

IMPACT OF MINERAL OILS ON DYNAMICS IN THE APHIDS PRESENCE AND VIRUS INFECTION OF TULIPS 'LEEN VAN DER MARK' CV. IN THE FIELD CULTIVATION

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ABSTRACT

The research material consisted of tulip plants of 'Leen van der Mark' cv. The effect of two preparations was evaluated: Sunspray 850 EC and Sunspray Ultra-Fine, in which the active substance is refined paraffin-based mineral oil. Preparations were applied at three concentrations: 1, 1.5, and 2%. Research plots were sprayed three times every 7 days. The first treatment was performed at the turn of the second and third decade of April. Starting from the beginning of May, observations of plant colonization by aphids were conducted every 14 days. The observations were terminated at the end of June. It was found that spraying using mineral oils Sunspray 850 EC and Sunspray Ultra-Fine of tulip 'Leen van der Mark' significantly affect the degree of virus infection and infection due to disease-causing pathogens in relation to the untreated plants. Plants sprayed with mineral oil at the lowest concentration (1%) were in better health condition and were less attractive to aphids. Increasing the concentration of oil and the number of treatments intensified the phytotoxic reaction of tulips manifested as chlorosis with varying intensity. They were not, however, apparent effect on plant growth and development. The chlorophyll content (expressed as greenness index) in the tulip leaves treated with mineral oil was lower as compared to untreated leaves, when plants were sprayed three times using Sunspray Ultra-Fine oil at a concentration of 2%.

Key words: bulbous plants, adjuvants, aphid competing, greenness index, phytotoxicity, decorativeness

INTRODUCTION

Cultivation of tulips is often unreliable due to viral diseases, which are a problem not only because of their harmfulness, but also the ease these plants get infected. There are also limited possibilities to combat these pathogens.

Aphids, as vectors of viruses, are of great importance in the field cultivation of tulips. The species composition and abundance of these insects during the growing season significantly affect subsequent health of collected bulbs. Protection of the bulbous tulip plantations against viral infection mainly consists of combating aphids using insecticides. How-

ever, their effectiveness in reducing the infection due to viruses transmitted by aphids in a transient way is indiscernible or there is no effectiveness at all [Milošević 1996]. In addition, concern for the environment forces to look for plant protection products that are safe for bees, and above all, safe for human and his environment.

An alternative to more toxic and more expensive, synthetic insecticides, are mineral oils. These preparations are nowadays highly refined, less toxic and are an important complement to traditional methods for combating pests and pathogens of horticultural

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crops [Furness 1981, Northover and Homeyer 1999, Wilcox and Riegel 1999, Wojdyła 2005, 2010, 2013, 2015, Moyer and Peres 2008]. They are widely used mainly in integrated pest management programs in the USA [Kallianpur et al. 2002], New Zealand [Beresford et al. 1996], and Australia [Nicetic et al. 2001, 2002, Northover and Timmer 2002]. Already in the 80s of the twentieth century, in France, the use of mineral oil for the protection of potato seed plantations during the growing season was regarded as a standard treatment [Kerlan et al. 1987]. Some of them are used for the protection of vegetables [Goszczyński and Tomczyk 2004, Stansly and Conner 2005, Wróbel and Urbanowicz 2007, Kraiss and Cullen 2008, Wróbel 2009, 2011, 2012, Olubayo et al. 2010], orchards [Finger 2000, Heng et al. 2002, Maciesiak and Olszak 2004, Fernandez et al. 2006, Taverner et al. 2011], and ornamental plants [Mizell 1991, Nicetic et al. 2001, Asjes and Blom-Bamhoom 2002b, Goszczyński et al. 2003, Soika et al. 2008] against aphids, mites, maggots, volutes, and pathogens. There are different opinions on the effects of mineral oils on aphids. Results reported by Powell et al. [1998], Wróbel [2006], as well as Wróbel [2009] indicate that oil influenced on the behavior of aphids during feeding time and peak flights. According to Qui and Pirone [1989], as well as Turska and Wróbel [1999], oil treatments do not always have a significant impact on the behavior of aphids.

High frequency of treatments using oil substances may pose the risk of phytotoxic symptoms on plants. This reaction was observed on rose [Goszczyński et al. 2003]. In the cultivation of some species, it may significantly impede or prevent the execution of

negative selection. Phytotoxic response of plants can also cause a decrease in the yield, which was observed on potato plants sprayed with oils several times during the growing season [Wróbel 2012].

Available literature is poor in information about the impact of mineral oils on aphids colonizing the ornamental plants in the field conditions.

The aim of the study was to evaluate the effect of two products containing mineral oil on the dynamics of the aphids' occurrence, virus infection, index of greening the leaves of 'Leen van der Mark' cv. tulips during the growing season and their impact on the quality of crops.

MATERIALS AND METHODS

The field studies were carried out in 2008–2010 at the Experimental Farm "Felin" belonging to the University of Life Sciences in Lublin (Poland, 51°23'N, 22°56'E) on the lessive soil containing approximately 1.6% of organic matter. The research material consisted of plant tulips of 'Leen van der Mark' cv. The effect of two preparations was evaluated: Sunspray 850 EC and Sunspray Ultra-Fine, in which the active ingredient is a paraffin-based mineral oil (tab. 1). Preparations were applied at three concentrations: 1, 1.5, and 2%. The plots were sprayed three times every 7 days (tab. 2). The preparations were applied by means of knapsack sprayer fitted with a spray Tee-Jet that consumes 400 liters of working liquid per hectare. The first treatment was performed, depending on the year of study, at the turn of the second and third decade of April. This was determined by varied weather circumstances (tab. 4).

Table 1. Physical properties of oil substances used in the experiment

Oil substances	Producer	Importer	Composition
Sunspray 850 EC	Sun Oil Company (Belgium) N.V.	Rol-Eko Sp. z o.o	85% mineral oil SAE
Sunspray Ultra-Fine	HollyFrontier GB	Rol-Eko Sp. z o.o	98.8% mineral oil SAE + 1.2% emulsifier

Table 2. Mineral oil treatments dates in respective research years

Year	Respective treatments date		
	1	2	3
2008	22 IV	29 IV	6 V
2009	15 IV	22 IV	29 IV
2010	24 IV	28 IV	8 V

Table 3. Observations of the aphid populations of tulips cultivar 'Leen van der Mark' (pcs · 10⁻¹)

Year	Individual dates of observation				
	1	2	3	4	5
2008	5 V	20 V	2 VI	15 VI	28 VI
2009	7 V	23 V	4 VI	18 VI	30 VI
2010	4 V	19 V	2 VI	13 VI	25 VI

Three days after each treatment, there was made an assessment of the impact of product applied to the tulip plants based on a 9-point scale valuation, where 1 – means no signs of damage, and 9 – completely destroyed plants. Characteristic symptoms of phytotoxic reaction after using the mineral oils, were necrosis and deformations of the leaf blade, and the scale only expresses the degree of their severity.

Starting from the beginning of May, every 14 days, observations upon the colonization of plants by aphids were performed (tab. 3). On each of the test plots, 10 plants were chosen at random and determined, where wingless (including larvae) and winged aphids were counted. Number of two most abundant aphid species on the tulip was specified: *Aphis fabae* Scopoli 1763 and *Myzus persicae* Sulzer 1776. Identification of aphids to species was carried out on the basis of permanent microscopic preparations. Assay employed detailed key of Blackman and Eastop [2000]. Observations were carried out on plots, where mineral oil treatments were made and on the control object, and were stopped at the end of June (deadline of tulip bulbs digging).

Just before topping of the test plants (approximately 15 May, depending on the study year), degree of virus infection of plants on the plots on the basis of visible symptoms on the perianth leaflets was assessed, also number of plants infected by other pathogens was counted (bacteria, fungi, physiological

diseases), at which damages were slightly different from those that testified to the presence of virus – deformation, necrosis, and physiological evidence in the form of mycelium raids or bacterial overhangs. Plants infested with the pathogens were counted together, because no additional phyto-pathological and physiological tests allowing an accurate assessment of the infection source, were performed. Evaluation of the viral infection was based on the use of visual criteria. The 'Leen van der Mark' variety has two-tone flowers (red with a white margin), and it is quite easy to observe changes caused by a viral infection (fig. 1). Moreover, the variety is characterized by relatively high susceptibility to viral infections. It was introduced to cultivation in 1968 [Baliuniene and Juodkaite 1991]. Due to the large decorative qualities and suitability for forcing, it is in second place in the Netherlands after the Strong Gold cv., in terms of acreage on reproductive plantations.

If during the observation, flowers had streaks or bands in an unusual color, petals were not fully stained, buds were deformed and not fully developed, and the leaves had chlorosis in the form of streaks along the leaf blade, and they were slightly smaller than leaves growing on properly developing plants, such plants were considered as virus-infected. After negative selection, diseased plants were dug together with bulbs and then burned in order to protect other plants from the spread of the pathogen.



Fig. 1. Virused of tulips cultivar 'Leen van der Mark' when making a negative selection



Fig. 2. Tulips cultivar 'Leen van der Mark': a – oil treated plot – brighter green leaves, b – plot without protection (control)



Fig. 3. Leaves tulips cultivar 'Leen van der Mark'. sprayed oil with lighter spots

During the observations in the first year of the study, plots treated with oil preparations contained test plants with leaves much lighter than those not sprayed with mineral oil (figs 2 and 3), while the flowers retained their natural varietal color. In the second and third year of studies, the assessment of the chlorophyll content in leaves of tested tulips, was carried out. Determination of the chlorophyll condition of test plants was based on the measurement of total chlorophyll on the basis of the so-called leaf greenness index by means of Field Scout CM1000 chlorophyll-meter (Spectrum Technologies, Inc., Plainfield, IL), in which the relative chlorophyll index (RCI) ranged within a scale of 0 to 999. The measurement was performed on 5 tulip plants randomly selected from each plot. The relative chlorophyll index was measured at the end of May, i.e. 26–30 days after the last plant spraying using oil preparations. Measurement was carried out non-invasively by holding the device at a distance of 30–40 cm from the test plant leaf in the morning, i.e. between 9:00 a.m. to 11:00 a.m.

The experiments were established in a randomized block pattern in 5 replicates. Each replicate con-

sisted of a plot with an area of 1m². Forty-five bulbs with a circumference of 8–10 cm were planted on the plot. The test results were statistically analyzed by means of variance analysis for three-factor experiments. The significance of differences between means were found using Tukey confidence intervals at a significance level of $\alpha = 0.05$.

Characteristics of meteorological conditions was based on data obtained from the Laboratory of Agrometeorology, UP in Lublin and from the website www.weatheronline.pl. The names of aphid species were accepted after Fauna Europaea [2014].

RESULTS AND DISCUSSION

Dynamics of aphids' population. Particular growing seasons in 2008–2010 were characterized by a very different course of weather (tab. 4). Varied weather conditions directly influenced on the size and dynamics of aphid development. Conducted observations revealed that the most frequent was *Aphis fabae* (tab. 5), the population of which was three times higher in comparison with *Myzus persicae* (tab. 6). The low number of *M. persicae* was also observed on

Table 4. Mean air temperature and total rainfall in respective decades April through June (2008–2010)

Year	April			May			June		
	I	II	III	I	II	III	I	II	III
Mean air temperature (°C)									
2008	7.8	9.4	10.8	11.3	13.3	13.6	18.0	16.4	18.8
2009	11.4	9.3	13.5	13.6	13.1	14.2	15.3	14.9	19.1
2010	8.5	9.5	10.2	13.6	14.5	15.2	19.0	17.9	17.1
Total rainfall (mm)									
2008	17.6	35.3	2.9	57.1	34.7	9.8	0	19.6	6.3
2009	1.1	1.8	0	3.6	34.8	32.9	28.2	32.7	64.6
2010	13.8	7.7	3.0	39.7	106.7	10.3	34.6	30.2	0.8

Table 5. Number of *Aphis fabae* aphids on tulip plants of the cultivar 'Leen van der Mark' in 2008–2010 (pcs · 10 plants⁻¹)

Years (A)	Observation dates (B) Decade / Month	Oil concentration (%) (C)							Means		
		Sunspray 850 EC			Sunspray Ultra-Fine			Control	A	B	
		1%	1.5%	2%	1%	1.5%	2%				
2008	I / 5 May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.9 A	10.60 C	II term
	II / 20 May	13.6	17.0	15.2	12.8	14.8	11.0	20.4			
	I / 2 June	14.2	26.8	14.6	15.2	13.6	14.6	47.2			
	II / 15 June	16.2	31.0	24.8	17.0	17.8	15.2	79.0			
	III / 28 June	21.2	47.0	16.0	28.6	22.0	25.0	70.6			
2009	I / 5 May	0.0	0.0	0.2	0.0	0.0	0.0	0.0	17.6 B	19.72 C	III term
	II / 20 May	1.6	2.0	1.2	1.2	1.2	1.8	15.4			
	I / 2 June	4.2	4.6	8.2	7.4	5.4	8.8	39.8			
	II / 15 June	13.6	9.4	5.8	8.8	10.4	12.2	91.6			
	III / 28 June	25.8	11.2	11.0	16.0	9.2	10.4	91.8			
2010	I / 5 May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.8 AB	40.82 A	IV term
	II / 20 May	1.8	4.4	3.0	1.6	5.8	3.2	29.4			
	I / 2 June	7.6	10.6	8.6	11.0	9.6	9.0	42.6			
	II / 15 June	11.2	9.6	23.2	9.4	8.4	11.8	71.6			
	III / 28 June	12.6	7.2	9.60	7.4	10.8	8.2	126.0			
Means C		9.5 C	12.0 B	9.4 C	9.1 C	8.6 C	8.7 C	48.4 A			

LSD_{0.05} for A = 4.33; B = 6.52; C = 9.17

Mean marked with the same letter do not differ significantly

Table 6. Number of *Myzus persicae* aphids on tulip plants of the cultivar 'Leen van der Mark' in 2008–2010 (pcs · 10 plants⁻¹)

Years (A)	Observation dates (B) Decade / Month	Oil concentration (%) (C)							Means				
		Sunspray 850 EC			Sunspray Ultra-Fine			Control	A	B			
		1%	1.5%	2%	1%	1.5%	2%						
2008	I / 5 May	0.0f	0.0f	0.0f	0.0f	0.0f	0.0f	0.4f	2.9 B	0.22 D	I term		
	II / 20 May	0.0f	0.0f	0.0f	0.0f	0.0f	0.0f	0.6f					
	I / 2 June	1.0f	0.2f	0.0f	0.2f	0.0f	0.0f	2.2ef					
	II / 15 June	2.2ef	2.6ef	2.2ef	3.0ef	2.8ef	4.0ef	8.0ef					
	III / 28 June	4.2ef	6.6ef	6.2ef	6.8ef	7.4ef	10.2de	17.2cd				0.27 D	II term
2009	I / 5 May	0.4f	0.2f	0.0f	0.2f	0.2f	0.0f	1.4f	2.5 B	3.54 C	III term		
	II / 20 May	0.2f	0.2f	0.2f	0.2f	0.2f	0.2f	1.6f					
	I / 2 June	1.2f	2.0f	1.2f	0.6f	1.2f	1.0f	2.0f					
	II / 15 June	2.0f	2.6ef	1.8f	2.4ef	1.4f	1.8f	5.8e					
	III / 28 June	3.8ef	4.4ef	2.4ef	2.6ef	2.2ef	2.8ef	17.6cd					
2010	I / 5 May	0.0f	0.0f	0.0f	0.0f	0.2f	0.2f	0.0f	7.7 A	6.75 B	IV term		
	II / 20 May	0.0f	0.2f	0.0f	0.2f	0.2f	0.0f	0.4f					
	I / 2 June	3.0ef	3.0ef	1.4f	6.4ef	4.0e	3.0ef	22.6c					
	II / 15 June	5.6ef	5.0ef	3.4e	3.6ef	5.2e	3.6ef	39.2b				11.26 A	V term
	III / 28 June	3.2ef	6.8ef	6.4e	3.8ef	6.0e	7.0ef	50.4a					
Means C		1.8 B	2.3 B	1.7 B	2.0 B	2.1 B	2.3 B	11.3 A					

LSD_{0.05} for A = 0.85; B = 1.28; C = 1.81; ABC = 8.19
Mean marked with the same letter do not differ significantly

Table 7. Effect of mineral oils on limiting infection virus with tulip plant cultivar 'Leen van der Mark' in 2008–2010 (pcs · 45 plants⁻¹)

Years (A)	Type of oil (B)	Concentration (C)				Means		
		1%	1.5%	2%	Control	A	B	
2008	Sunspray 850 EC	3.2	5.2	3.4	14.2	6.65 B	6.73	Sunspray 850 EC
	Sunspray Ultra-Fine	5.8	6.2	5.6	9.6			
2009	Sunspray 850 EC	2.4	4.2	3.8	9.0	5.37 B	6.80	Sunspray Ultra-Fine
	Sunspray Ultra-Fine	4.8	5.2	3.6	10.0			
2010	Sunspray 850 EC	6.0	8.2	4.6	16.6	8.27 A	6.80	Sunspray Ultra-Fine
	Sunspray Ultra-Fine	5.6	7.2	6.0	12.0			
Means C		4.6 B	6.0 B	4.5 B	11.9 A			

LSD_{0.05} for A = 1.40; C = 1.77
Mean marked with the same letter do not differ significantly

pepper plants grown in Spain [Martin-Lopez et al. 2006], and also in the cultivation of potatoes in Kenya [Olubayo et al. 2010]. For *Aphis fabae*, the year 2008 was optimal: not very high air temperatures and little precipitation in the period from April to June contributed to a large number of these insects. The first aphids *Myzus persicae* were noted on May 5, and they were observed only in the control plots. And the first specimens *Aphis fabae* occurred 2 weeks later, i.e. on May 20. The heat during the day and light rains contributed to the development of insects. This arrangement of weather conditions caused that during the peak flights of aphids (end of June) [Karczmarz 2010, 2012] almost 4 times more individuals on 10 observed plants were recorded as compared to the first dates of observations (2010). During this research period, moderate air temperature also favored the development of both aphids. However, fairly dry April and June, and torrential rains in May, especially in the mid, could be the cause of their population decline. In 2009, there were droughts in April and heavy rainfalls in other months, especially at the end of June, and this resulted in significantly the smallest aphid number as compared to the first season of research. Regardless of the study year, both paraffin oils used at concentrations from 1 to 2% significantly reduced the number of aphids *Aphis fabae* on tulip plants as compared to the control combination. Only in the case of using the Sanspray 850EC at concentration of 1.5%, greater number of aphids was recorded in relation to other combinations, in which paraffin oils were used, but it was associated with a large aphid population in the first year of the study. The number of aphids of this species increased gradually during subsequent measurements and their greatest intensity was recorded at the end of June.

Oils used in the experiment, regardless of the concentration applied, remarkably limited the size of the aphid *Myzus persicae* population as compared to the control plants. It was also shown the interaction between oil concentration and date of measurement in individual years of the study. In 2008, significantly more these aphids occurred in the control combination only at the end of June. In 2009, significantly more aphids on plants untreated with oil occurred in the mid and end of June. However, in the last year of the study

(2010), in which the number of *M. persica* was the highest, significantly less aphids on plants protected with mineral oil were observed from the beginning to the end of June. That year, also the highest pressure of infectious viruses was found (tab. 7). The peak colonization of plants by aphids throughout the whole study period was recorded at the end of June. At that time, very strong limiting effect of mineral oil on the number of aphids, was observed.

Plants protected with oils in 2010 significantly better survived the effects of drought and were in better shape (full turgor) than control plants (some plants faded – stronger transpiration). This was probably closely depended on a layer of oil on the leaves, which can affect the gas exchange at plants [Finger 2000, Goszczyński et al. 2003, Goszczyński and Tomczyk 2004, Wróbel 2008]. Also the number of aphids on untreated tulips was high as compared to plants sprayed with oil, regardless of its type and concentration. It can be assumed that these plants, because of the lack of an oil film on the leaf surface, were more attractive to insects.

In all years of the study, significant limitations in abundance of both aphids' species on the leaves and shoots of tulips on plots where mineral oil was used, were reported. In the second – the third decade of June, i.e. during the peak flights, the population decreased by approximately 80% as compared to the combination unprotected with oil. Heng et al. [2002], Wróbel [2006], as well as Wróbel [2008] confirmed these dependencies by reporting that treatment applying 1, 2, and 3.75% mineral oil solution every 7–10 days reduced the aphids populations by 80–60%. Olubayo et al. [2010] also found high efficiency of mineral oils in reducing the number of aphids on potato plants. Decrease in the pests number can occur through physical effects on the respirator system of insects, rarely by chemical interaction [Wróbel 2006]. The film produced by oil on the surface of a leaf or shoot of plants can also act as a repellent for insects. An attempt to explain this phenomenon may be observations made by Nicetic et al. [2001]. Authors of that publication, when assessing the effects of mineral oil on the spider mite *Tetranychus urticae* Koch in a greenhouse cultivation of roses, found that starting the treatments of oil protection before the

first appearance of this pest on roses reduced their number below the hazard threshold later in time. The oil applied to roses already occupied by the spider only stabilized the population growth, without reduction in their number to the level below the threshold of economic harm.

Infections due to viruses and other pathogens

Viruses are most often tested as pathogens. However, the presence of many of them has a beneficial effect on the host, providing necessary conditions for the proper development. This is a form of viral symbiosis, that was first discovered at various organisms, including bacteria, fungi, plants, and animals (insects) [Roossinck 2011].

Despite of this, information on symbiosis of viruses with a tulip is lacking in available literature. Therefore, the primary factor determining the tulip quality is the health of produced bulbs and flowers obtained by forcing in an aspect of viral diseases. Commercial reproduction of bulbs based on the clonal propagation leads to the accumulation of viruses. Among 22 viruses occurring on the tulips, the most common and most dangerous are: tulip breaking virus – TBV, tobacco necrosis virus – TNV, lily syncytial virus – LSV, cucumber mosaic virus – CMV, and tobacco rattle virus – TRV. Accordingly, the tulip plantations protection is closely related to treatments limiting the infection of bulbs by viruses. One of the methods which somewhat reanimates tulip varieties, e.g. destroyed due to virus, is the micro-propagation method (*in vitro*), which provides the virus-free plants [Podwyszyńska 2005, Podwyszyńska et al. 2005, Podwyszyńska and Sochacki 2010]. Another method, which is used for bulbous plants, e.g. narcissus, is chemotherapy [Sochacki and Podwyszyńska 2012]. However, these plants are not resistant to virus. According to Boiteau et al. [2009], increased plant protection can be achieved by setting up a proper insulation surrounding the plants susceptible to the virus. They are grown altogether with plants resistant to these pathogens. With this treatment, the plants are returned to the crops and the market. Virus protection is also based on the fight against aphids using insecticides. In the case of viruses transmitted by aphids in a transient way, like

TBV (tulip breaking virus) and LSV (lily syncytial virus), insecticides are no longer as effective, especially in a situation of the presence of virus sources on the plantation or in the vicinity [Martín-López et al. 2006, Rolot et al. 2008]. That is why, other substances that limit the spread of the virus have been sought for many years, and in particular TBV, which proved to be one of the most frequently detected viruses in the Polish tulip plantations [Sochacki 2013]. One of them is a mineral oil. Its use for plant protection is becoming increasingly important in the world, especially in terms of reducing the use of traditional pesticides in favor of ecology [Turska and Wróbel 1999, Finger 2000, Asjes and Blom-Barnhoom 2001, 2002a, 2002b, Iovieno et al. 2005, Wróbel 2006, 2009, 2012, Rolot et al. 2008, Boiteau et al. 2009, Olubayo et al. 2010].

The studies have shown that repeated use of mineral oils to protect against the risk of viruses differently reduced the virus-infected plants (tab. 7). Significantly greater effect in reducing the viruses was obtained on objects treated with oily preparations as compared to untreated plots (control). There was no effect of the oil concentrations on reducing the infections due to viral diseases. Despite of the lack of statistical difference in the effectiveness of relevant formulations, it can be observed a tendency in limiting the spread of viruses when preparations are sprayed at the lowest (1%) and highest concentrations (2%).

Statistical analysis showed no significant differences in the two mineral oils actions.

The strongest effect in reducing the spread of the virus was obtained in the first (2008) and second year of the study (2009). Plants protected with oils in the third year of the experiment (2010) were much stronger infected.

Available literature indicates that besides fungicides [Wojdyła and Łyś 2000, Rongai et al. 2009, Wojdyła 2009], bio-preparations [Wojdyła and Orlikowski 2008], and fertilizers [Wojdyła 2004], also mineral oils can be useful for competing the fungal-origin plant diseases. It has been demonstrated the high effectiveness of the oils used for a direct control of fungi which cause powdery mildew [Wojdyła 2002, Jee et al. 2009]. In the case of other pathogens that cause leaf spot [Northover and Schneider 1993, Wojdyła 2010], rust

[Wojdyła and Janiewicz 2004], and gray mold [Wojdyła 2003], the efficacy data are relatively scarce, and the resulting effect of combat fluctuates around a dozen to several dozen percent.

When analyzing the results of own research, we have found a significant effect of applied concentrations of oil substances in reducing the infection by

other pathogens (tab. 8). It has been proved that the oil formulations applied at the lowest concentration (1%) efficiently protected plants against other pathogens. The increase in concentrations of the mineral oils was associated with a reduction in their effectiveness in reducing the infection of tulip plants with other pathogens.

Table 8. Effect of mineral oils on limiting infection with other plant pathogens of the tulip plant cultivar 'Leen van der Mark' in 2008–2010 (pcs · 45 plants⁻¹)

Years (A)	Type of oil (B)	Concentration (C)				Means	
		1%	1.5%	2%	Control	A	B
2008	Sunspray 850 EC	3.0	3.6	2.8	4.2	3.5 A	2.3
	Sunspray Ultra-Fine	1.8	2.4	4.8	5.0		
2009	Sunspray 850 EC	1.0	0.0	1.6	2.4	1.4 B	2.7
	Sunspray Ultra-Fine	1.4	1.6	0.4	2.8		
2010	Sunspray 850 EC	1.6	2.4	1.0	4.2	2.7 A	Sunspray Ultra-Fine
	Sunspray Ultra-Fine	1.2	2.4	3.6	5.2		
Means C		1.6 C	2.1 B	2.4 A	4.0 A		

LSD_{0.05} for A = 1.27; C = 1.61

Mean marked with the same letter do not differ significantly

Table 9. Evaluation of the phytotoxicity of mineral oils used on tulip plants cultivar 'Leen van der Mark' in 2008–2010

Year – Preparations	Concentration	Evaluation of phytotoxic reaction after successive applications		
		1	2	3
2008				
Sunspray 850 EC	1%	1.0	1.0	1.0
	1.5%	1.0	1.1	1.2
	2%	1.1	1.3	1.4
Sunspray Ultra-Fine	1%	1.0	1.0	1.0
	1.5%	1.0	1.0	1.0
	2%	1.1	1.2	1.3
2009				
Sunspray 850 EC	1%	1.0	1.0	1.0
	1.5%	1.0	1.0	1.1
	2%	1.1	1.3	1.5
Sunspray Ultra-Fine	1%	1.0	1.0	1.0
	1.5%	1.0	1.0	1.1
	2%	1.1	1.3	1.4
2010				
Sunspray 850 EC	1%	1.0	1.0	1.0
	1.5%	1.0	1.3	1.4
	2%	1.1	1.3	1.5
Sunspray Ultra-Fine	1%	1.0	1.2	1.2
	1.5%	1.0	1.3	1.5
	2%	1.2	1.4	1.5

1° – no damage; 9° – completely destroyed plants

Table 10. Effect of mineral oils on chlorophyll content in leaf of the tulip plant cultivar 'Leen van der Mark' in 2009–2010 (relative chlorophyll index (RCI) from 0 to 999)

Concentration	Sunspray 850 EC			Sunspray Ultra-Fine			Control	Mean
	1%	1.5%	2%	1%	1.5%	2%		
2009	202.2a	196.8a	189.4ab	189.6ab	168.6abc	165.8abc	199.1a	187.3A
2010	151.0c	175.6bc	151.8c	171.6abc	175.0abc	156.0bc	177.7abc	165.5B
Mean	176.6AB	186.2A	170.6AB	180.6AB	171.8AB	160.9B	188.4A	

LSD_{0.05} for A = 8.1; B = 22.8; AB = 36.7

Mean marked with the same letter do not differ significantly

Statistically significant differences in the way the two mineral oils acted, have not been demonstrated.

The strongest effect in reducing the spread of other pathogens was achieved in the second year of the study (2009). Plants protected with oils in the first (2008) and third (2010) year of observation were much stronger infected.

Greenness index. Measurements of leaf greenness index showed differences in the color of the test plants. The authors of numerous scientific papers report that the greenness index of plant leaves depends among other on irrigation and mineral fertilization [Niewiadomska and Sawicka 2010, Jaroszewska 2011, Podsiadło and Jaroszewska 2013, Radkowski 2013], growth regulators [Dobrowolska et al. 2004, Janowska et al. 2012], plant density [Jaśkiewicz 2009], habitat conditions [Kulig et al. 2012], application manner, and concentration of the agent used [Schroeter-Zakrzewska et al. 2013]. The scientists Asjes and Blom-Barnhoorn [2001], wheat treating the tulips of 'Halcro' and 'Apeldoorn' cv. with the oil preparations, observed that leaves of these plants were much brighter than untreated ones. Such situation has raised suspicion that the oil preparations can decompose the chlorophyll in the leaves of plants. Our study confirmed this relationship and showed that another factor influencing on the level of green pigment in plant leaves is the use of mineral oils. The highest concentration of total chlorophyll was found in plants treated with Sunspray 850 EC at 1.5% concentration, and in the control combination, and the lowest level of green pigment was found in the leaves of tulips treated with 2% Sunspray Ultra-Fine (tab. 10). The chlorophyll content in tulip leaves in

other combinations was comparable to that for the control plants. In opinion of Zabkiewicz [2000] as well as Skórska and Swarczewicz [2005], mineral oils, as compared with herbicides, penetrate deeper into the inner leaf tissue of treated plants, which can be explained by the decrease in adhesion strength onto the leaf surface. Such a phenomenon may be the main cause of physical damage to the leaf tissue [Finger 2000], and thus chlorophyll and, therefore, lowering its level in the plant.

In the first year of the study (2009), the total chlorophyll concentration in the leaves was comparable to plants sprayed with oils and constituting a control object. It was noted only a slight decrease in chlorophyll content in plants treated with oil Sunspray Ultra-Fine at concentrations of 1.5–2%. In the second study year (2010), level of green pigment was significantly lower in all combinations in relation to the previous year. The smallest content of chlorophyll was determined in the leaves of plants sprayed with Sunspray 850 EC at the lowest and highest concentration, as well as with Sunspray Ultra-Fine at the highest concentration, but these differences were not statistically significant.

Application of mineral oils at concentrations higher than 1.5% to protect tulip plants is unjustified, since it does not significantly affect the reduction in pests' number and improvement of plant health and even causes much stronger phytotoxicity reactions and diminished the level of chlorophyll in leaves. This condition may lead to a reduced rate of photosynthesis and, consequently, reduction in the condition of a plant [Wood and Payne 1986, Finger 2000, Urbanowicz and Wróbel 2005, Wróbel and Urbanowicz 2007].

Decorative value of plants. Mizell [1991], when sprayed 30 tree seedlings in Florida and 17 shrub seedlings in Georgia using 2% solution of Sunspray Ultra-Fine at 30°C, did not observe any phytotoxic changes on test plants.

However, in our study, the tulip plant of 'Leen van der Mark' cv. were characterized by a diverse reaction to the applied mineral oils (tab. 9). After application of oily substances at the lowest concentration (1%), no phytotoxic response of tulip plants (the maximum average value of 1.2 in a 9-degree scale) was found. After applying the Sunspray 850 EC, plants did not differ in their appearance from those in the control object. However, with increasing concentration, phytotoxic reaction also increased, which manifested with chlorosis of different intensity (yellowish, lighter green leaves). Symptoms of phytotoxic reaction intensified with increasing number of treatments. One can speculate that it was related to the "overlapping" symptoms after previous spraying. They were not, however, apparent effect on plant growth and development. Opinion that mineral oils can cause slight symptoms of phytotoxic reaction was also confirmed [McKenna 1999, Nikolov 2000, Wójdyła 2002, Iovieno et al. 2005, Urbanowicz and Wróbel 2005, Wróbel and Urbanowicz 2007]. It is also possible that the brighter green color seen on the leaves of tulips after applying the mineral oils is related to the distribution of chlorophyll. This assumption was confirmed by evaluation of the greenness index of the test plants leaves (tab. 10).

CONCLUSIONS

1. The use of mineral oil greatly limited the number of aphids present on the tulip plants, especially during their peak flights.

2. Much of the effectiveness of the mineral oils in reducing the infection of tulip bulbs by viruses in a transient way indicates the usefulness of preparations for tulip plantation protection.

3. The increase in concentrations of the mineral oil was associated with a reduction in their effectiveness in decreasing the tulip plant infection by other pathogens.

4. Level of chlorophyll in the test plants leaves was lower as compared to those of untreated plants, when they were used at a concentration above 1.5%. The plant response varied in different years and was dependent on the weather conditions.

5. It is recommended to apply three spray treatments with mineral oils at concentrations of 1–1.5%, so that the first treatment preceded the appearance of aphids on the plantation.

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