicals were measured at different time intervals in days. After completing the experiment, the data collected was analysed. In addition results include the effect of post pasteurization of tomato juice when the chemicals were applied alone or in combination. The following figures and tables show the results collected during this study (see Table 4 and 5, and Figure 5 and 6).

4. Table. Measured pesticide concentrations in tomatoes (µg/kg)

		Movento	Ridomil Gold
Treatment	**Sample**	**spirotetramat**	**metalaxyl-M**
Movento alone	control	0,17	0,15
	just after the treatment	208,17	2,77
	day 2	198,09	1,12
	day 4	180,82	0,30
Ridomil alone	control	0,20	0,11
	just after the treatment	0,83	450,73
	day 2	0,46	87,53
	day 4	0,33	45,85
	day 8	0,22	14,50
Ridomil + Movento in combination	control	0,30	26,36
	just after the treatment	206,49	141,35
	day 2	174,08	92,88
	day 4	95,32	91,85
	day 8	43,12	23,57

4.1. Residue concentrations of spirotetramat

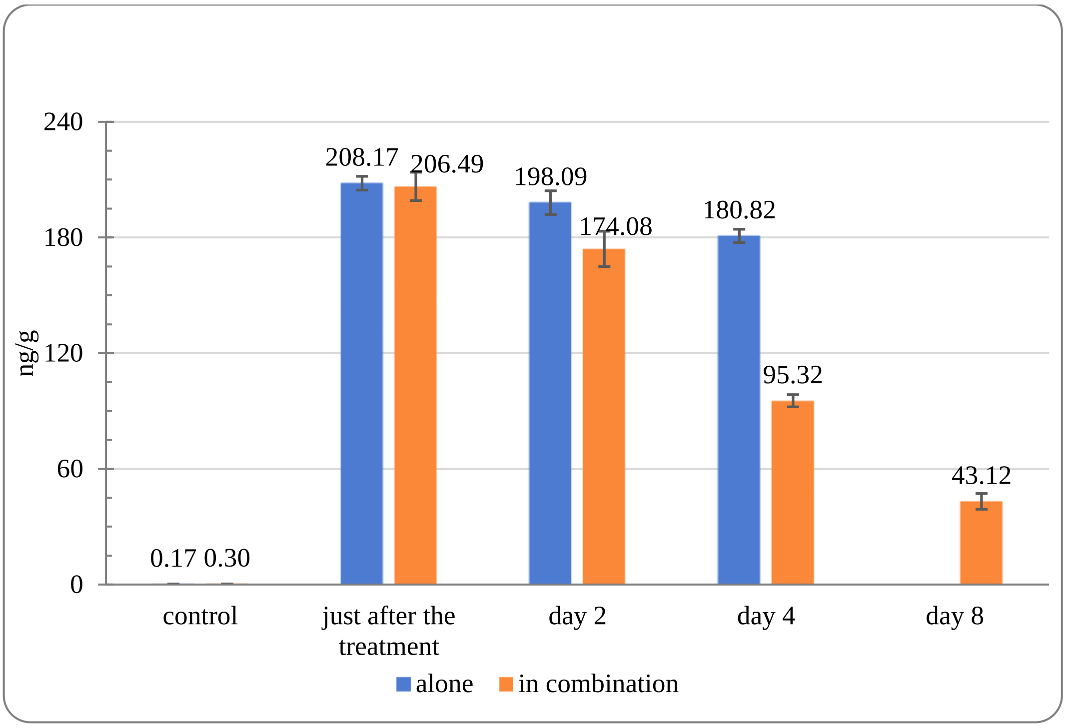
[Table 4] can be divided into three blocks. The first of the three blocks is for Movento with the active ingredient spirotetramat. It shows when Movento was applied alone, and residue levels were measured right after application, on day 2, and day 4. The second block comprised when matalaxyl-M was applied alone. The final block represents the residue values measured of spirotetramat and matalaxyl-M applied in combination while measured over the next 8 days.

The first block of [Table 4] shows the average values measured when spirotetramat was applied alone. The residue levels of spirotetramat, was 208,17 µg/kg, right after chemical application

[Table 4] & [Figure 5]. At day two WP, these levels decreased from 208,17 µg/kg, down to 108.83 µg/kg on day 4, which is a decrease in roughly 10 µg/kg per day.

The last block of [Table 3], column 3, illustrates the average levels measured when spirotetramat was applied in combination with Ridomil. At the time of application, these measured levels were found none-significant regarding spirotetramat applied alone, which is better illustrated in [Graph 5].

5. Figure. Residue concentrations of spirotetramat alone and in combination



[Graph 5] depicts the measured levels during the application of spirotetramat alone (blue colour), versus when it was applied in combination with metalaxyl-M (red colour). The graph illustrates that there was no considerable significance in the levels detected right after application. The observed standard deviation bars overlap, implying it is not statistically significant. This was confirmed by performing a statistical test. The calculated P-value regarding right after treatment was 7.641E-01.

The error bars observed are relating to the Real Standard Deviation (RSD). Some of the points have a longer error bar than others, as shown on 2 days post-application, at the X-axis. This represents the scattered data collected for that set. On day 2 until day 4 post-application, there is an increase in P-value, comparing spirotetramat applied alone versus in combination.

Between day 2 and the end of day 4, we saw the most important decline of spirotetramat (red column) when applied in combination with Ridomil.

When measured on day 2 after the application, the levels of Moventos' parent compound spirotetramat, when it was applied alone (blue column) was 198,09 µg/kg, and 174,08 µg/kg (red column) when combined with Ridomil. On day 4, the residue levels of spirotetramat, when applied with Ridomil, were 95,32 µg/kg. It is believable that this is because of the dissipation between the two chemicals and should be taken into consideration when applying Movento in combination with another chemical. The dissipation might affect the success rate for the chemical. The reason for this, as previously mentioned, is that the majority of pesticides today are not tested as to how the chemical would react when used simultaneously with another molecule.

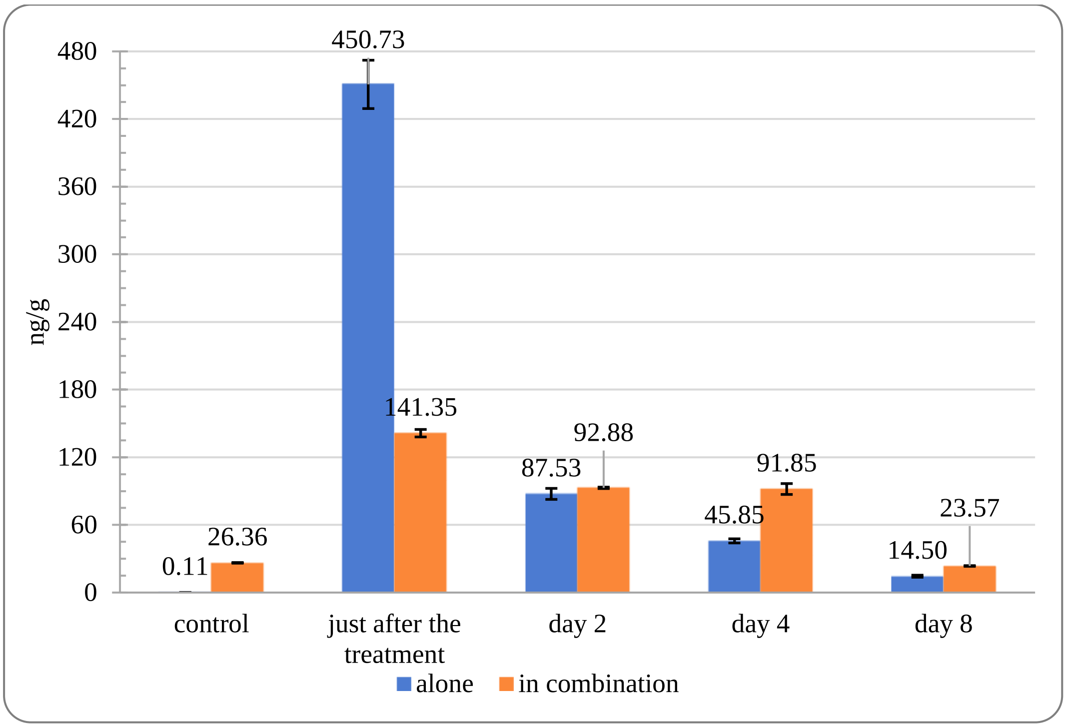
[Graph 5] shows that the amount of spirotetramat measured when combined with Ridomil, shows a lower residue concentration, then when it was applied alone, giving support to a quicker dissipation when applied together. This was also confirmed by the P-values.

By calculating the P-value, from previous data, right after application had a P-value of 7.641E-01, which is not significant. However, on day 2 and day 4 post-treatment, we were able to determine that there was a significant difference regarding spirotetramat being applied combined versus alone. The P-value of 2 days post-application is 5.710E-03 < 0.05. While the P-value calculated for day 4 is 8.695E-15 < 0.05.

4.2. Residue concentrations of metalaxyl-M

[Figure 6] represents the levels of metalaxy-M, the active ingredient found in Ridomil Gold. Below shows the measured concentrations when applied alone versus in combination with Movento over 8 days. Despite having a withdrawal period of 7 days, was the final measurement was performed at 8 days (post-treatment). This was due to keeping the days consistent when measuring the residue concentrations.

6. Figure. Residue concentrations of metalaxyl-M alone and in combination



Depicted in [Figure 6] is the average residue concentration (y-axis) measured at each time interval found on the X-axis. First investigating when metalxyl-M has applied alone, the measured residues right after application, had an average of 450,73 µg/kg (0.450 µg/kg). Looking at day 2 post-application, we see a fivefold decrease (from 450,73 down until 87,53). From there, we see a steady decrease until the last day of measurements, on day 8.

Comparing the residue levels of metalaxyl-M with when it was measured in combination with Movento, you can clearly see a significant difference, especially just after the treatment.

By looking at [Figure 6], it is possible to see that when metalaxyl-M has applied alone and measured on the day of application, the measured concentration was a lot higher, then if both Ridomil and Movento were applied simultaneously. Applied alone, the residue concentration measured right after application, shows that Ridomil was 450,73µg/kg. However, when applied together with spirotetramat, found in Movento, these levels were 141.35µg/kg. That is a decrease of 31.36 percent. By calculating the P-values, which is 1.302E-08 gives support that there is a significant difference in the dissociation between applied alone or together.

Despite the large difference at the first measurement, between alone and applied in combination, day two shows no such change. Matter of fact, metalaxyl-M was slightly higher on 2, 4, and 8 days post-treatment, when applied in combination. On day 4, we see the second biggest difference, where the measured residue concentrations for metalaxyl-M combined was higher than alone. Further studies should be carried out as to why.

The calculated P-value for 2 days post-application was 1.405E-01, which is not significant.

Regarding day 4 and day 8, the calculated P-values are 7.878E-08 and 9.032E-08, respectively. These two numbers do show an increased significance.

As described in chapter 2.2.2, Ridomil has unfavourable ecotoxicological effects, despite low measured concentration.

Further studies should be carried out to see if there are any adverse effects between the dissipation of chemicals applied collectively with pests.

5. Table. Measured pesticide concentrations in tomato juices (μg/kg)

			ng/g (=μg/kg)	
			Movento	**Ridomil Gold**
	Treatment	**Sample**	**spirotetramat**	**metalaxyl-M**
untreated	Movento alone	control	0,51	NQ.
		day 4	0,52	NQ.
	Ridomil alone	control	0,55	ND.
		day 8	0,62	ND.
	Ridomil + Movento in combination	control	0,44	0,29
		day 8	0,51	ND.
heat treated	Movento alone	control	0,60	0,37
		day 4	0,48	NQ.
	Ridomil alone	control	0,57	0,28
		day 8	0,60	ND.
	Ridomil + Movento in combination	control	NQ.	0,28
		day 8	NQ.	0,30

N.D. non-detectable: no peak appeared at the corresponding part of the chromatogram
N.Q. non-quantifiable: there was a peak at the corresponding part of the chromatogram; however, it represented a smaller amount of the compound than the lowest calibration point

[Table 5] shows that levels of spirotetramat and methylaxyl-M detected in the tomato juice before and after heat treatment, when applied alone and in combination. The juice that contained Movento on day 4 did show a slight decrease after heat treatment. However, looking at the control group of tomato juice, there was an increase from 0.51 µg/kg to 0.60 µg/kg. However, due to the very low concentrations measured, we decided not to compare these results.

An unexpected finding was the presence of spirotetramat and metalaxyl-M in the control groups when neither chemical was used. This might be because no walls were separating the tomatoes, causing contamination. Especially since it is well documented that after a pesticide is applied, it can travel far distances with the wind (Tharp, u.d.). Another possible scenario is that the sprayers they used were not properly cleaned between the different chemicals used, or that the scientists had residues on their protective gear that touched the other tomato plants. Or if there was a draft in the greenhouse during application. Optimally there should have been one spray used only for Movento, another one for Ridomil, and a third one only used when the chemicals were applied simultaneously.

5. Conclusions

Overall, it can be concluded that the dissipation is influenced when spirotetramat and metalaxyl-M were combined. This is an important finding, due to the fact once a chemical is authorised, they may result in an unexpected case if applied in combination, without the consumer knowing.

A previous study on terrestrial field dissipation (TFD) has proved that the rate of dissipation of pesticides, even when applied alone in the field, can differ from those observed in a laboratory setting (Corbin, Eckel, Spatz, & Thurman, 2006). Studies similar to this is, therefore, important shining light on possible risk assessors and how more in-depth field studies should be carried out.

However, considering the positive pollinators such as bees, it might be positive that our results showed that chemicals dissipate quicker when applied together. The reason for this is that the residual toxicity might be greater for ``long-lasting pesticides than those that dissipate quicker´´ (May, Wilson, & Isaacs, 2015).

Another point of interest while using a pesticide, is to spray non-flowering plants since this decreases the threat towards bumblebees (Grube, Donaldson, Kiely, & Wa, 2011).

Other methods to protect crops from unwanted diseases, fungi, and other pests, or even pesticides, include different strategies, used according to available resources. We plant different crops with varying life circles and use crop rotation. We plant annual cereal grasses, different legumes, quick-growing vegetable (Morton & Staub, 2008) to optimize production and decrease the amount of pesticides.

The competent authority has fulfilled their role to ensure safety in regards to the MRLs measured in greenhouse tomatoes of these two compounds in this experiment.

All residue levels measured were found to be below the legal limits set forth by the EC, at the end of the withdrawal period. This included the experiments where Movento and Ridomil where applied alone and in combination, as well as the experiment of the pasteurized tomato juice.

In regards to the maximum legal levels for spirotetramat in food and feed, which is 2 mg/kg or 2000 µg/kg, the levels measured were never found above, not even right after application, as shown in [*Table 2*] and [*Figure 5*].

It should still be mention that even though the levels were below the legal limits, and safe to consume, my scepticism stands. If a chemical is safe to consume at 10 µg/kg, but toxic at 100 µg/kg, and you consume this 10 times, you are in danger. Today the majority of the food we consume contains some form of residue; it slowly adds up. Luckily studies, have documented that cooking, steaming, and pasteurization will decrease the number of pesticides in a product.

6. Summary

The aim was to investigate the residue concentrations of spirotetramat and metalaxyl-M in greenhouse tomatoes when applied alone or in combination. To determine if there is a change in dissipation measured, followed the interaction when both chemicals were applied. This study also aimed to investigate tomato juice residues of spirotetramat and metalaxyl-M post-heat treatment, when applied alone versus in combination.

A literature review was performed to gain in-depth knowledge regarding the aim of the study and the two pesticides used. Spirotetramat is the active ingredient in Movento, and metalaxyl-M in Ridomil Gold. Previous studies have proved the dissipation of a chemical, but there is a vast array regarding our topic when spirotetramat and metalaxyl-M are used in combination.

The experiment was carried out using greenhouse tomatoes, and the chemicals, spirotetramat and metalaxyl-M, were applied alone and in combination before measuring the residue concentrations — techniques such as Sigma-Aldrich. Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) kits I-II, IKA T-25 Ultra-Turrax® Labrotory homogenizer, by a Phenomenex Kinetex C18, Electrospray (ESI) ionization, and LC-MS measured the pesticide concentration.

To summarize our results regarding spirotetramat and metalaxyl-M there was a significant dissipation when the chemicals were applied together versus alone. Spirotetramat showed significant P-values of 5.710E-03 at 2 days post-treatment and increased significance at 4 days post-treatment of 8.695E-15. Metalaxyl-M showed significant P-values right after application with 1.302E-08, and not again before 4 days post-treatment had a significant P-value of 7.787E-08 and on day 8 post-treatment a P-value of 9.032E-08. Regarding the pasteurization of tomato juice, no significant change was observed worth mentioning.

From analysing our data, it was evident that the presence of another pesticide influenced the dissipation, which is an important finding. Especially regarding pesticide products, that are approved without being applied in combination. This is why further experiments should be carried out regarding pesticides applied together before measuring the dissipation.

7. Literature list

Bayer CropScience. (2016). *User Guide, October 2016.* Bayer CropScience. Hentet April 21, 2019 fra file:///C:/Users/candv/AppData/Local/Temp/2962-bcs-veg-ug-movento-1016-v4.pdf

Bayer CropScience. (2019). *Movento*. Hentet fra CropScience: https://cropscience.bayer.co.uk/our-products/insecticides/movento/

Corbin, M., Eckel, W., Spatz, D., & Thurman, N. (2006). *NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies.* 31: March. Hentet fra https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/nafta-guidance-document-conducting-terrestrial-field

EC. (2019, August 7). *Priority substances under the Water Framework Directive.* Hentet fra https://ec.europa.eu/environment/water/water-dangersub/pri_substances.htm

Eddy, D. (2010). *Movento: It's Back*. Hentet fra Growing Produce: https://www.growingproduce.com/vegetables/movento-its-back/

EFSA. (2013). *Conclusion on the peer review of the pesticide risk assessment of the active substance spirotetramat.* European Food Safety Authority, European Food Safety Authority. Parma: European Food Safety Authority. Hentet fra https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2013.3243

EFSA. (2013). *Conclusion on the peer review of the pesticide risk assessment of the active substance spirotetramat.* Hentet fra https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2013.3243

EFSA. (n.d.). *How Europe ensures pesticides are safe.* Hentet fra European Food Safety Authority: https://www.efsa.europa.eu/en/interactive-pages/pesticides-authorisation/PesticidesAuthorisation

EPA. (2008). *Pesticide Fact Sheet Spirotetramat.* Office of Prevention, Pesticides and Toxic Substances. Environmental Protection Agency. Hentet April 21, 2019 fra https://drive.google.com/drive/search?q=spirotetramat

European Commission. (2016). *Spirotetramat.* PLANTS EU Pesticides database. Hentet April 21, 2019 fra https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.detail&language=EN&selectedID=1890

FAO. (2006). *InfoCrop: A dynamic simulation model for the assessment of crop yields, losses due to pests, and environmental impact of agro-ecosystems in tropical environments. I. Model description.* Hentet fra FAO: http://agris.fao.org/agris-search/search.do?recordID=US201301091197

FAO. (2016). *Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed.* Rome: FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. Hentet August 15, 2019 fra http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Manual/FAO_manual_3rd_edition_Final.pdf

FAO. (u.d.). *5.22 SPIROTETRAMAT (234)* . Hentet fra FAO: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report08/Spirotetramat.pdf

Funk, S. (2011). *SPIROTETRAMAT (234).* US Environmental Protection Agency & FAO. Hentet April 21, 2019 fra http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation08/Spirotetramat.pdf

Graziano da Silva, J. (2011). *Save and Grow A policymaker's guideto the sustainable intensificationof smallholder crop production.* Hentet fra FAO: http://www.fao.org/3/i2215e/i2215e.pdf

Grube, A., Donaldson, D., Kiely, T., & Wa, L. (2011). *Pesticides Industry Sales and Usage 2006 and 2007 Market Estimates.* Biological and Economic Analysis Division Office of Pesticide Programs. Washington, DC: U.S. Environmental Protection Agen. Hentet fra http: //www.epa.gov/oppbead/ pesticides/99 pestsales/market-estimates.pdf

Mahfoud, R., Maresca, M., Garmy, N., & Fantini, J. (2002, June 15). *The Mycotoxin Patulin Alters the Barrier Function of the Intestinal Epithelium: Mechanism of Action of the Toxin and Protective Effects of Glutathione.* Hentet fra sciencedirect.com: https://www.sciencedirect.com/science/article/pii/S0041008X02994172

May, E., Wilson, J., & Isaacs, R. (2015). *Minimizing Pesticide Riskto Bees in Fruit Crops.* Michigan: Department of Entomology, Michigan State University. Hentet August 21, 2019 fra https://www.canr.msu.edu/uploads/resources/pdfs/Minimizing_Pesticide_Risk_to_Bees_in_Fruit_Crops_(E3245).pdf

Mogerman, J. (2009). *NRDC*. Retrieved from Big Win for Bees: Judge Pulls Pesticide: https://www.nrdc.org/media/2009/091229

Moore, D., Robson, G., & Trinci, A. (2019). 14.1 Fungal diseases and loss of world agricultural production . I *21st Century Guidebook to Fungi,* (SECOND EDITION. utg.). Manchester, UK: Cambridge University Press. Hentet October 2, 2019 fra http://www.davidmoore.org.uk/21st_Century_Guidebook_to_Fungi_PLATINUM/Ch14_01.htm?fbclid=IwAR1orQInQQZ8X9gcAfymOO0uTOmXnaFTWN_mssJ4etmb5Q2CEJV66A6ufPs

Morton, V., & Staub, T. (2008). *A Short History of Fungicides*. Hentet fra APSnet: https://www.apsnet.org/edcenter/apsnetfeatures/Pages/Fungicides.aspx

Nauen, R., Reckmann, U., Thomzik, J., & Thielert, W. (2007, November). *Biological profile of spirotetramat (Movento)—a new two way systemic (amimobile) insecticide against sucking pests*. Hentet fra Reaserchgate.net: https://www.researchgate.net/publication/267198550_Biological_profile_of_spirotetramat_Movento-a_new_two_way_systemic_amimobile_insecticide_against_sucking_pests

NPIC. (1999). *DDT.* Oregon: National Pesticide Information Center. Hentet fra http://npic.orst.edu/factsheets/ddtgen.pdf

Pimentel, D. (2009). *Chapter 3: Pesticides and Pest Control.* Hentet August 21, 2019 fra Springer.com: https://page-one.springer.com/pdf/preview/10.1007/978-1-4020-8992-3_3

Seidl Johnson, A., Jordan, S., & Gevens, A. (2015). *Efficacy of Organic and Conventional Fungicides and Impact of Application Timing on Control of Tomato Late Blight Caused by US-22, US-23, and US-24 Isolates of Phytophthora infestans.* Department of Plant Pathology, University of Wisconsin-Madison. Madison : PubMed. doi:0.1094/PDIS-04-14-0427-RE

Syngenta. (2017, February 6). *Ridomil Gold MZ 68 WG*. Hentet fra Syngenta: https://www.syngenta.co.ke/product/crop-protection/fungicides/ridomil-gold-mz-68-wg

Syngenta AG. (2015). *Ridomil Gold Mz 68WG*. Hentet 2019 fra Syngenta: https://www.syngenta.ie/sites/g/files/zhg366/f/ridomil_gold_product_label_5.pdf?token=1471876893

Syngenta AG. (2019, July 30). *RIDOMILGOLD®480SL*. Hentet fra Syngenta: https://www.syngenta.ca/pdf/labels/RIDOMIL_GOLD_480SL_28474_en_pamphlet.pdf

Syngenta Canada Inc. (2014, December 31). *MATERIAL SAFETY DATA SHEET*. Department of Regulatory & Biological Assessment. Guelph, ON: Syngenta Canada Inc. Hentet August 21, 2019 fra Syngenta: https://www.syngenta.ca/pdf/msds/Ridomil_Gold_Bravo_26443_en_msds.pdf

Tharp, C. (u.d.). *Avoiding Pesticide Drift*. Hentet fra Montana State University: http://www.pesticides.montana.edu/reference/drift.html

The Aspen Institute. (n.d.). *What is Agent Orange?* Hentet fra The Aspen Institute: https://www.aspeninstitute.org/programs/agent-orange-in-vietnam-program/what-is-agent-orange/

Unsworth, J. (2010, May 10). *History of Pesticide Use*. Hentet fra IUPAC: http://agrochemicals.iupac.org/index.php?option=com_sobi2&sobi2Task=sobi2Details&catid=3&sobi2Id=31

Ware, G., & Whitacre, D. (2004). An Introduction to Insecticides. I *The Pesticide Book*. Radcliffe's IPM World Textbook. Hentet April 10, 2019 fra Radcliffe's IPM World Textbook: https://ipmworld.umn.edu/ware-intro-insecticides

WHO. (2008). *PESTICIDES*. Hentet fra WHO: https://www.who.int/ceh/capacity/Pesticides.pdf

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9. Appendices

Appendix I. Authorization and monitoring of pesticide products

Appendix II Further concerns in connection with Monsanto's acquisition by Bayer

Appendix III. HuVetA Declaration

Appendix I.

Authorization and monitoring of pesticide products (EFSA, n.d.)**.**

1: An Active substance is approved

2: How pesticide are authorized

3: How a new pesticide is monitored

1: An active substance is approved

An application is submitted to the Member state for approval of a new active substance. Occasionally, a renewed or modified substance, produced by the company can be submitted too. This application is comprised of the companies' scientific studies and other important information, giving support for this new substance.

The application is then assessed by the member state before the EFSA will critically evaluate the document. The application will be discussed with other member states, before the conclusion of the EFSA is delivered to the European Commission. Calculated risk management is often incorporated in this document.

The active substance will be authorized, depending on the conclusion of the EFSA. This is done after the European Commission, together with the member states, reach a mutual understanding. An application will either be approved or not, based on the proposal submitted by the European Commission. After the submission, the representative legislatures of the member state will make a public vote to approve or not. A substance approved lasts for 10 years, while a renewal might be granted for 15 years.

2: How Pesticides are Authorized

After the active substance is approved in step 1, the pesticide attended for the market containing the chemical, will be described in dept, in an application produced by the company. It is crucial that the intended usage is included, such as what crops it can be applied too, along with the amount to be used per hectare for optimal effect, and so forth.

Once the Member State receives the application, the information will be evaluated along with the proposed MRLs. If there is a pre-existed legislation of the MRL, this application will fast forward to the European Commission. However, if it is not, a few steps are included in the authorization of this pesticide.

The EFSA evaluates the suggested MRL levels before being forward to the European Commission together with any important notes. During this step, a full risk assessment of the intended use is carried out by the EFSA, before being passed for the decisions to be made by the European Commission.

The pesticide might be approved by the Member State if the MRL were accepted by the European Commission. As previously mentioned, MRL of 0.01 mg/kg is used by default, if otherwise not stated.

Note: Three zones are acknowledged within the EU, in regards to where pesticides can be used. North, South, and Centre zones are created so that areas with similar agriculture, plant health, furthermore environmental conditions have been grouped.

In other words, when a pesticide is authorized in one zone, this chemical can also be used by the other Member States, belonging to the same zone.

3: How pesticides are monitored

The European Food Safety Authorities (EFSA) stated that: ``A method for residues in body fluids and tissues is not required as the active substance is not classified as toxic or very toxic`` (EFSA, 2013).

Once the pesticide is approved, the company will release it on the market. Now the Member States will monitor the pesticide, ensuring that the decision-making was appropriated in terms of the regulations. It is vital that the chemicals are used according to the instructions. Two annual monitoring programs are implemented by all EU member states so that no residues of the chemicals exceed the legal limits.

An annual report is produced by the EFSA in regards to different pesticide residues found in food. Included in this report, might be new legal MRLs after monitoring programs were analyzed. Along with dietary exposure assessments conducted by the EFSA, the already approved MRL might be up for re-evaluation before the European Commission and the Member States make their final decision if to change the MRL or not.

It is comforting to know that multiple directives are a part of the assessment and monitoring of pesticides. These include; Water framework directive, priority substances directive, groundwater directive, and even drinking water directive (EC, 2019).

Additional Safety Studies

Mandatory safety studies can be included in the pesticide application giving support to the chemical in question (EFSA, 2013). Bees, fish, and birds are frequently used as non-target systems, in terms of the toxicity and environmental behaviour the chemical might have. Specialized laboratories are used so that the EFSA and the rest of the competent authorities can be ensured that the right conditions were followed during the trials after there is a possibility of risk assessment, which can be extracted from the data received.

Appendix II.

Further concerns in connection with Monsanto's acquisition by Bayer

The fact that Monsanto, an American company specializing in agrochemical production, as well as for biotechnology, has produced many chemicals that were later banned due to its toxic effect. This is why it understands that people are sceptical about new products from Monsanto-Bayer. Looking at Monsanto's history, they have produced a lot of chemicals that have had a negative and not to talk about the poisonous effect on humans, animals, and the environment.

Agent Orange, during the 1960s, contained the poisonous chemical dioxin. This was used during the Vietnam War. Thankfully in 1971, this chemical was banned due to the horrible toxicity. It has been linked to causing everything from diabetes to birth defects, numerous types of cancer, and other disabilities (The Aspen Institute, n.d.).

Monsanto has produced several chemicals that later have been banned. Monsanto sold DDT, PCB's, Growth hormones for cattle (that are banned in Europe) and Aspartame sweetener, possibly linked to cancer (Andreatta et al., 2008). Another chemical worth mentioning is the glyphosate herbicide, better known as Roundup. International Agency for Research on Cancer (IARC) performed a study independently found this chemical as a ``probable human carcinogen´´ while the EFSA did not.

The fact that independent savant peer-reviewed research is not needed; only the research was done by the owner of the product is used (Landrigan and Belpoggi, 2018). The list goes on, and it often seems that Monsanto would do anything, ethically or not, to protect their company. Early November 2014, Monsanto donated many millions in the No campaign, hoping the win would mean that genetically engineered products, were not to be labelled (Srinivas, 2014). Should not this be up to the individual consumer to decide? Monsanto says otherwise; that mandatory labels would inaccurately put fear in the heart of consumers.

Appendix III. HuVetA

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Name: Cecilie Marie ANDVORD

Contact information (e-mail): CAndvord@gmail.com

Title of document (to be uploaded): Cocktails or not: Dissipation of some pesticides from greenhouse tomatoes applied alone or in combination treatment.

Publication data of document: 05.12.2019

Number of files submitted: One

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