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Equisetum arvense (horsetail) Extract: The First Approved Basic Substance Allowed for EU Crop Protection

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ABSTRACT

Equisetum arvense is an herbaceous perennial plant traditionally used in human medicine (i.e. for organic silicon supplementation). It is an approved basic substance with fungicidal properties under Article 23 of agricultural EU pesticide regulation (EC) No 1107/2009 (Anonymous, 2009) since 2014 and in Organic Production since 2016. Currently, its approved uses are for arboriculture, horticulture, ornamentals and viticulture through initial approval and two use extensions granted in 2016 and 2017. Further use extensions to allow cereal use have been submitted to the EU Commission for evaluation in 2018. Thus, fungicide usages for the cereals are being evaluated by the EU regulatory assessment organisation, with ESA outcome in 2020 and further discussion at Commission level since. Horsetail extract has recently been shown to have antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, antidiabetic, antitumor, cytotoxic and anticonvulsant activities. Initial main field trials to demonstrate its plant protection properties were coordinated in France by the "*4P*" project "Protection des Plantes Par les Plantes" (Plant Protection by Plants) between 2010 and 2013 but additional field trials were later collected. We detail in this study the results obtained and all the uses of this decoction as a plant protection product. In addition, we describe the full approval processes for the basic substance *Equisetum arvense* and the extensions of use, covered by the Article 23 of the plant protection regulation in EU.

KEYWORDS: Basic substance, biorational, *Equisetum arvense*, fungicide horsetail, plant protection, Regulation 1107/2009

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1. INTRODUCTION

Outside of the medicinal purpose, considering its properties, several people have tried to use plant extracts as plant protection product since plant diseases are an important struggle in crop production (Maiti, 2016). Effectiveness in vineyard protection against downy mildew was previously shown (Cohen, 2006). Antifungal properties were also described to explain this potential efficiency (Arif et al., 2011). It is for this field trial purpose that the Organic Food and Farming Institute (ITAB) conducted the "*4P*" experimental program. This work designated to test plant extracts as complementary solutions was undertaken to be able to cope with reduction for the use of copper per hectare and per year during renewal procedures at general EU pesticide regulation, and reduction of copper quantities in Organic Production (Anonymous, 2002) from 6 kg ha^{-1} $year^{-1}$ to currently 4 kg ha^{-1} $year^{-1}$ since 2019 (Anonymous, 2018a).

Later, ITAB worked on the approval of the *Equisetum arvense* extract as plant protection means under the EU plant protection regulation (EC) No 1107/2009 as basic substance (Marchand, 2016) which made the approval of plant extracts possible as "basic substances" in accordance with Article 23, these are active substances not normally used as plant protection products, but which can be useful also economic value for the approval of such substances may be limited. At present, 24 basic substances that have been identified as having neither immediate nor delayed harmful effects on human and animal health nor unacceptable effects on the environment are approved. They are permitted for the protection of conventional crops and granted without maximum residue limits (Charon, 2019), and are likely to be included in Annex I of the Regulation (EC) 2021/1165, most of them (22) in organic production also (Marchand, 2015, 2016, 2017a, 2021).

The application files were partly based on the results of the "*4P*" research program (Marchand et al., 2014) which involved analyses of diverse plant content including horsetail (*Equisetum arvense*) (Andreu, 2018). This project focused on the search for alternatives to the chemical pesticides and copper uses based on the evaluation of herbal preparations against bio-aggressors regarding their "fungicidal" potential (Deniau, 2019). *Equisetum arvense* decoction contains also various inactive compounds, together with the potential active components, like organic silicic acids. Several studies have also shown that silicic acids own antifungal activity (Reynolds et al., 1996; Fauteux, 2006).

Horsetail decoction has long been used as a fungicidal extract. The interest for this substance as natural resource for plant protection is multiple: it is useful for by acting as an elicitor of the resistance and defence mechanisms in the plants (Daiana et al., 2011; Wang et al., 2017); moreover, indirect antifungal properties were proved through these field trials. This extract is environmentally friendly since the supposedly active molecules are not exhibiting biocides properties as in line for human consumption (Anonymous, 2016b). The recipe (decoction of the plant parts in boiling water) is included in the later review report (Anonymous, 2017) as previously published (Marchand, 2016). ITAB is currently working on extending the usages of this approved basic substance since the actual claimed uses are limited in grapevine against *Plasmopara viticola* (downy mildew) and *Erysiphe necator* (powdery mildew), in arboriculture for apple trees targeted toward foliar fungi like scab disease *Venturia inaequalis* and powdery mildew *Podosphaera leucotricha*, and for peach trees against foliar fungi like *Taphrina deformans*. *Botrytis cinerea* and *Penicillium expansum* are also important pathogens for these crops, mainly contaminating post-harvest fruits. Horsetail decoction has been shown to have antifungal activity against both fungi (Andreu et al., 2018). Horsetail decoction inhibits the germination and sporulation of fungi seeds and therefore the spread of fungal diseases without really killing the fungi as expected for chemical pesticides.

2. MATERIALS AND METHODS

Research period of the CAS DAR Project "4 P" took place between 2010 and 2013 (36 months). Initial main field trials to demonstrate its plant protection properties were coordinated in France in different areas located in the most diverse location possible from south to north and from west to east (62750 Loos-en-Gohelle, latitude: 50.457611, longitude: 2.792593; 66000 Perpignan, latitude: 42.69853; longitude: 2.895312; 01250 Ceyzériat, latitude: 46.191456, longitude: 5.323815; 78730 Arrondissement de Rambouillet, latitude: 48.5711, longitude: 1.9395; 33290 Blanquefort, latitude: 44.919423, longitude: -0.645096; 84 911 Avignon latitude: 43.5430, longitude: 4.52.55; 29600 Suscinio, latitude: 48.620826, longitude: -3.84045), but additional field trials were collected in other French locations.

2.1. *Composition of the extract*

Full phenolic composition of *Equisetum arvense* horsetail decoction was described during the "*4P*" project (Andreu et al., 2018).

2.2. *Horsetail extract recipe for fungicide uses*

The recipe is described in the GAP table part for many fungi diseases (Anonymous, 2017).

2.3. *In vitro action and field trials during the "4P" project*

The "*4P*" project was also dedicated to experimental usefulness trials. Experiments suggest an optimal dosage of

0.5 mg l^{-1} for this extract. Investigations were conducted to show the range of activities of plant protection properties against bio-aggressors in arboriculture, market gardening and viticulture. This "*4P*" project focused on the fungicidal properties together with the eco-toxicity characterisation of these extracts (i.e. studies *versus* non target organisms). The agronomic efficacy studies are based on field experiments carried out on a plots network and the eco-toxicity of the extract was evaluated in laboratory. However, the results presented here are only part of the "*4P*" program dedicated to *Equisetum arvense* horsetail decoction.

2.4. *In vitro effects of Equisetum arvense* horsetail decoction *on grapevine and orchard phytopathogen*

In vitro tests were carried out on grapevine downy mildew (*Plasmopara viticola*): three types of tests were executed on loose leaves, from Aquitaine vineyard: anti-germinative, curative and preventive. Inoculation protocols were conducted as follow: the pathogen (*Plasmopara viticola*) population used for the 2012 trials comes from late season (Sept. 2011) leaf sporulation samples from Château Dillon plots. Whole leaves were frozen and stored in bags. Seasonal mildew samples have completed this stock. The sporocysts of *P. viticola* are leached from the leaf sporulation with distilled water and a brush and accumulated in a beaker placed in ice. The density of the inoculum is controlled with a Malassez cell from a sample. The inoculum must reach a density of about 10×10 5 sporocysts ml^{-1}. The detached vine leaves (vegetal material for inoculation) used in the in vitro tests came from the parcels of Château Dillon and come from Merlot Noir grape varieties. Inoculation was done by drop at the rate of one drop of 15 µl of spore solution for one disc. After inoculation, the disks were placed in the dark for a minimum period of 5 hours, and then the drops of inoculum are aspirated using a pipette (Marchand, 2014). The leaves were then kept in the culture chamber. In general, the first sporulation appears 5 days after inoculation. The biological activities of plant extracts were also tested in the laboratory for their antifungal activity on different species of phytopathogenic fungi (*Penicillium expansum* and *Botrytis cinerea*) with identical protocol.

2.5. *Field trial protocols*

The starting postulates were adopted for all trials: the limitation of the total quantities of copper metal was set up to a maximum of 6 kg ha^{-1} $year^{-1}$ as foreseen by the current EU Regulation in Organic Production, and of the maximum number of treatments was limited to 10.

Field trials were done according to Vegephyl-Anses C.E.B. (Commission des Essais Biologiques) methods (No. 7, No. 14, No. 22, No. 33 and for No. 53 *Plasmopara viticola, Venturia inaequalis, Erysiphe necator, Taphrina deformans and Guignardia bidwellii* respectively) (Anonymous, 2022).

2.6. *In campo activity of Equisetum arvense* horsetail decoction *on grapevine and orchards phytopathogen*

2.6.1. *In vineyards*

2.6.1.1. *P. viticola*

In vineyards, work was carried out over three years against the grapevine downy mildew pathogen (*P. viticola*), with testing carried out in association with a low dose of copper, in comparison to a regional copper reference (400 to 600 g of eq. copper metal per hectare), an untreated control and low-dose copper modality (150 g copper metal per hectare). For each modality, the frequency and the intensity of late downy mildew on the leaves and fruit clusters were measured. Secondary observations were also monitored on black rot (*Guignardia bidwellii*).

Postulates adopted after meeting with grapevine responsible for all tests: limitation of the total quantities of copper metal to 6 kg ha^{-1} $year^{-1}$ as foreseen by the current EU Regulation in organic farming; limitation to 10 of the number of treatments. This number may be lower depending on climatic conditions and disease pressure of the year. We will simply make sure, for a given test, to do the same number of treatments in all the modalities so that the comparisons between the modalities can be possible. Terms of treatments are described in Table 1.

The product chosen for all the tests and modalities is Kocide opti® copper hydroxide (Dupont company) because of its formulation in dispersible granules and of its recent

Table 1: Modalities of copper* treatments

Modality	m0	m1	m2	m3
Depiction	control	Regional reference £	"low copper only" $	Low copper+infusion"
Description	untreated	between 400 and 600 g / treatment	250 g / treatment under controlled conditions 150 g / treatment under natural field conditions	dose defined in modality 1 i n association with willow leaves infusion

Quantities of "copper" are considered as mass of copper metal; £ Doses of copper commonly used in the region; $ In this modality copper is used in this modality alone and at low dose.

homologation at 750 g ha^{-1} of copper metal is 2500 g of product ha^{-1}. Note that for a given modality we work at constant dose of copper / treatment throughout the season.

Basic plots trials: 5 grapevine feet minimum as described in C.E.B. protocols (Anonymous, 2022): feet # 1 and # 5 are out of the test and feet # 2, 3, 4 are monitored and misted. 4 repetitions per modality were observed with renewal of treatments every 8 to 12 days after each fogging; every ten days in regional reference (Table 1. £) in the absence of rain; after a rain (the next day) if it is greater than or equal to 20 mm, but within the limit of the expected rate of 8 to 12 days.

Notations were done with three counts minimum: at inflorescence / fruit set leaves and bunches (grey rot); at the beginning of veraison leaves and clusters (brown rot) and at harvesting leaves only (or defoliation). Notations must comply with the C.E.B. protocols (Anonymous, 2022), and relate to intensity and frequency (leaves and clusters). In all cases the counts are carried out either on all the clusters+100 leaves, or on 100 clusters and 100 leaves according to the size of the elementary parcel, avoiding the edge vines, by alternating the positions of notation and while maintaining the same observer throughout the same repetition. Anti-powdery mildew: treatment with Thiovit® or Microthiol® if necessary to provide effective protection against powdery mildew. Anti-powdery mildew treatments were decoupled from anti-mildew treatments; the same on all modalities including cookies, and with the same commercial product throughout the season. Volume of mixture to spray on crops is 120 to 200 l ha^{-1} with a pneumatic backpack sprayer to be adapted, if necessary, with the vegetation stage. It was ensured that the presence of a weather station or the possibility of rainfall recordings was available within a radius of 5 km around the test.

2.6.1.2. *E. necator*

The starting postulates adopted for all tests were: no limitation of the quantities of sulphur and no limitation on the number of total treatments (however, make the same number of treatments for all modalities). Terms and modalities are described in Table 2.

Table 2: Modalities of sulphur* treatments

Modality	m0	m1	m2	m3
Depiction	Control	Regional reference £	"low copper only" $	"low sulphur + infusion
Description	Untreated	5000 g / treatment	1600 g / treatment for Burgundy 2000 g for regions with high fungi pressures	Dose defined in modality 1 in association with willow leaves infusion

Quantities of "sulphur" are considered as mass of pure sulphur; £ Doses of sulphur commonly used in the region; $ In this modality sulphur is used in this modality alone and at low dose.

The product selected for all tests and modalities is Microthiol Special Disperss® (Cerexagri Company). Basic plots with 5 feet minimum defined by C.E.B. protocols (Anonymous, 2022): feet # 1 and # 5 are out of the test and feet # 2, 3, 4 are noted and misted. 4 repetitions per modality were collected and delays between treatments were from 8 to 12 days. Notations were collected with two counts minimum (with the possibility to make three) and with counting only on clusters but possible to make foliage logs when it is obvious. In all cases the counts are carried out either on all the clusters+100 leaves, or on 100 clusters according to the size of the elementary parcel, avoiding the edge vines, by alternating the positions of notation and by keeping the same note-takers throughout the same repetition. Anti-mildew cover treatment if necessary was done with a copper hydroxide or a copper sulphate to provide effective protection against late blight, within the limits of 6 kg ha^{-1} $year^{-1}$ provided by the organic farming regulation (allowed before 2019). Anti-mildew treatments were decoupled from anti-powdery mildew treatments; make them identical on all modalities including controls, and with the same commercial product throughout the season. Volume of slurry to spray was defined from 120 to 200 l ha^{-1} to be adapted, if necessary, with the stage of vegetation. Ensure the presence of a weather station or the possibility of rainfall readings within a radius of 5 km around the test.

2.6.2. *In orchards*

2.6.2.1. *T. deformans*

In arboriculture (peach trees): the objective was the protection of fruit trees and the search for alternatives to the use of sulphur and copper compounds against peach leaf curl (*T. deformans*). For peach trees, the experimentations were performed in an organic peach orchard, with the Spring Lady variety: an early and sensitive to the leaf curl pathogen. The trial plot was converted to organic farming since 2010. The orchard has a leaf curl inoculum occurrence from medium to high (in 2010). The plant extracts were sprayed in addition to the producer's treatments. The producer's pathway for fungal diseases consists of a mixture of sulphur, potassium bicarbonate, terpene alcohol (heliosol®) and leaf fertilizer (UFAB Biotham), including lithothamne, silica and copper. These treatments were applied on all trees. The schedule of treatments and observations was conducted as such: two treatments were carried out (1 treatment / week, 03 and 9 July) in prevention to the development of leaf curl on fruit. On the other hand, the producer has carried out several fungicide treatments since the beginning of the year, including two sulphur-based treatments, 15 days before the first treatment and the day after the second treatment, respectively. Harvesting (16/07/2012) was carried out one week after the last treatment and 2 days before the first harvest of the producer. The observations correspond to estimate of the frequency of tree fruits: count of the number of fruits per 100 fruits observed at human height (because hail 8 days before harvest and top fruits damaged) the day before harvest and estimation of the frequency of the attack of leaf curl on a sample of 12 healthy fruits and without wounds during the sampling, corresponding to 60 fruits by modality and preserved at ambient temperature. Frequency is the number of fruits affected compared to the total number of fruits.

2.6.2.2. V. inaequalis

Choice of varieties was focused on apple trees with very strong sensitivity variety against scab: Jugala. A true control (no fungicide treatment) was implemented on the orchard according to an included scheme. Fisher type block device with 4 repetitions was fooled with orchard size of 50×25 m^2; experimental elementary plots consisted of 3 trees. The reference was the included control, and five other modalities were studied. All treatments were done with a predictive positioning with a pace of 7 days maximum. A renewal of protection was realized when crossing the 20 mm threshold precipitation. Predictable period of primary contaminations was 25 March–30 June whereas effective period of primary contaminations: 27 April–28 June. The treatments were made using a STIHL SR400 atomizer. The volume of slurry was 500 l ha^{-1}. Other orchard maintenance treatments like fungicides applied on the plot were suspended throughout the test period on the experimental ranks and guard rows. Several ratings were performed on each modality. The first was done from the first exit stains from the first contaminations. Then every week after the first scoring up the end of primary contaminations, a follow-up of the evolution of secondary contaminations was pursued at the same frequency. A last rating has occurred when the crop has finished. In view of the main protection program for apple scab, the periods of notations are based on observations made for scab. The first one after the exit first spots from the first contaminations (before flower) then every week after then first rating up to the end of contaminations and the program of field observations, was mainly to observe the fruit decays. The ratings involved the observation of 210 leaves on the 3 trees of each parcel total of 840 sheets for each modality. An equitable distribution of different leaf areas was respected according to the following schema: leafy stage (Tree 1 Tree 2 Tree 3); top 2 shoots of 10 leaves 2 shoots of 10 leaves 2 shoots of 10 leaves; medium 3 shoots of 10 leaves 3 shoots of 10 leaves 3 shoots of 10 leaves; low 2 shoots of 10 leaves 2 shoots of 10 leaves 2 shoots of 10 leaves. The leaves were divided into two classes as follows: 0: leaves free from scab /1: leaves having at least one scab spot. On fruits, the ratings involved all the fruits observable on the 3 trees of each parcel. The fruits were classified in 2 categories: 0: fruit free of blemishes/1: fruits having at least one scab spot.

2.7. Ecotoxicity assessment

2.7.1. On bees

SupAgro Montpellier laboratory has performed toxicity tests of *Equisetum arvense* extracts on bees (*Apis mellifera*), by both feeding and contact methods. The protocols used for the oral toxicity test are carried out according to C.E.B.-Anses methods No. 230 (Anonymous, 2022). Each test is composed of the following modalities:

- control treatment carried out with sugar distilled water (500 g l^{-1}),

- treatment by plant extracts, bark and horsetail extract at 5 concentrations (regular application rate D, and dilutions: D/10, D/5, 2D/5 and 3D/5).

The concentration of the preparation to be tested represented 10% of the final volume of feeding syrup.

In order to evaluate the deleterious effects of the substances by contact, a tissue with a surface area of 100 cm² was deposited in the boxes of contention; the tissue being sprayed by the substance under controlled conditions using a Potter tower (Potter, 1952). The solutions were applied in such a way that 1.5 mg +/- 0.2 mg of the substance was deposited per cm^2 of tissue.

Each test exhibited the following modalities:

- the control treatment was performed with distilled water.

- the treatment with the bark extracts at 5 concentrations (see above).

2.7.2. On aquatic organisms

Finally, a toxicological study of the preparations, not previously published was carried out by the University of Perpignan on aquatic organisms, two classical models were used: the brine shrimp artemia (*Artemia salina*) and the water flea daphnia (*Daphnia pulex*) using the OECD 202 protocol (Anonymous, 1984).

3. RESULTS AND DISCUSSION

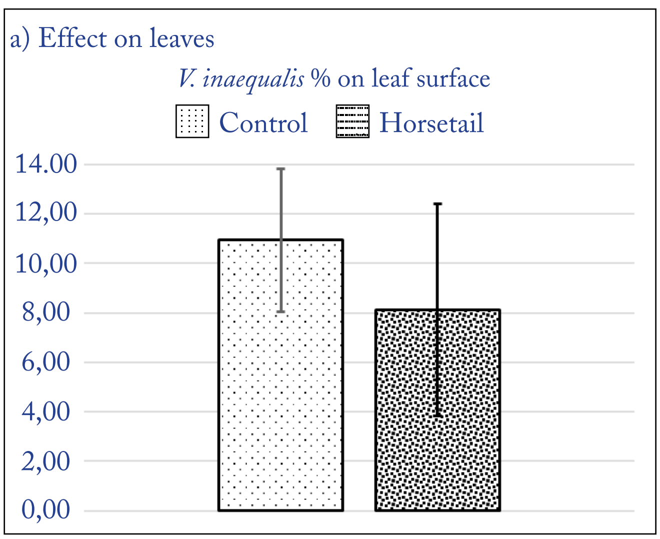
3.1. Horsetail as anti-germinative substance against fungi

The analyses of the *Equisetum arvense* preparation only showed effective result against on the germination of *P. viticola*. For the characterisation of the inhibitory effects on the various peach and vine pathogenic fungi, the horsetail extract has an interesting activity (Marchand, 2014).

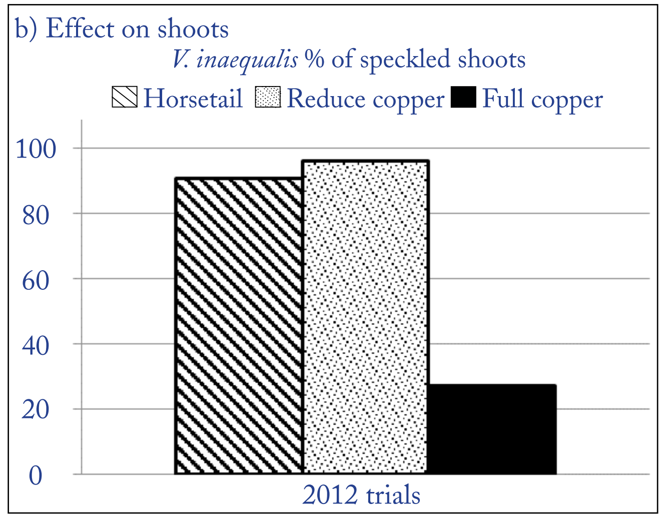
3.2. Horsetail as a fungicidal means on grapevine, peach and apple trees

3.2.1. Horsetail as a fungicidal on apple trees

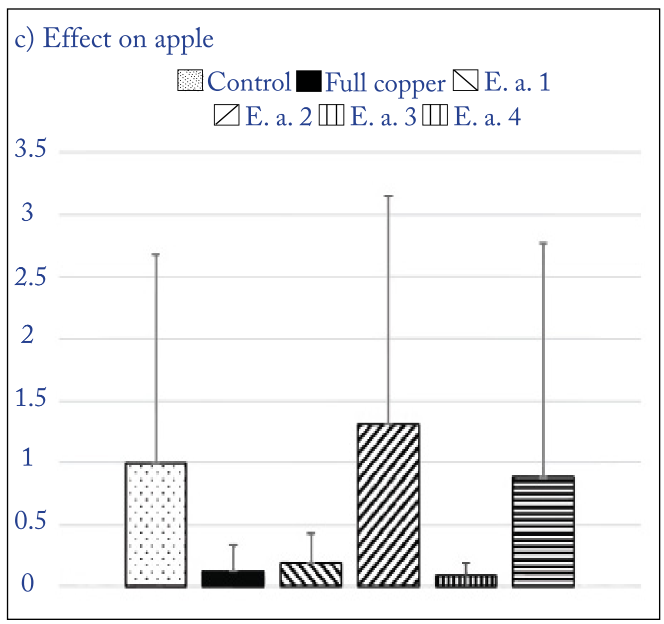
The untreated control apple trees in the field experimental conditions exhibit a *V. inaequalis* attack percentage of about 11%. Treated modalities are labelled "*E. arvense* 1 to 4" are corresponding to different trials with horsetail infusion. Light fungicidal effect of the horsetail extract was measured on apple trees with only 8% of the leaves surface infested with apple scab disease (Figure 1a.). Trees treated with horsetail infusion show a reduction of more than half of the *V. inaequalis* attacks as compared to the untreated controls. Light fungicidal effect was also measured on specked shoots (Figure 1c.) compared to full and reduced copper doses treatments. Depend on modalities, *E. arvense* decoction seems to have a similar effect to "Full Copper" on fruits (Figure 1c).



	Control	Horsetail
	8	7.00
	14.00	2.75
	12.75	10.00
	9.00	12.75
Mean	10.94	8.13
SD	2.89	4.28



					Mean	SD
Horsetail	65	100	100	98	90.75	17.2
Reduce copper	89	100	100	96	96.25	5.2
Full copper	8	23	45	32	27.00	15.55



	Control	Full copper	E.a 1	E.a 2	E.a 3	E.a 4
Mean	0.993	0.124	0.191	1.309	0,089	0.878
SD	1.680	0.210	0.240	1.840	0.100	1.890

Figure 1: Fungicidal efficacy of *Equisetum arvense* decoction against apple scab disease *V. inaequalis* a) on the fruits b) on the leaves and c) on the shoots

3.3. Horsetail as a fungicidal means on grapevine

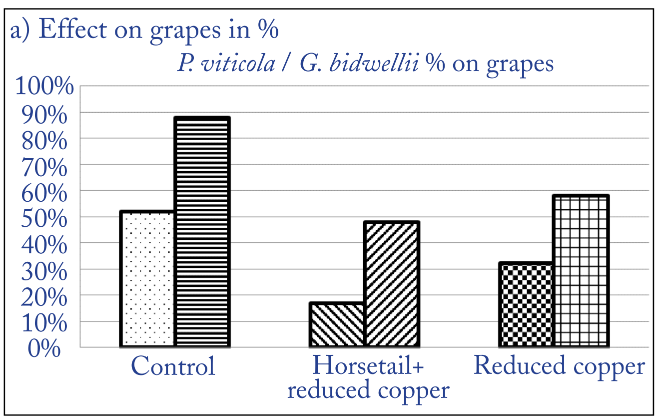
Concerning the vineyards, the trials show a small fungicidal effect for horsetail herbal tea, extract treatments on grapes against *P. viticola*, the grapevine downy mildew agent (figure 3a). The silicon in horsetail extract is known for the antigerminative property of the extract and was exhibited during the "4P" CASDAR project (Marchand, 2014). Main observations of the fungicidal resulting effect were managed on downy mildew expansion since the effect was reported, but secondary observations on black rot were also achieved and reported on Figure 2.

3.4. Horsetail extract on peach trees

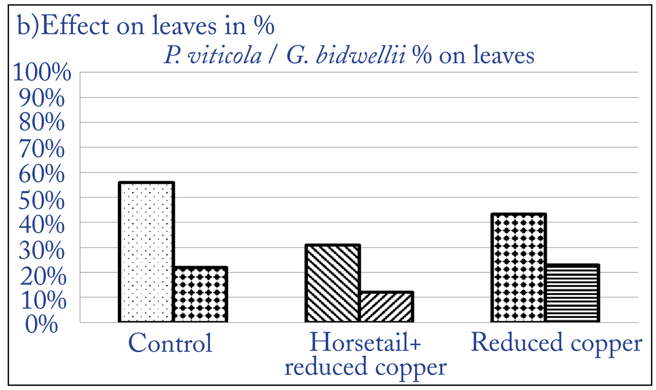
Effect on *Monilia* contaminations was recorded in orchards. Compared to *Salix* cortex extract; any fongistatic effect is observed regarding average result (Figure 3).

3.5. Equisetum arvense extract is nontoxic to bees and aquatic organisms

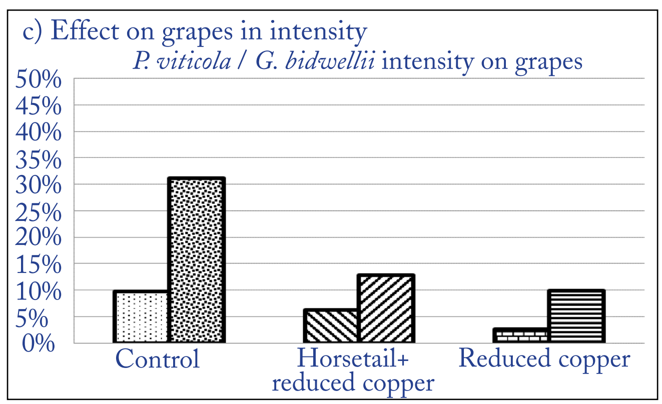
Regarding the toxicity by feeding, the extract of horsetail has no observable lethal or acute effect on bees for the five concentrations tested, in fact, the induced mortality did not



Grapes						
P. viticola	assay 1	assay 2	assay 3	assay 4	Mean %	±SD
Control	36	72	20	80	52	28.66
Reduced copper	44	9	24	52	32.3	19.5
E.a+Reduced copper	8	20	32	8	17	11.49
G. bidwellii						
Control	80	80	100	92	88	9.8
Reduced copper	48	56	76	52	58	12.43
E.a+Reduced copper	48	60	52	32	48	11.77

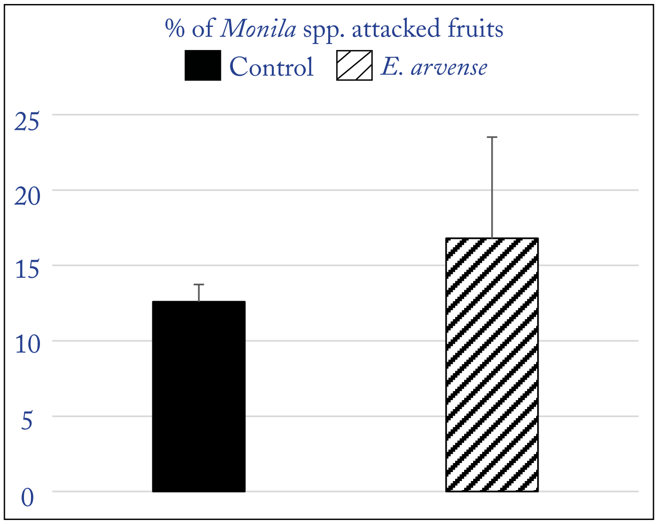


leaves						
P. viticola	assay 1	assay 2	assay 3	assay 4	Mean %	±SD
Control	40	64	24	96	56.0	31.30
Reduced copper	80	17	40	36	32.3	19.50
E.a+Reduced copper	20	40	56	8	31.0	21.26
G. bidwellii						
Control	20	20	24	24	22	2.31
Reduced copper	52	0	20	20	23	21.51
E.a+Reduced copper	12	16	16	4	12	5.66



Intensity						
P. viticola	assay 1	assay 2	assay 3	assay 4	Mean %	±SD
Control	5.00	17.35	2.50	14.00	9.71	7.09
Reduced copper	9.19	1.00	4.50	10.40	32.30	19.5
E.a+Reduced copper	0.69	3.65	6.04	0.24	2.66	2.72
G. bidwellii						
Control	21.92	23.12	36.60	43.00	31.16	10.32
Reduced copper	7.81	8.58	28.50	6.40	12.82	10.49
E.a+Reduced copper	4.48	20.00	10.85	4.28	9.90	7.39

Figure 2: Effect of *Equisetum arvense* (E.A) application against *Plasmopara viticola* (downy mildew) a) on the grapes b) on the grapevine leaves. Secondary observations were done on *Guignardia bidwellii* (black rot).



	Mean %	±SD
Control	12.6	1.14
E. arvense	16.8	6.72

Figure 3: Fungicidal usefulness of *Equisetum arvense* extract for peach tree protection

reach 10% after 96 h. For contact toxicity tests after 96 h, the deaths observed were lower by contact than by ingestion for all the extracts tested. The treatment with *Equisetum* did not induce a clear excess mortality among the bees for all the concentrations tested as reported in Figure 4.

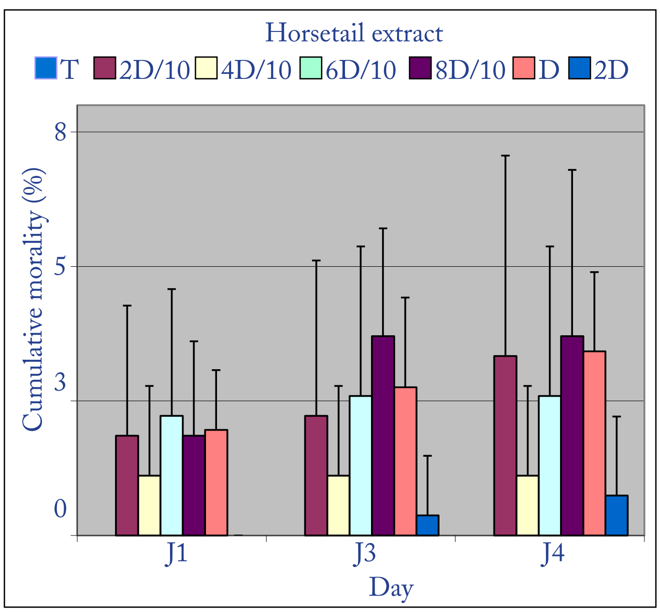


Figure 4: Toxicity of the horsetail extract preparation tested on the bees

The results obtained INRAE (Avignon) with horsetail, mugwort and willow (Marchand, 2014), show that the excess mortality at 4 days for bees never exceeds 5%, while a mortality is not significant if <15%. Although the toxicity of the preparations is not high, different toxicity profiles can be observed: a triphasic bell curve with horsetail was observed. Thus, toxicity is higher at the D 8/10 dose than at higher doses D and 2D. The results obtained show a low toxicity on bees of the infusions of horsetail even when used at the double dose.

For aquatic organisms, the effective concentration of horsetail is much higher than references cited in literature, so the horsetail extract was less toxic. On Artemia salina (Marchand, 2014), horsetail extract seems to be the less toxic extract because of the highest effective concentration (Table 3).

Table 3: Aquatic toxicity of horsetail extracts. Effective concentration (EC50) (immobilisation at 48 hrs) against aquatic organisms (in mg ml^{-1})

Species	Aliphatic nitrated fungicide	Triazole benzoylureas reference	*Equisetum arvense*
On *Artemia salina*	> 0.05		8.9
On *Daphnia pulex*		0.051 to 0.0000225	55.0

3.6. Horsetail as an activator of plant defence mechanisms

The presence of silicon (Si) and various other compounds makes horsetail extract interesting for plant defence. Si seems to play a role in the regulation of defence genes expressions. Indeed, it has been shown that in response to salt stress, silica will modulate the expression of genes through transcript factors as well as genes hormone-related genes (Zhu, 2019). Horsetail extract, composed of molecules such as flavonoids, glycosides, plant acids improves the development of seedlings (Lisjak, 2015).

3.7. Horsetail as a fungicide

As we said, Si has a role in genes expression but also in extracellular environment. Si acts as physical barrier against many pathogen fungi such as *Fusarium spp.* and *Pseudomnia synringuae*. Applied before the infection, Si enhance rigidity and reinforce cell-wall become against pathogen fungi. However, depend on the localisation of the application (Heine et al., 2007), Si could be induced systemic resistance (Liang et al., 2005), on roots, or increase cucumber resistance to powdery mildew via a foliar application. The physical barriers inhibit pathogen penetration and make plant cells less susceptible to enzymatic degradation caused by fungal pathogen invasion. (Wang et al., 2017*).*

3.8. Horsetail extract in association

Results showed that the association of horsetail extract with two other basic substances and a reduced dose of copper showed interesting effects against powdery mildew. Indeed, associated with nettle and a reduced dose of copper, horsetail extract shows an efficiency higher than 50% on mildew compared to copper doses alone (Marchand, 2014). The extract of horsetail associated with a reduced dose of copper and willow, also part of the 4P project (ref merchant, the journal), showed a rather good efficiency on mildew compared to doses of copper alone, although the impact of the fungus remained important.

3.9. From general regulation point of view

3.9.1. Equisetum arvense L. as a basic substance for plant protection.

Initial Basic Substance Application (BSA) for *Equisetum arvense* L. was constituted following the corresponding Guideline (DG Health and Food Safety, 2014) and our previous work (Marchand, 2015). We were aiming for the approbation of *Equisetum arvense* L. by the European Union as "basic substance", (Marchand, 2017c) according to provisions of article 23 laid down by EC regulation 1107/2009 on placing plant protection products on the market. *Equisetum arvense* L. was thereby submitted as a basic substance application (BSA) and was declared eligible in 2014.

After the eligibility and the assessment by Europe, the European Food Safety Authority (EFSA) issued a positive opinion on the "*Equisetum arvense*" dossier. Indeed, *E. arvense* meets the characteristics of a basic substance because *Equisetum arvense* decoction is made by boiling dried aerial parts plant during 45 min. Moreover, the substance has neither an immediate or delayed harmful effect on human or animal health nor an unacceptable effect on the environment (Anonymous, 2016b).

The Member States through Commission draft proposal approved *Equisetum arvense* as a basic substance in accordance with Article 23 of Regulation (EC) No 1107/2009, stating that *Equisetum arvense* is a part of plant found throughout the environment and the additional exposure of humans, animals and the environment is negligible compared to the expected exposure in an ordinary natural context (Anonymous, 2017). Voting at standing Committee on Plants, Animal, Food and Feed (PAFF committee) was done according to the EU official vote procedure (Marchand, 2015). The substance was then registered in EU pesticide database and the MRL fixed at 0 in Annex IV of EC Reg. 396/2005 Art. 18 (EC, 2005). This shows that initial work from "4P" program was not only successful but incentive to other developments.

3.10. Previous extensions at EU general regulation

The procedure for extending the uses of basic substance has already been described by our Institute (Marchand, 2017b). The application was undertaken accordingly for *Equisetum arvense* L. which would reduce the effects of excessive water around plants that would lead to fungus due to its component, Si. It would act also as an activator of plant defence mechanisms. Many extensions of uses were therefore constituted, submitted in October 2016, July 2017 and June 2019 with all information including the recipe modifications and the new GAP table. Each one was evaluated by the PAFF Committee.

3.10.1. Extending the uses of Equisetum arvense: Horsetail used in mulch

In November 2015, an extension of use was submitted to the European Commission. On 7 October 2016, the Appendix II of the Review Report in EU pesticide database (Anonymous, 2017) has been amended to include the use of horsetail in mulch for tomato (*Lycopersicum esculentum*), cucumber (*Cucumis sativus*) and ornamentals (*Prunus spp*., *Rosa spp.*) protecting against powdery mildews; *Pofodphaera xhantii*, *Phytium spp*., common root rot. Such extension of use has been evaluated and it has been established it can still satisfy the requirements of Article 23 of Regulation (EC) No 1107/2009.

3.10.2. Extending the uses of equisetum arvense: horsetail used on strawberry, raspberry and potato

One year later, on July 2017, the Appendix II of the Review Report in EU pesticide database (Anonymous, 2017) has been amended to include the uses on strawberry, raspberry and potato to control several fungal diseases (*Mycosphaerella fragariae*, *Aspergillus flavus*, *Fusarium vertillioides* and *Phytophthora infestans*). Trials showed the horsetail extract produces a high concentration of glyceollin phytoalexins in soybean cotyledons, which have a role in the resistance against fungi pathogens. (Guimaraes, 2015; Lygin, 2013) Such extension of use has been evaluated and it has been established it can still satisfy the requirements of Article 23 of Regulation (EC) No 1107/2009.

3.10.3. Extending the uses of Equisetum arvense: Horsetail used on on strawberry, raspberry and potato

One year later, on July 2017, the Appendix II of the Review Report in EU pesticide database has been amended to include the uses on strawberry, raspberry and potato to control several fungal diseases (*Mycosphaerella fragariae*, *Aspergillus flavus*, *Fusarium vertillioides* and *Phytophthora infestans*). Trials showed the horsetail extract produces a high concentration of glyceollin phytoalexins in soybean cotyledons, which have a role in the resistance against fungi pathogens. (Guimaraes, 2015; Lygin, 2013) Such extension of use has been evaluated and it has been established it can still satisfy the requirements of Article 23 of Regulation (EC) No 1107/2009.

3.10.4. Specificity of these extensions of use

The previously extensions of use were voted at PAFFF Committee, and any Technical Reports were promulgated by EFSA during the procedure nor Implementing Regulation.

3.10.5. Ongoing extension of uses for Equisetum arvense

In June 2019, an extension of use was submitted for horticulture and vegetable crops to control fungi diseases caused by *Plasmopara viticola*, *Fusarium spp* and *Erysiphe necator*. On 19 May 2020, the EFSA published its technical report on the outcome of the consultation with Member States which highlighted a lack of precision on the chemical compounds of horsetail extract, a lack of data on the safety of horsetail on non-target organisms and little toxicological data on mammals as well as the lack of evaluation of the extract for human consumption and health. However, for the previous applications for extension of use, there was not so much opposition even for the initial basic substance application (Anonymous, 2014, 2017). In March 2021, a version updated with bibliographical references has been sent to EFSA before the vote that should take place soon.

3.11. From Organic production regulation point of view

Organic farming also allows some plant extract uses in the fertilizer compartment (Annex II) of the production regulation (Anonymous, 2018a). Some plant extracts are already allowed in the plant protection compartment (Annex I of 2018/848). Later basic substances were included as specific category (Anonymous, 2016a ; Marchand, 2017a). Recently, *Equisetum arvense* became the head of Part 1 with all basic substances of Annexe I described in Article 24 of 2018/848 (Anonymous, 2018b).

4. CONCLUSION

This work showed the efficacy in field as plant protection means with fungicide properties of the approved under EU pesticide regulation and in organic farming basic substance *Equisetum arvense* and the absence of concern in the environmental compartments (groundwater and non-target organisms). Trials were conducted to investigate and deposit new possible agricultural usages. The efficacity of horsetail extract in association with other basic substance may promote these low concern natural substances. Only a vote at EU level is now needed.

5. FURTHER RESEARCH

Some of the useful applications and usages in fields are already approved at EU level or under evaluation (for field and tree nurseries usages); however, further development and field trials are under investigation to expand the beneficial aspect of this plant protection substance.

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