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DEVELOPMENT OF HIGH PURITY PARAFFIN OIL FOR THE SUSTAINABLE CONTROL OF POWDERY MILDEW IN GRAPE

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ABSTRACT

Mineral oils are currently used as winter treatment in arboriculture to limit wintering pests populations such as aphids, mites, mealybugs or psyllids. Their fungistatic effects are also proven and their use in the prevention of fungal diseases such as powdery mildew makes sense. Given the challenge of controlling powdery mildew in the vineyard, Koppert and its supplier committed four years ago to develop a paraffin oil, coded DEV1502. With a high degree of purity, Koppert study this oil in order to improve the selectivity of the product on the crop and to improve its effectiveness against the disease. In this article, two recent and representative test were selected from about thirty trials to demonstrate theselectivity and the efficacy of DEV1502against powdery mildew on grapes . The first test shows a good selectivity of the oil at different stages of the grapes (young leaves, flowers and bunches) with sulfur framing at the interval of 7 days and 12 days. The second test demonstrates an efficacy equivalent to sulfur when the oil is applied around flowering. In addition to its efficacy and its selectivity, the DEV1502 product meets the most stringent technical requirements and environmental standards. Thanks to its mode of action, the occurrence risk of resistance is non-existent. As this product has a wide range of targets (other developments in progress, particularly in summer bio-insecticide), DEV1502 can be integrated into complete programs and fulfill several tasks guaranteeing sustainable viticulture.

Keywords: paraffin oil, powdery mildew, grape, bio-fungicide, efficacy, selectivity.

RÉSUMÉ

DEVELOPPEMENT D’UNE HUILE PARAFFINIQUE HAUTEMENT PURIFIÉE POUR LE CONTROLE DE L’OIDIUM DE LA VIGNE

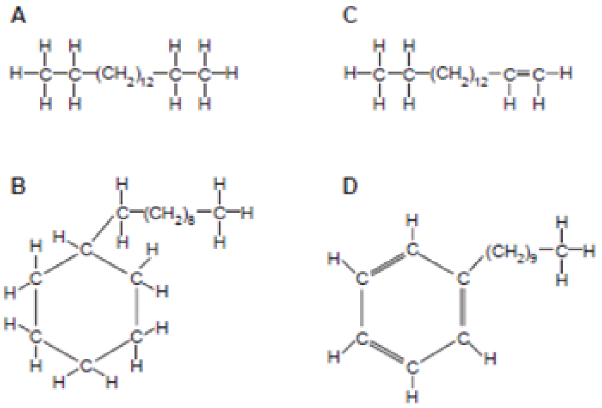
Les huiles minérales sont actuellement utilisées comme traitement en arboriculture pour limiter les populations de ravageurs hivernants telles que les pucerons, les acariens, les cochenilles ou les psylles. Leurs effets fongistatiques sont également prouvés et leur utilisation dans la prévention des maladies fongiques telles que l'oïdium a tout son sens. Compte tenu du défi que représente le contrôle de l'oïdium chez la vigne, Koppert s'est engagé il y a quatre ans à développer, avec son fournisseur, une huile de paraffine codée DEV1502. De haut degré de pureté, Koppert étudie le fonctionnement de cette huile pour améliorer la sélectivité du produit sur la culture et pour améliorer son efficacité contre la maladie. Dans cet article deux essais, récents et représentatifs, ont été selectionnés parmis une trentaine d’expérimentations, pour démontrer la selectivité et l’efficacité du DEV1502 contre l’oïdium de la vigne. Le premier essai montre une bonne sélectivité de l’huile sur différents stades de la vigne (jeunes feuilles, fleurs et grappes) avec un encadrement avec une préparation à base de soufre avec un intervalle de 7 et 12 jours. Le deuxième essai démontre une efficacité équivalente au soufre lorsque l’huile est appliquée en encadrement de floraison. Outre son efficacité et sa sélectivité, le produit DEV1502 répond aux exigences techniques et aux normes environnementales les plus strictes. De plus, de par son mode d'action, le risque d'apparition de résistance est nul. Le DEV1502 ayant un large éventail de cibles, tant d’un point de vue de biofongicide que bio-insecticide, il peut donc être intégré dans des programmes complets et garantir une viticulture durable.

Mots-clés : Huile paraffinique, oidium, vigne, bio-fongicide, efficacité, sélectivité.

INTRODUCTION

The latest generation paraffin oil is one of the many products derived from fractionation by distillation of petroleum. The process from which paraffin oil is derived aims to separate the different fractions that makes up oil but also and above all to eliminate aromatic compounds which have the disadvantage of being phytotoxic (see figure 1).

Figure 1: Chemical structure of paraffinic oil's compounds: A-Paraffin, B-Naphten, C-Olefin, D: Aromatics (Jacques and Kuhlmann 2002). (Structure chimique des composés d'une huile paraffinique: A-Paraffine, B-Naphtène, C-Oléfine, D-Aromatique)



Many discoveries make it possible to optimize oils derived from petroleum for a more appropriate use in crop protection. In 1915, De Ong demonstrated that sulfuric acid can remove phytotoxic compounds from oils that are in fact aromatic compounds and unsaturated compounds. Thus, the sulphonation index makes it possible to measure the refining degree of an oil, the higher it is, the more the oil is purified; belowan index of 92% the oil is potentially phytotoxic. Khulmann and Jacques proposed in 2002 a standardized classification and declined the oils derived from petroleum in three groups, with increasing degrees of refining:

- Mineral oils with a boiling temperature of 232 ° C ;
- Agricultural mineral oils (minimum sulphonation index of 92% , minimum of 60% of carbon) ;
- Horticultural mineral oils having the same properties as agricultural mineral oils but the length of the carbon chains must be between 21 and 25 carbon.

Since then, several theories have been accepted to explain the mode of action of mineral oils according to their chemical composition:

- Saturated heavy fractions (>nC23) form a physical barrier to respiration by blocking spiracles and kill the insect by asphixia ;
- Liquid light unsaturated fractions (<nC19) penetrate the tissues and dissolve the fatty substances destroying the cellular structure.

According to van Overbeek and Blondeau (1954), the unsaturated compounds of mineral oils are capable of solubilizing the lipidic membranes that makes up the cuticles of insects. This property helps to promote the desiccation of the insect since the cuticle prevents breathing. Thanks to this ability, oils are also used as an adjuvant for synthetic pesticides which help to penetrate the normally impermeable cuticle.

The fungicide mode of action of mineral oils has also been studied since they are widely used for control of Sigatoka leaf spot (*Mycosphaerella musicola* Leach). According to Mc Whorter (1927), Wilson (1961) and Northover & Schneider (1996), mineral oils have an anti-sporulating effect on powdery mildew in grapes, rose and celery septoria. In Northover & Scheider study (1996), three oils showed moderate efficacy on the powdery mildew in grape in preventive stategy and the oils showed an excellent anti-sporulating action, even if the surfactant used in the formulation seems to play a role in the effectiveness. Thanks to the evolution of refining processes, to the understanding of their mode of action and their lack of selectivity, modern mineral oils are increasingly adapted to be used in crop protection, as much in winter as in summer period.

MATERIAL AND METHOD

1) SELECTIVITY TEST OF DEV1502 AT DIFFERENT SCHEDULE AND BBCH STAGES ON GRAPEVINE WITH A SULFUR COMPOUND (2018).

Given the potential risk that the vine expresses phytotoxicity with the use of oil in summer, we elaborated a protocol to evaluate the risk. We choose to test the selectivity of oil alone on three sensitive stages: young leaves, flowers and clusters, respectivelly BBCH stage 14-15/ stage 63-65/ stage 75-77 . The second objective was to evaluate the compatibility of DEV1502 and Sulfur applications with two intervention intervals: 7 days and 12 days.

Biological material

To test the phytotoxicity on grapes, the chosen variety was “Grenache” because it is one of the most sensitive variety. The vineyard was vigorous and homogeneous, it didn’t show physiological stresses.

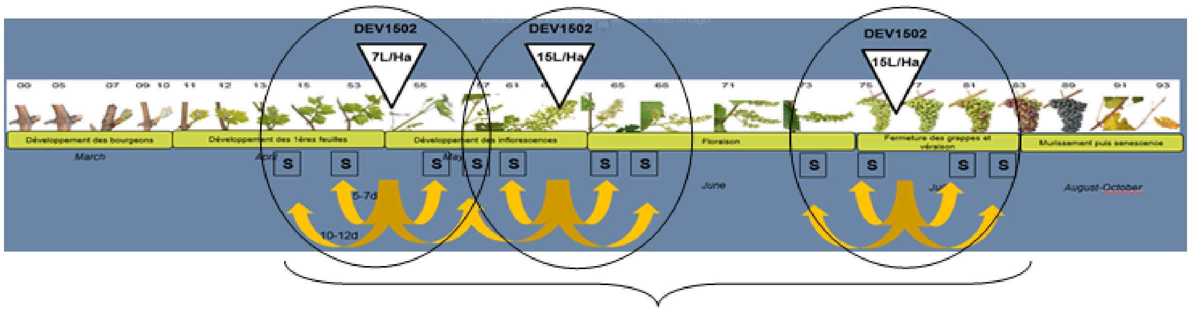
Experimental design and device

The trial set up was a randomized block in three repetitions and fourteen modalities, the number of plants per plot is ten and the plot size is 25m². The spraying liquid is applied to the plants by means of a pneumatic airblast sprayer with a centrifugal pump.

Treatments

Treatments were applied with a water volume between 200L/Ha and 300L/Ha. DEV1502 applications (7L/Ha and 15L/Ha) were made at BBCH stage 14-15/ stage 63-65/ stage 75-77. Sulfur applications were made with Sulfur(7.5Kg/Ha) 5-7 days before and after each DEV1502 treatments and 10-12 days before and after DEV1502 treatments (see figure 2).

Figure 2: Diagram of the experimental protocol: S=sulfur treatments; (Schéma du protocole d’expérimentation,S=soufre)



	Sulfur 12days	Sulfur 7 days	DEV1502	Sulfur 7 days	Sulfur 12 days
Untreated					
Check DEV1502 solo			X		
Check Sulfur solo (7d)		X		X	
Sulfur/DEV1502/Sulfur (7d)		X	X	X	
Check Sulfur solo (12d)	X				X
Sulfur/DEV1502/Sulfur (12d)	X		X		X

Assessment

No efficacy assessment was made, only phytotoxicity was observed 3 days after each treatment then 10 days, 21 and 28 days after each treatment. If phytotoxicity was observed, the type and the part of the damaged plant was reported (using a scale from 0 to 10, respectively no phytotoxicity to burning symptoms) and pictures were token. Side effect on beneficials was assessed if presence and product’s solubilization was visually observed during mixing.

Statistical analysis

All statistical analyses were performed using the ARM software. The analysis of variance was made using parametric test (ANOVA) on the percentage of phytotoxicity at each observation date. Then identification of the statistical groups using the Newman-Keuls (parametric) or Mann-withney (nonparametric) test.

2) EFFECTIVENESS ASSESSMENT OF DEV1502 AT THE BEGINNING AND END OF THE PROTECTION PROGRAM AGAINST POWDERY MILDEW IN COMPARISON WITH A SULFUR REFERENCE (FRANCE, 2017)

Experimental design and device

A Fisher block device with four repetitions is adopted and a control is included in the device. The elementary parcel has eight consecutive useful grapevines on a row. The four repetitions are placed on four rows of grapevine separated from each other by an untreated guard row, to increase the parasite pressure and to protect from any drift.
The treatment (water volume variable from 150 to 250 L / ha depending on the vegetative development), are applied using a motor sprayer (atomizer).

Treatments

The evaluation is carried out on the basis of a comparison of five treatmentsmodalities to an untreated control. The products making up the protocol were applied at a rate of 8-10 days at the beginning and end of the program and 12-14 days in the middle of the program. Applications are made between stage BBCH 14 (4 leaves spread) and stage BBCH 77 (early closing of cluster). This test was set up on the Carignan grape variety, in a vineyard usually showing significant symptoms of powdery mildew attack (see table I).

Table I: Application protocol (Protocle d'application)

		Treatment	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
		BBCH stage	14	55	55	55	57	61	71	75	77
Modality	Active substances	Rate/Date	07/04/2017	17/04/2017	20/04/2017	25/04/2017	04/05/2017	18/05/2017	01/06/2017	15/06/2017	23/06/2017
1 UNTREATED											
2	Sulfur	Evolutive (kg/ha)	6	8		10				12.5	12.5
	Fluopyram+Trifloxystrobin	0.15 L/Ha					X				
	Metrafenone	0.2 L/Ha						X			
	Proquinazid	0.25L/Ha							X		
3	DEV1502	Evolutive (L/ha)	7	10		12				15	15
	Fluopyram+Trifloxystrobin	0.15 L/Ha					X				
	Metrafenone	0.2 L/Ha						X			
	Proquinazid	0.25L/Ha							X		
4	DEV1502	Evolutive (L/ha)	7	10		12					
	Fluopyram+Trifloxystrobin	0.15 L/Ha					X				
	Metrafenone	0.2 L/Ha						X			
	Proquinazid	0.25L/Ha							X		
	Sulfur	12.5 Kg/ha								12.5	12.5
5	Sulfur	Evolutive (kg/ha)	6	8		10					
	Fluopyram+Trifloxystrobin	0.15 L/Ha					X				
	Metrafenone	0.2 L/Ha						X			
	Proquinazid	0.25L/Ha							X		
	DEV1502	15 L/ha								15	15
6	DEV1502	15 L/Ha	15		15						
	Fluopyram+Trifloxystrobin	0.15 L/Ha					X				
	Metrafenone	0.2 L/Ha						X			
	Proquinazid	0.25L/Ha							X		
	Sulfur	12.5Kg/Ha								12.5	12.5

Assessment

Two assessments were made on leaves and clusters, allowing an optimal evaluation of the strategies implemented throughout the cycle of the grapevine. The first observation on leaves and clusters occurred on June 14th and the final rating was on July 4th at the closing cluster stage. Frequency of attack was assessed evaluating the presence of the disease on 100 leaves or on 100 clusters. The intensity of attack was measured by estimating the attacked surface on 100 leaves and on 100 clusters.

Statistical analysis

Statistical analysis relate to the raw data, the averages of each factor are compared by the Newman-Keuls test to the 5% risk threshold, and the resulting statistical groups are identified by letters. In addition, the modalities are compared to the reference using the Dunnett test.

RESULTS

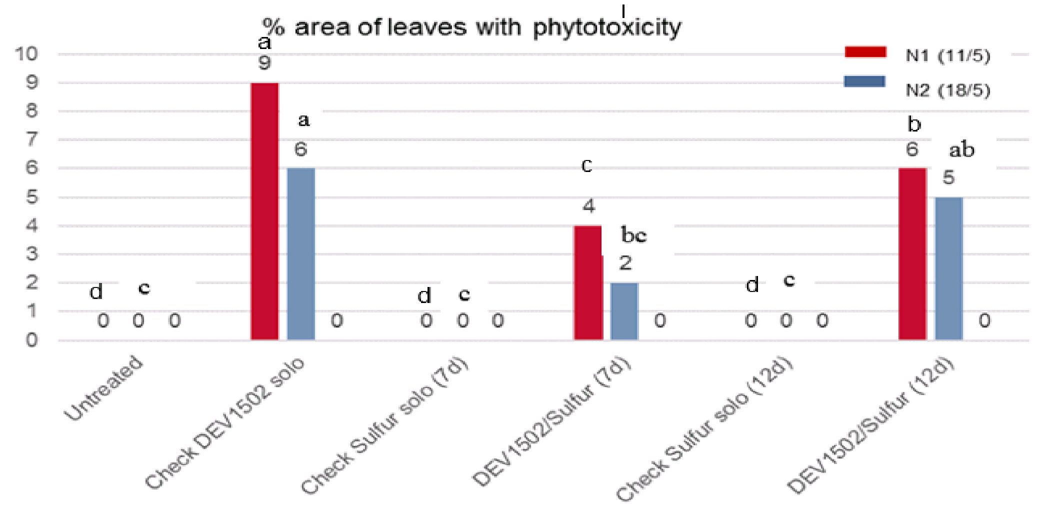
1) SELECTIVITY TEST OF DEV1502 AT DIFFERENT SCHEDULE AND BBCH STAGES ON GRAPEVINE WITH A SULFUR COMPOUND, 2018.

No side effect on beneficials were observed, the solubilization of the product was satisfactory.

<u>Young leaves</u>

The first DEV1502 application was made the 8th of May, 3 days after (N1) and 10 days (N2) after observations were made. Symptoms of phytotoxicity were observed in the modality DEV1502 alone whereas in modalities sulfur alone (7 days and 12 days) no symptom was observed. Application of DEV1502 framed by sulfur (at 7 and 12 days) caused phytotoxicity (see figure 3).

Figure 3: Phytotoxicity assessment on young leaves after application of DEV1502 framed or not by sulfur applications (7 and 12 days). (Observation de la phytotoxicité sur jeunes feuilles de vigne après une application d'huile paraffinique encadrée ou non par des traitements au soufre-7 et 12 jours).



Even if symptoms were present, they were qualified as very acceptable. As showed in Figure 4,. it corresponds to small oily and darker spots.

Figure 4 : Picture of phytotoxicity symptoms 3 days after application of DEV1502 on young leaves of Grenache variety. (Photographies des symptômes de phytotoxicité 3 jours après l'application du DEVI502)

In all modalities, symptoms were acceptable and disappeared 20 days after DEV1502 application,.

Flowers

No phytotoxicity symptoms were observed on flowers.

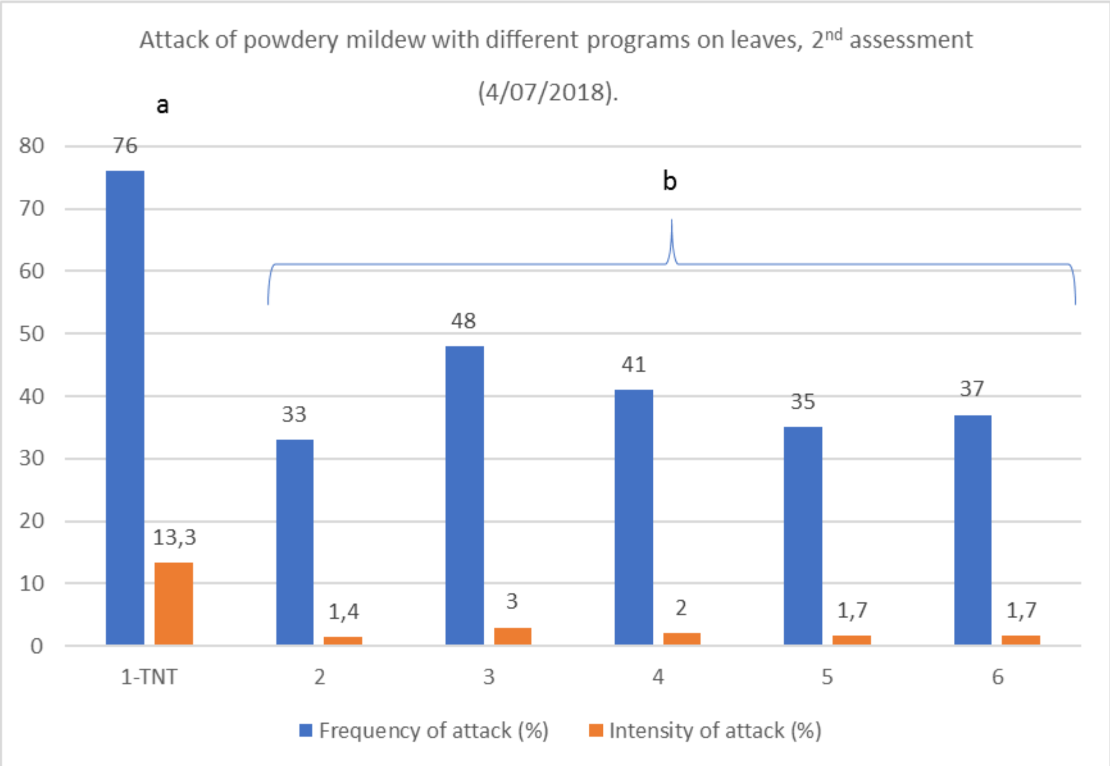
Bunches

No phytotoxicity symptoms were observed on bunches.

2) Effectiveness Assessment of DEV1502 at the beginning and end of the protection program against powdery mildew in comparison with a Sulfur reference (France, 2017).

The first observation on leaves occurred on June 14th, showing low disease pressure (4.1% in intensity and 57% in frequency). Efficacy of the different modalities were not statistically different, except for the control.

Figure 5 : Attack of powdery mildew in different programs on leaves, 2nd assessment (4/07/2018). (Attaque d'oidium sur feuilles selon différents programmes, 2ème notation, 04/07/2018).

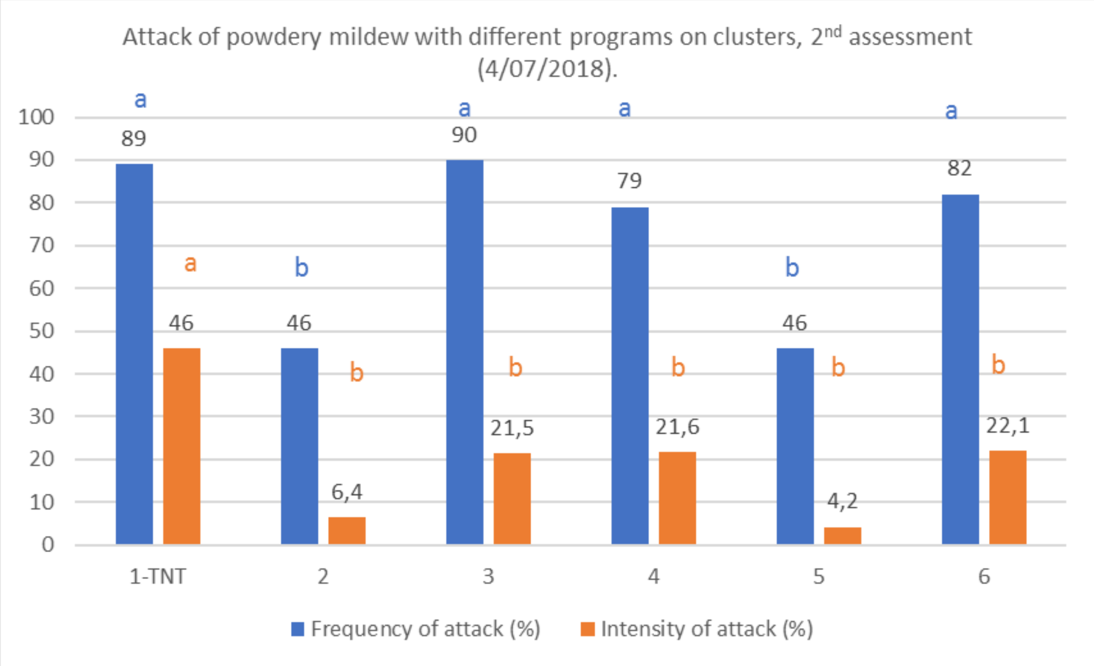


At the second assessment on leaves (04/07/2018), the attack increased in the control with an intensity which reached 13.3% and 76% in frequency. Regarding the intensity of the attack, when the control is excluded, modality 3 (DEV1502 at the beginning and at the end of the program) is not statistically different from the modalities 4, 5 and 6 but statistically different from the sulfur reference (modality 2), with a lower efficacy. This is confirmed by the Dunett test (see figure 5)

For the frequency of attack, all modalities are different from the control but there is no significant statistical difference between terms.

During the first cluster assessment, carried out on the same date as that on leaves, powdery mildew was already strongly implanted, with an intensity of 23.0% and a frequency of 81% of affected clusters. This first notation does not allow to distinguish the modalities between them, neither in attack intensity nor frequency.

Figure 6: Attack of powdery mildw indifferent programs on clusters, 2nd assessment (4/07/2018). (Attaque d'oidium sur grappes selon différents programmes, 2ème notation, 04/07/2018).



This second assessment on clusters done at the end of the test shows the increase of the powdery mildew pressure, with 89% of the grapes affected and a consequent intensity of 46% in the Control. This level is already extreme, the harvest can be considered totally unsuitable for vinification with such a level of powdery mildew on bunches.
For intensity, modalities 3, 4 and 6 present positive results (respectively 21.5%, 21.6%, 22.1%) but they seem less effective than modalities 2 and 5 . Modality 5 seems to be the most effective. However, the Newman-Keuls and Dunnett tests show that the differentiation is not statistically significant. Indeed, for the Newman-Keuls test, the results are equivalent. For the Dunnett test, there is no modality that differs from the reference.
The increase in the frequency of attack allowed the differentiation of the strategies. Indeed, modalities 2 and 5 (46% attack) get the best efficacies. This is confirmed by the Newman-Keuls test. According to the DUNETT test, modalities 3, 4 and 6 have a higher attack frequency than the reference (see figure 6)..

DISCUSSION

1) SELECTIVITY TEST OF DEV1502 AT DIFFERENT SCHEDULE AND BBCH STAGES ON GRAPEVINE WITH A SULFUR COMPOUND (FRANCE, 2018).

Grenache variety was chosen because it is known to be sensitive to phytotoxicity and choosing three different BBCH timings of application allow us to test different organs and different climatic conditions. Moreover, we chose to frame DEV1502 applications with sulfur because some incompatibility exist in tank mix. The objective of this test is to refine the recommendations of use and the positioning of the product in a compete program.
In the trial, phytotoxicity appeared only at the stage BBCH 14-15 when oil was applied alone and also when it was framed by sulfur. Sulfur alone didn't induce symptoms so we can conclude that this very light symptoms are caused by oil. The dose of oil at this stage was 7L/Ha compared to 15L/Ha at stage 63-65 and 75-77 with the volume of water (200L/Ha). The volume of oil deposited is, according to Ebeling (1932), a factor influencing its effectiveness and its phytotoxicity. The concentration, the emulsifier, the spraying equipment, the dose per tree and the treated surface are parameters that will play on this volume. May be the phytotoxicity observed in this case is due to the low leaf area at stage 15. Indeed, between stage 15 and stage 63 or 75, leaf area double or triple. If the product dose is not adapted to the leaf area, it was maybe too high at the first application of DEV1502. In addition to this

consideration, symptoms were very mild and acceptable. Symptoms were not increasing when the oil was surrounded by sulfur. We can conclude that sulfur can be applied 5-7 days after DEV1502 on the grenache variety under the experimental conditions of the test.
Concerning the other stages, no symptoms were observed even with dry and hot climatic conditions during flowering and clustering. We can conclude that DEV1502 is selective on Grenache and the sulfur can be applied 5-7 days before and after DEV1502 on the grenache variety under the experimental conditions of the test.

2) Effectiveness assessment of DEV1502 at the beginning and end of the protection program against powdery mildew in comparison with a Sulfur reference (France, 2017)

At the selectivity level, no symptoms of phytotoxicity appeared, DEV1502 turns out to be totally selective on the culture.
Under the extreme pressure conditions of this powdery mildew test on grapevine, evaluation of the effectiveness of DEV1502 applied around floweringcompared to a reference sulfur applied around flowering allowed to put forward certain hypothesis . Indeed, DEV1502 seems toto maintain the intensity and frequency of the attack on leaves as on clusters at the level of the reference in the case where it is applied at the end of the program as in modality 5. A modality with no treatment after flowering could have made it possible to validate this hypothesis. Indeed, in this modality, the results remain close to the reference strategy (modality 2) throughout the notations on leaves and on clusters. In addition, a significant reduction of the attack is observed compared to the control, with nearly 50% attack reduction. Conversely when the DEV1502 is used at the beginning of the program (modalities 3, 4 and 6), the pressure of the attack is not as well controlled as modality 2 and 5 with, according to the DUNETT Test, a frequency of attack more important compared to the reference but an equivalent intensity.

CONCLUSION

Paraffinic oils are commonly known for their insecticide winter uses. However, in this paper are exposed two types of trial which illustrate how a mineral oil can performed as a biofungicide and how it can be used during summer period. The two challenges to demonstrate are the selectivity of the DEV1502 for grapevine as well as the anti-powdery mildew efficiency.

Several studies have investigated the effect of mineral oils on the physiology of plants. Two types of effects are reported by Hodgkinson *et al.* (2002): acute phytotoxicity and chronic effect. In these tests (and many others in grapevine), no effect on plant growth is observed and negligible effects were observed on leaves. So DEV1502 can be considered as selective to grape. Because some mild oily stains were observed in early stages, the test suggests that it could be interesting to study the effects of DEV1502 on leaf with Leaf Wall Area system. This trial also suggests that sulfur can be applied at 7days interval with the oil without causing damages on leaves, flowers and bunches.
Several studies prove the interest of mineral oils for controlling ectoparasite as powdery mildew in many crops. Indeed, Northover & Scheinder (1996) showed a moderate efficiency of three oils on powdery mildew in grapevine in preventive strategy. All these experiments make it possible to refine the positioning of DEV1502 in conventional programs. It must therefore be positioned after flowering when periods of risk have been avoided. The DEV1502 can bring a complementary action in the control of powdery mildew in vineyards. According to the results of Nicetic et al (2002), mineral oils show a potential to significantly reduce the use of synthetic fungicides used against powdery mildew in tomato crops. The moderate action of DEV1502 could be used to decrease the use of chemicals. Thus, it could be interesting to position DEV1502 in tank mix with conventional products and study the potential reducing of doses. The optimization of this oil has made it possible to use it into integrated protection programs for several crops as vineyard and during summer. Of natural origin, DEV1502 will be listed in France on the list of biocontrol products, it presents no risk for resistance and no residue, It meets all the requirements for safe and sustainable agriculture.

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