Végéphyl – 12e CONFÉRENCE INTERNATIONALE SUR LES MALADIES DES PLANTES
TOURS – 11 ET 12 DÉCEMBRE 2018

DEVELOPMENT OF A YEAST *METSCHNIKOWIA FRUCTICOLA* FOR THE CONTROL OF PRE AND POST HARVEST DISEASES IN STONE FRUITS, VINE AND SOFT FRUITS

K. BOULY[1], S. DE WEERT[2] ET T. VERFAILLE[3]
[1] Koppert France, 147 Avenue des Banquets 84200 Cavaillon, France, kbouly@koppert.fr
[2] Koppert BV , Box 155 2650 AD Berkel en Rodenrijs, The Netherlands, sdweert@koppert.nl
[3] Koppert France, 14 rue de la communauté, 44860 Pont St Martin, France, tverfaille@koppert.fr

ABSTRACT

Chemical pesticides are widely used to control pre- and post-harvest diseases, particularly in orchards, vineyards and vegetables. Public concern about consumer exposure to toxic residues has led to research for safer alternatives, therefore several biocontrol solutions have emerged. The yeast *Metschnikowia fructicola* NRRL Y-27328 was isolated in Israel from table grapes. A production process was developed to produce the biofungicide and used to control pre-harvest and post-harvest diseases in stone fruit, grape and soft fruits. This yeast acts by antagonism and competition for space against the microorganisms responsible for diseases like *Botrytis*, *Aspergillus*, *Monilia*, *Rhizopus*, *Sclerotinia*. The laboratory results were validated by field trials for the control of *Botrytis* in grapevine and strawberry and control of peach moniliosis. The product is formulated in dispersible granules and has a shelf life of two years stored between 4 and 8°C. It can be applied with a conventional sprayer and is compatible with most chemical pesticides. As soon as the registration is finalised (scheduled for 2019), this new biocontrol solution will be usable in Organic Agriculture (OA), it will reduce TFI (Treatment Frequency Index) and fulfill the demand for zero residues.

Keywords: Antagonist yeast, biocontrol, pre- and post-harvest diseases, residues, Integrated Pest Management.

RÉSUMÉ

DEVELOPPEMENT D'UNE LEVURE *METSCHNIKOWIA FRUCTICOLA* POUR LE CONTROLE DES MALADIES PRÉ- ET POST-RECOLTE EN FRUITS À NOYAUX, VIGNE ET FRUITS ROUGES

Les pesticides chimiques sont largement utilisés pour la prévention des maladies pré- et post-récolte notamment en arboriculture, en viticulture et en maraîchage. L'opinion publique réclamant de plus en plus de sécurité vis-à-vis des résidus chimiques, un certain nombre de solution de biocontrôle ont vues le jour. La levure *Metschnikowia fructicola* NRRL Y-27328 a été isolée en Israël sur des grains de raisin de table. Le processus de production a été travaillé durant plusieurs années pour aboutir au produit codé KM1110, biofongicide à usage maladies prés et post récolte en fruits à noyaux, vigne, fraisiers et fruits rouges. Cette levure agit par antagonisme et par compétition spatiale et nutritionnelle avec les micro-organismes responsables des maladies type Botrytis, Aspergillus, Monilia, Rhizopus, Sclerotinia. Les résultats de laboratoire ont été validés par des essais d'efficacité en plein champ pour le contrôle du botrytis de la vigne, de la fraise et de la moniliose des pêchers. Le produit est formulé en granulés dispersibles et se conserve deux ans à une température comprise entre 4 et 8 °C. Il peut être appliqué avec un pulvérisateur classique et est compatible avec la plupart des pesticides chimiques. Dès l'obtention de l'Autorisation de Mise sur le Marché (AMM, attendue pour 2019), cette nouvelle solution de biocontrôle sera utilisable en Agriculture Biologique (AB) et permettra de diminuer les Indices de Fréquence de Traitement (IFT) et de répondre à la demande de zéro résidu.

Mots-clés : Levure antagoniste, biocontrôle, maladies pré- et post-récolte, résidus, Protection Biologique Intégrée.

INTRODUCTION

What makes *Metschnikowia fructicola* interesting for biocontrol industry is its antagonistic properties against postharvest pathogens was first suggested by its discoverer (Kurtzman and Droby, 2001). It was patented in 2001 as a biological control agent by the agricultural research organization of Israel and developed into a commercial product.

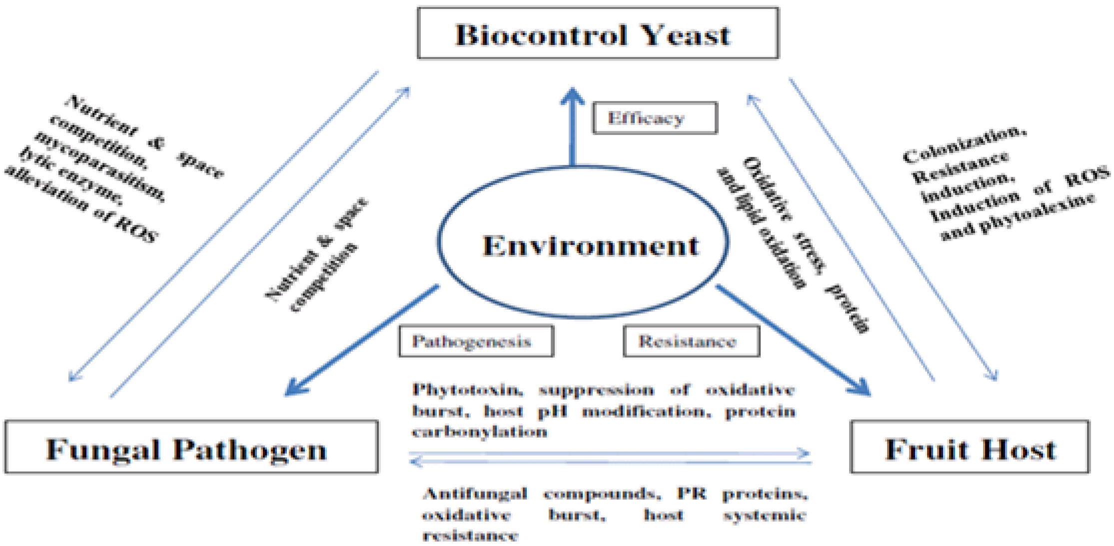
- Description of the organism

Metschnikowia fructicola (accession codes: AF360542, NRRL. Y-27328, CBS 8853) is a yeast from the order of the *Saccharomycetales* that is closely related to *Metschnikowia pulcherrima* (also an antagonistic yeast).

- Antagonistic yeast

Chemical pest control has its issues with public relations as demonstrated by the negative public opinion on the matter and the biological farming movement. Chemical control is also increasingly regulated and has a negative impact on the environment. This opens up opportunities to explore biological disease control. As a result the use of an antagonistic yeast for the control of postharvest pathogens has sprung up as a major area of study (Droby *et al.*, 2009 ; Liu *et al.*, 2013). Although it remains unclear as to how exactly the antagonistic properties emerge from biological interactions there has been significant progress into the subject of antagonistic yeasts. Researchers have proposed several modes of action to explain these antimicrobial properties, these include but are not limited to: nutrients and space competition (Filonow, 1998 ; Droby *et al.*, 2009) ; mycoparasitism (Hershkovitz *et al.*, 2012 ; Banani *et al.*, 2015) ; Induction of plant defenses (Xu *et al.*, 2008 ; Macarisin *et al.*, 2010); Production of Reactive Oxygen Species (ROS) (Xu *et al.*, 2008); Production of lytic enzymes (Xu *et al.*, 2008 ; See Figure 1 for a schematic overview of the different interactions).

Figure 1: Diagram of the interactions and responses between host, biocontrol yeast and fungal pathogen (schéma des interactions et réponses entre l'hôte, la levure de biocontrole et le champignon pathogène.)



In *Metschnikowia fructicola* specifically research into chitinase production and its effects on the pathogens *Monilinia fructicola* and *Monilinia laxa* has been performed (Banani *et al.*, 2015). The gene involved into chitinase production, MfChi, was tested for its efficacy against the pathogens and it was determined that MfChi has a strong deleterious effect on the germination percentage and germ tube lengths of *Monilinia fructicola* and *Monilinia laxa.* Another interaction that has been described for *Metschnikowia fructicola* is the production of ROS and the effects it has on the transcription regulation in grapefruit peel. It was discovered that *M. fructicola* produces ROS and that grapefruit peel inoculated with *M. fructicola* shows a significant increase in ROS. This is relevant because ROS are a part of the intrinsic immune response of plant (Mehdy *et al.*, 1996). This indicates that *M. fructicola* is in some way modulating the immune response of the host plant while at the same time producing its own effectors against fungal pathogens (or competitors).

- Efficacy of *M. fructicola* as crop protection

Assessing the usefulness of an organism as a pest control mechanism requires field trials and co-inoculation studies. There is litterature describing the efficacy of *M. fructicola* as a postharvest treatment and as a preventative measure during the growing season. Field trials on strawberries in Israel and Turkey have demonstrated that spraying the plants with *M. fructicola* during the growing season can control pathogen induced postharvest decay of strawberries similarly to fenhexamid-based fungicide (Karabulut *et al.*, 2004). Researchers also discovered that efficacy was dependent on high yeast densities on the fruit, this however required weekly treatments during fruiting period. Trials on grapes spraying only once (24 hours before harvest) were successful in reducing pathogen induced spoilage by approximately half compared to control, and yielded similar results to surface sterilization with ethanol and sulfur dioxide in most cases (Karabulut *et al.*, 2004). This report will be focusing on proving the efficacy of *M. fructicola* as crop protection in field trials more specifically in peach and in strawberry.

MATERIAL AND METHODS

1) BIOLOGICAL EFFICACY EVALUATION OF *M. FRUCTICOLA* AGAINST FRUIT ROT, *BOTRYTIS CINEREA*, ON STRAWBERRY (2013, BELGIUM)

The aim of this trial is to evaluate the efficacy of *M. fructicola* (KM1110) against fruit rot (*Botrytis cinerea*) on strawberries.

Biological material

The strawberries were planted in 2012. The variety is Elsanta.

Experimental design and device

The trial design is set up as a randomized block in 4 repetitions, the number of plants per plot is 33.3 and the plot size is 5m^2. The spraying liquid is applied to the plants by means of a motor backpack sprayer, model Sthil SR 430.

Treatments

The fungicides were applied with a water volume of 1000 L/Ha treated strawberry field.

Table I : Dates of applications on strawberries, weather conditions and phenological stages. (Dates d'application, conditions météorologiques et stades phénologiques sur fraisiers.)

	Date	Number of days between treatments	Temperature	Relative humidity (%)	Sunshine	Rainfall (l/m^2) between Treatments	Phenological stage (BBCH)
A	14/05/13		15.0	82	0h30		60
B	21/05/13	7	15.0	88	0h06	21.4	61
C	28/05/13	7	20.0	49	9h42	38.0	65
D	04/06/13	7	11.0	76	9h27	15.0	65-67
E	12/06/13	8	18.0	85	2h40	0.0	67-81

Table II: Tested substances and doses on strawberry (Substances testées et doses sur fraisiers)

Obj. No.	Fungicide	Formulation	Active ingredient		Formulated product	
			g/100 m treated strawberry band	g/ha treated strawberry field (TSF)	g, ml/100 m strawberry band	g,ml/ha strawberry field
1.	Check					
2.	Frupica **ACE** (mepanipyrim)	50.0 WP	3.00	300.0	6.0	600.0
	Teldor **BD** (fenhexamid)	50.0 WG	7.50	750.0	15.00	1500.0
3.	KM1110 **A**	56.0 WG	11.20	1120.0	20.00	2000.0
	Frupica **B** (mepanipyrim)	50.0 WP	3.00	300.0	6.00	600.0
	Teldor **C** (fenhexamid)	50.0 WG	7.50	750.0	15.00	1500.0
	KM1110 **DE**	56.0 WG	11.20	1120.0	20.00	2000.0
4.	Frupica **B** (mepanipyrim)	50.0 WP	3.00	300.0	6.00	600.0
	Teldor **C** (fenhexamid)	50.0 WG	7.50	750.0	15.00	1500.0
5.	Frupica **A** (mepanipyrim)	50.0 WP	3.00	300.0	6.00	600.0
	Teldor **B** (fenhexamid)	50.0 WG	7.50	750.0	15.00	1500.0
	KM1110 **CDE**	56.0 WG	11.20	1120.0	20.00	2000.0
6.	Frupica **A** (mepanipyrim)	50.0 WP	3.00	300.0	6.00	600.0
	Teldor **B** (fenhexamid)	50.0 WG	7.50	750.0	15.00	1500.0
7.	KM1110 **ABCDE**	56.0 WG	11.20	1120.0	20.00	2000.0

Assessment

Activity against fruit rot: at each picking date, the number of infected fruits is counted per plot. The yield of sound fruits is weight. From the 4 replicates, 100 fruits were chosen randomly and weighed to determine the fruit weight average. In this way, the percentage of infected fruits and the corrected efficacy (Abbott value) of the test substances can be calculated. When the botrytis infestation is too low at harvest, the sound fruits are stored in a cooled room at 6°C and regularly evaluated on fruit rot symptoms.

Determination of yield: The trial was harvested at several picking times. At each picking time the total yield in Kg/plot is mentioned. This give a figure of possible side effects of the treated objects on yield.

Statistical analysis

All statistical analyses were performed using the Unistat statistical package, version 6.0. A completely randomized block design is used as standard for the statistical analysis. Prior to the analysis of variance, the homogeneity of variance is checked with Bartlett's Chi-Square and Bartlett Box F test. The original or transformed data are analysed with the GLM (General Linear model) procedure with different output options (Anova, table of means, etc). Treatment means are separated in a standart way by Duncan's multiple range test (5 % level).

2) Efficacy of KM1110 for the control of monilia on peach

Monilia disease is one of the most important disease in stone fruits. In conventional farming, several products exist to protect efficiently crops against fungi but the current context is to reduce TFI and chemical residues detected in fruits. This trial's objective is to evaluate the efficacy of a yeast-based biofungicide against conservation's diseases.

Biological material

The variety of peach is Couloured. Trees are planted at 6m*3.5m

Experimental design and device

The trial is implemented in randomized Fisher blocks with 4 repetitions with 42m^2 plots' surface.Treatments

Treatments were realized with backpack sprayer STHIL n°2 and 5, nozzle of 1.6. Application volume: 600 then 700 L / ha.

Table III: Tested substances and doses for the control of monilia on peach (Substances testées et doses sur moniliose de la pêche)

Fungicide	Active substance	Formulation	Concentration
KM1110	Metschnikowia *fructicola*	WG	490-550g/kg
Squad (adj)	Triglycéride ethoxylé	EC	790 g/L
Luna Experience	Fluopyram + tebuconazole	SC	200 g/L + 200 g/L
Signum	Boscalid + pyraclostrobine	WG	267g/kg + 67 g/kg
Impala	Fenbuconazole	EW	50 g/L

Table IV : Dates of applications, phenological stages for the control of monilia on peach. (Dates d'application et stades phénologiques sur moniliose de la pêche)

Modality / Date	09/08 : Harvest -16days	17/08 : Harvest -8days	22/08 : Harvest -3days	Harvest (H) destruction
1- Untreated plot	/	/	/	
2- Ref	SIGNUM 0.75 kg/ha	LUNA 0.5 L/ha	IMPALA 1 L/ha	No
3- KM1110	SIGNUM 0.75 kg/ha	LUNA 0.5 L/ha	KM1110 2kg/ha + SQUAD 0.5 L/ha	Yes

Assessment

Before treatment, 10 branches (about 10 fruits) are marked. Then, different assessment are done: observation of all fruits on marked branches and counting of fruits with symptoms per branch (2 harvest periods). After harvest, 45 fruits per tree are picked and stored at 3°C for 48 hours before being transferred to a room at 21 ° and high humidity. The evolution of the number of attacked fruits is counted every 2 to 3 days.

Statistical analyses

Tests performed: Analysis of variance using parametric test (ANOVA) or non-parametric test (Kruskal-Wallis) on the cumulative percentage of rotten fruit during storage at each observation date. Then identification of the statistical groups using the Newman-Keuls (parametric) or Mann-withney (nonparametric) test.

RESULTS

1) BIOLOGICAL EFFICACY EVALUATION OF *M.FRUCTICOLA* AGAINST FRUIT ROT, *BOTRYTIS CINEREA*, ON STRAWBERRY (2013, BELGIUM)

Total Yield

The total yield per plant was 0.600kg for the control, 0.619 kg for the modality 4 and between 0.659 kg and 0.726kg for the others modalities.

The mean number of fruits per plant was 43.2 in the control plot, 42.5 for the modality 4 and from 45.1 to 50.5 fruits per plant in others modalities with respectively a fruit weight mean of 13.9 g, 14.6 g and from 14.4 g to 14.8 g.

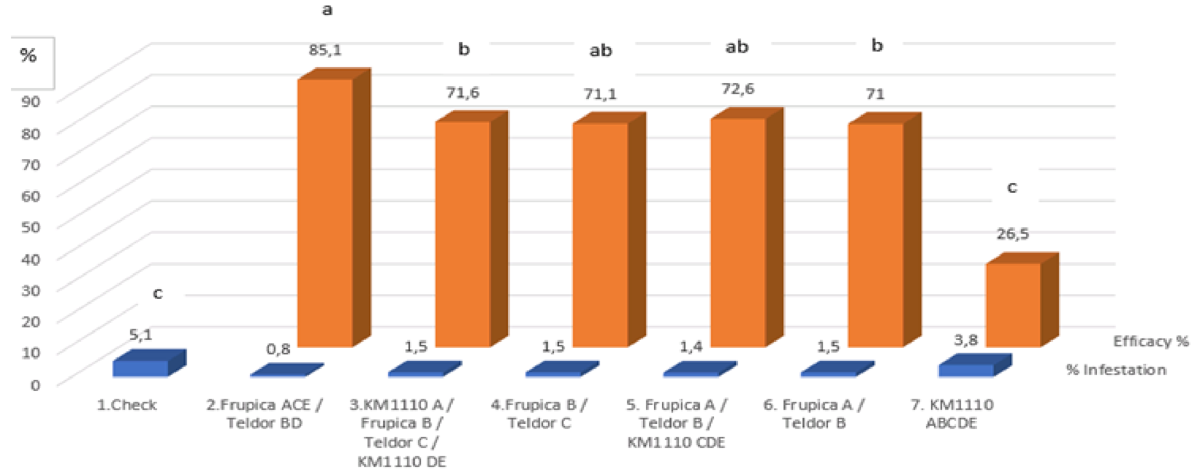
Table V : Total yield and number of fruits per plant and average fruit weight (AFW). Observation dates: 21/06, 28/06, 02/07, 09/07/2013. (Rendement total et nombre de fruits par plante et poids moyen des fruits. Dates d'observation : 21/06, 28/06, 02/07, 09/07/2013)

Obj. No.	Fungicide	Formulation	Active ingredient g/ha TSF	Plot			Mean		
				Total yield/ plant (kg)	Total fruits/ plant	AFW g	Total yield/ plant (kg)	Total fruits/ plant	AFW g
1.	Check			0.458	33.4	13.70	0.600	43.2	13.9
				0.669	45.5	14.69			
				0.693	50.7	13.67			
				0.580	43.1	13.45			
2.	Frupica **ACE**	50.0 WP	300.0	0.690	49.4	13.96	0.726	50.5	14.4
	Teldor **BD**	50.0 WG	750.0	0.850	59.1	14.36			
				0.713	48.6	14.66			
				0.650	44.8	14.52			
3.	KM1110 **A**	56.0 WG	1120.0	0.721	48.5	14.88	0.691	46.7	14.8
	Frupica **B**	50.0 WP	300.0	0.645	45.7	14.11			
	Teldor **C**	50.0 WG	750.0	0.707	46.0	15.38			
	KM1110 **DE**	56.0 WG	1120.0	0.593	41.7	14.21			
4.	Frupica **B**	50.0 WP	300.0	0.660	43.9	15.02	0.619	42.5	14.6
	Teldor **C**	50.0 WG	750.0	0.607	40.4	15.05			
				0.588	43.0	13.66			
				0.623	42.7	14.58			
5.	Frupica **A**	50.0 WP	300.0	0.534	36.8	14.51	0.659	45.1	14.6
	Teldor **B**	50.0 WG	750.0	0.656	47.2	13.88			
	KM1110 **CDE**	56.0 WG	1120.0	0.829	55.2	15.03			
				0.617	41.3	14.92			
6.	Frupica **A**	50.0 WP	300.0	0.611	43.0	14.21	0.664	45.3	14.6
	Teldor **B**	50.0 WG	750.0	0.733	50.7	14.47			
				0.641	42.0	15.27			
				0.669	45.7	14.64			
7.	KM1110 **ABCDE**	56.0 WG	1120.0	0.570	40.5	14.09	0.666	46.3	14.4
				0.679	49.0	13.84			
				0.716	48.5	14.77			
				0.700	47.2	14.84			
8.	KM1110 AG	56.0 WG	1120.0	0.657	45.6	14.42	0.668	45.4	14.7
	ABCDE			0.671	46.2	14.54			
				0.740	50.2	14.74			
				0.604	39.7	15.21			

There are no statistical differences at a probability level better than 95 % between the above treated objects.

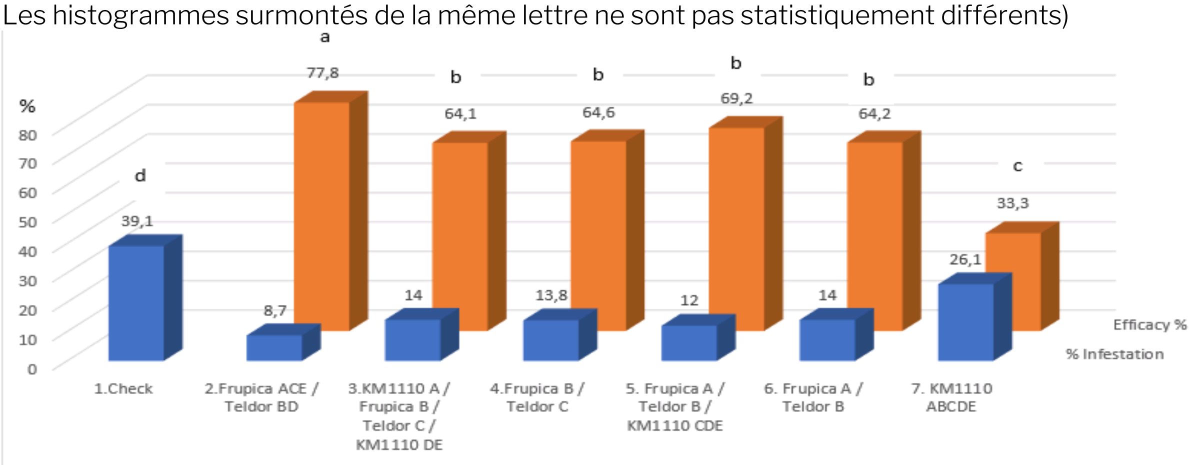
Fruit rot infestation at harvest

Figure 2 : Fruit rot infestation (*B. cinerea*) at harvest: total of four pickings. Histograms marked with the same letter are not statistically different (Niveau d'infestation à la récolte, total des 4 récoltes. Les histogrammes surmontés de la même lettre ne sont pas statistiquement différents.)



Fruit rot infestation after storage

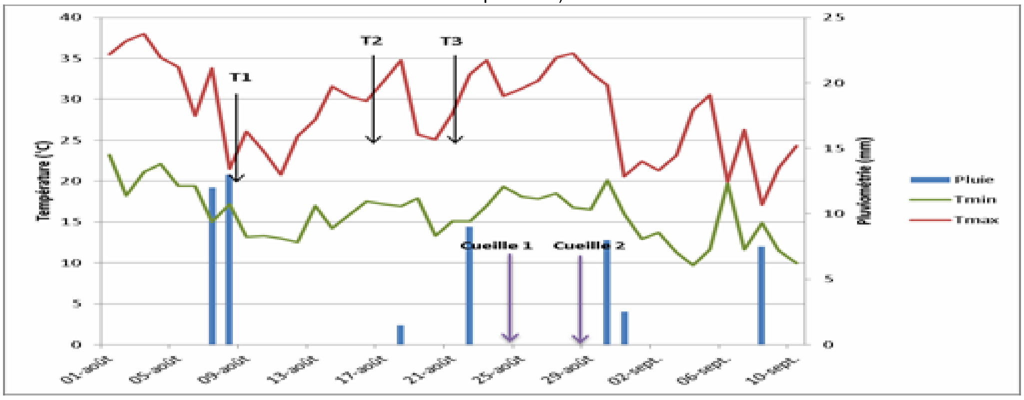
Figure 3 : Fruit rot infestation (*B. cinerea*) after storage: first picking. Histograms marked with the same letter are not statistically different (Niveau d'infestation après conservation, première récolte. Les histogrammes surmontés de la même lettre ne sont pas statistiquement différents)



2) Efficacy of KM1110 for the control of Monilia on peach

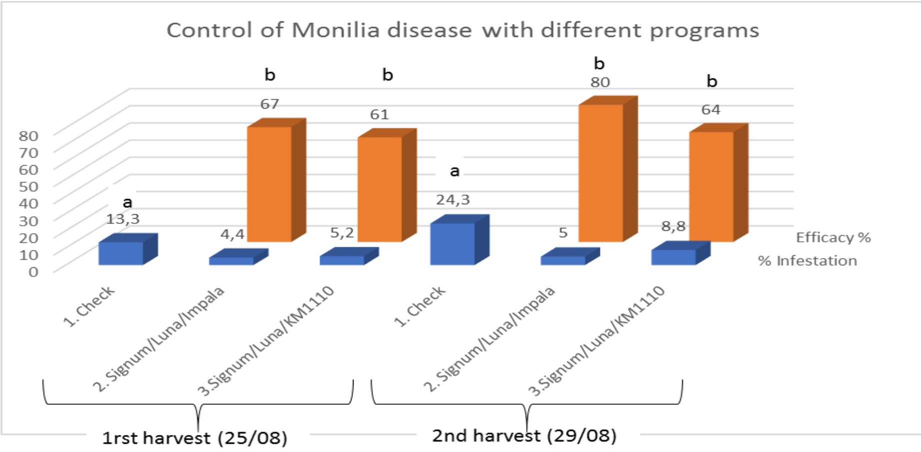
Weather conditions were very hot and quite dry during the trial period. Two rainy episodes preceded the trial and a small rain occurred on the evening of treatment 3. The most contaminating period could be therefore early this year (T1) or after T3.

Figure 4 : Weather conditions and calendar of the trial of monilia on peach. (Conditions climatiques durant l'essai sur la moniliose de la pêche)



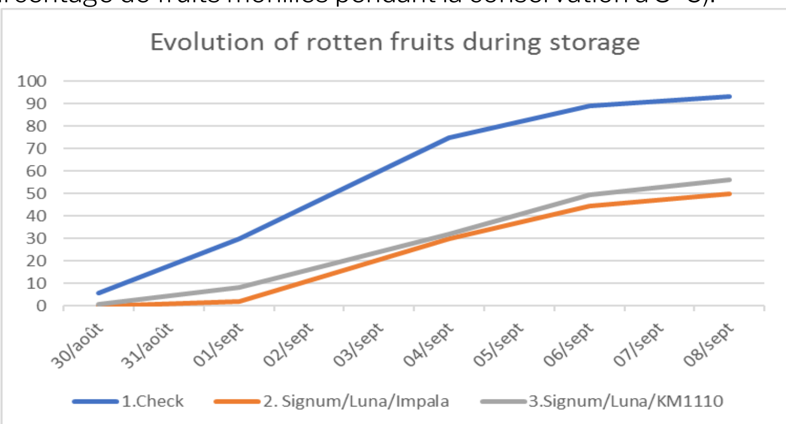
Two observations were done, one at the first harvest time (25/08) and the second at the last harvest (29/08). Figure 5 below shows the means by modality for the two harvests, the product efficacy and the statistical groups.

Figure 5: Assessment on trees, evaluation of the control of Monilia disease at 2 timing of harvest (Observation sur arbres, évaluation du controle de la moniliose à 2 périodes de récolte)



Fruits were taken from the trees which were observed the 25/08 and placed in honeycomb trays. They were placed in a cold room at 3°C for 48 hours before being transferred to a room at 21°C and high humidity on 28/08 and stay there for two weeks. The notation relates to the percentage of fruits affected by monilia, with distinction with other diseases of conservation.

Figure 6: Evolution of rotten fruits (%) during storage at 3 °C.
(Evolution du pourcentage de fruits moniliés pendant la conservation à 3°C).



DISCUSSION

1) Biological efficacy evaluation of *M.fructicola* against fruit rot*, Botrytis cinerea*, on Strawberry (2013, Belgium)

In this trial, only the first assessment after storage was workable as at the further storage too many fruits were infested for reliable conclusion. In strawberries, the most important period for infection with botrytis is during flowering. Moist is necessary for the spores to germinate and infect flower parts. After infecting the flower, the fungus eventually invades the maturing fruits and causes rot. This year during the blossom period in May, the weather was rainy and sulky. Favoured by this weather conditions, yet the Botrytis infestation in the field was restricted (5.1%), but the expression of the disease was delayed till after storage. In the control, Botrytis infestation was 39.1%. At the assessment in the field, the whole program *M.fructicola* (KM1110 applied at 2kg/Ha in 7 days treatment schedule, modality 7) showed a low reduction of Botrytis rot fruit, yet not significantly different from the untreated plot. Conversely, after storage, the KM1110 whole program showed a significant lower Botrytis infestation compared to the untreated object.

At both assessment, in the field and after storage, an alternating fungicide schedules with 3 Frupica applications within between two Teldor applications (object 2) showed significantly the best Botrytis control (resp. 85.1% and 77.8%). For the fungicide schedules with only one Frupica and one Teldor treatment with differences in application periods before picking (object 4 and 6), the Botrytis control was significantly lower as compared to the object (2) with five chemical treatments. A slightly better result of KM1110 (object 5) in a schedule which started with Frupica followed by a Teldor application was observed. These differences were however not significantly expressed. These results of KM1110 was not observed/confirmed in the schedule starting with treatment with the micro-organims *M.fructicola,* followed by a Frupica and a Teldor application and then to end up with back two applications with KM1110 (object 3).

2) Efficacy of a KM1110 for the control of monilia on peach

The field damage rate during harvest on the control is high and allows to observe products efficacy in a high disease/monilia pressure context. The chemical modality (object 2) and the program including KM1110 product (object 3) are statistically different from untreated control. The modality including the product KM1110 associated to the adjuvant Squad shows an efficacy of 60% at the two harvest periods. This is a good control level in this context of strong pressure especially because there was a rain after T3, episode that it is thought to be a factor of contamination. Nevertheless, it is difficult to conclude on the real interest of this strategy (since the last treatment may have been useless if there was no contamination). This interest should be supported by a treated window (Signum / Luna / only) in another study.

The results from the orchard are confirmed in storage. The level of pressure is very important because in two weeks, more than 90% of the fruits of untreated controls are affected by monilia. The chemical program gives the expected results by making it possible to significantly reduce by up to 50% the level of unripe fruits after 2 weeks in storage. The modality with KM1110 and Squad (3) is statistically similar to the reference.

CONCLUSION

These two trials are reprensentative of trials performed for several years. Indeed, 37 field trials have been evaluated forassess the efficacy of KM1110 to control *Botrytis* on strawberry, 35 trials to control *Monilia* on stone fruits and 33 to control *Botrytis* on vinegrapes. All of these experiments lead to the same recommendations: KM1110 should be used at 2 kg/Ha, in a preventive strategy and preventively in IPM program in the case of low or medium pressure of pre- and post-harvest diseases. Althought a good chemical program prior KM1110 is necessary, *M.fructicola* can replace the last chemical sprays(s). Biocontrol products are used differently from chemicals especially when it comes to micro-organisms which need favourable environmental conditions to develop. Studies have been done to measure and monitor the evolution of the amount of yeast on berries over time and according to treatments. It could be interesting to measure the quantity of yeast cells and to test the efficacy of *M. fructicola* if treatments are applied with the help of a Decision-Making-Tool.

KM1110 is a biofungicide for the control of gray mold (*Botrytis cinerea*) in grapes and in soft fruits and for the control of pre- and post-harvest diseases in stone fruits. It prevents a wide range of rot diseases, it presents very low risk for resistance and zero day pre-harvest interval. It meets all the requirements for safe and substainable agriculture.

BIBLIOGRAPHY

Banani H., Spadaro D., Zhang D., Matic S., Garibaldi A. & Gullino M.L., 2015. Postharvest application of a novel chitinase cloned from Metschnikowia fructicola and overexpressed in Pichia pastoris to control brown rot of peaches. *International journal of food microbiology* 199, 54-61.

Droby S., Wisniewski M., Macarisin D. & Wilson C., 2009. Twenty years of postharvest biocontrol research: is it time for a new paradigm?. *Postharvest Biology and Technology,* 52, 2, 137-145.

Filonow A. B., 1998. Role of competition for sugars by yeasts in the biocontrol of gray mold of apple. *Biocontrol Science and Technology*, 8, 2, 243-256.

Hershkovitz V., Ben-Dayan C., Raphael G., Pasmanik-Chor M., Liu J., Belausov E., Aly R., Wisniewski M. & Droby S., 2012. Global changes in gene expression of grapefruit peel tissue in response to the yeast biocontrol agent Metschnikowia fructicola. *Molecular plant pathology,* 13, 4, 338-349.

Karabulut O.A., Tezcan H., Daus A., Cohen L., 2004. Control of preharvest and postharvest fruit rot in strawberry by Metschnikowia fructicola. *Biocontrol Science and Technology,* 14,5, 513-521.

Karabulut O.A., Smilanick J.L., F.M. Gabler, Mansour M. & Droby S., 2003. Near-harvest applications of Metschnikowia fructicola, ethanol, and sodium bicarbonate to control postharvest diseases of grape in central California. *Plant Disease* 87, 11, 1384-1389.

Kurtzman C.P. & Droby S., 2001. Metschnikowia fructicola, a New Ascosporic Yeast with Potential for Biocontrol of Postharvest Fruit Rots. *Systematic and applied microbiology.*

Liu J., Sui Y., Wisniewski M., Droby S., Liu Y., 2013. Review: utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *International journal of food microbiology*, 167, 2, 153-160.

Macarisin D., Droby S., Bauchan G. & Wisniewski M., 2010. Superoxide anion and hydrogen peroxide in the yeast antagonist–fruit interaction: a new role for reactive oxygen species in postharvest biocontrol?. *Postharvest Biology and Technology,* 58, 3, 194-202.

Mehdy M.C., Sharma Y. K., Sathasivan K. & Bays N.W., 1996. The role of activated oxygen species in plant disease resistance. *Physiologia Plantarum*, 98, 2, 365-374.

Xu X., Guozheng Q. & Shiping T., 2008. Effect of microbial biocontrol agents on alleviating oxidative damage of peach fruit subjected to fungal pathogen. *International journal of food microbiology,* 126, 1, 153-158.