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Post harvest control of *Phytophthora cryptogea* of witloof chicory with different fungicides and possible occurrence of resistant strains

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Abstract

Mefenoxam applied on chicory roots at 4.8 g active ingredient $100\,l^{-1}$ achieved a very effective control of *Phytophthora cryptogea,* one of the main pathogens of witloof chicory. In seven trials, less than 10% of the roots treated with mefenoxam showed necrosis. The mefenoxam efficacy was better than that of propamocarb-HCl at $180\,g\,100\,l^{-1}$ (89–100% of infected roots) or mancozeb at $300\,g\,100\,l^{-1}$ (97–100% of infected roots). The efficacy of fosetyl-Al appeared to be irregular (2–98% of infected roots). Sensitivity to mefenoxam and azoxystrobin of some *P.cryptogea* strains was studied on amended media. Among the six strains tested, one was resistant to mefenoxam and two were moderately sensitive to azoxystrobin. The risk of occurrence of resistant strains in practice has to be considered; management of resistance to the two fungicides by application scheduling is proposed.

Keywords: *Cichorium intybus*; *Phytophthora cryptogea*; Mefenoxam; Azoxystrobin; Resistance

1. Introduction

In northern France, the root of the witloof chicory (*Cichorium intybus* L.) is harvested for its apical bud: put in the dark and irrigated with nutrient solution (forcing period in artificial conditions), it can grow etiolated and then produce a white salad, the chicory heart called "chicon" in France and Belgium. One of the main pathogens of the root is *Phytophthora cryptogea* (Pethybridge and Lafferty). This Oomycete can cause necrosis of the root, resulting in a large decrease in yield of chicory hearts (Forlot et al., 1966). Symptoms have also been described on roots in the field, in Arizona (Stanghellini and Kronland, 1982), but in Europe, Mestdagh (1998) confirms that necrosis appear in most cases during the forcing period, when the temperature (16–22 °C at root level) and the humidity are compatible with the formation and spreading of spores. The development of the disease is very rapid, especially when hydroponic systems are used.

1.1. Disease control on witloof chicory

Recent studies have shown the efficacy of fluazinam used before the storage of the roots and azoxystrobin applied before the forcing period (Benigni and Bompeix, 2004). This has enabled compounds with these two fungicides to be used in France on witloof chicory. However, in spite of their relatively satisfactory protection against *P.cryptogea* there are a few factors that could limit their use in practice:

- The efficacy of fluazinam decreases after three weeks of storage: between 23 and 37 days in the trials (Benigni et al., 2000). Some growers keep the roots in storage at least two months. They need fungicides with lasting efficiency.

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- Residues in roots treated with fluazinam do not allow their use for cattle food, a method used to remove roots after the production of the chicory heart.
- Azoxystrobin, also employed in the field to control foliar diseases (rust, powdery mildew), could favor emergence of resistant strains of *P.cryptogea* if the pathogen was present in the plot.

So, trials were carried out with the objective of evaluating the effectiveness of mefenoxam, the active enantiomer of metalaxyl (Nuninger et al., 1996) and the possibility of alternating with azoxystrobin. Indeed, since the end of the 1990s, the manufacturer (*Syngenta*, formerly *Novartis*) has replaced metalaxyl with mefenoxam in all its formulations. The use of compounds containing metalaxyl is not allowed in France since December 2003. The efficacy of metalaxyl in controlling *P.cryptogea* on witloof chicory was shown, in the 1980s (Saindrenan et al., 1981), but the fungicide had never been registered in France for this use. The efficacy of mefenoxam has been assessed on several Oomycetes: *Plasmopara helianti* (Zadra et al., 2002), *Phytophthora erythroseptica* (Peters et al., 2003) and also *Phytophthora gummosis*, on Citrus trees (Matheron and Porchas, 2002). Meanwhile, several cases of resistance to mefenoxam have been reported with *Phytophthora capsici* on bell pepper (Parra and Ristaino, 2001), *Phytophthora erythroseptica* on potato (Taylor et al., 2002) and *Phytophthora cactorum* on strawberry (Jeffers and Schnabel, 2004). In these crops, mefenoxam is the primary fungicide used to manage diseases caused by Oomycetes and frequent fungicide applications are required to control the pathogens. During witloof chicory production, there are only two fungicide applications:

- when harvesting before root storage (root dipping, drenching or spraying),
- when planting roots, before the forcing period (root collar drenching or spraying).

The risk of selecting resistant strains seems limited but sensitivity to mefenoxam and azoxystrobin of some strains of *P.cryptogea* has been studied on amended media.

2. Material and methods

2.1. Fungicide sensitivity

The effect of azoxystrobin (Ortiva®, 250 g a.i. l^{-1}) and mefenoxam (Santhal®, 480 g. a.i. l^{-1}) on mycelial growth in vitro was determined on malt agar medium.

2.1.1. Concentrations of fungicides

Mefenoxam was incorporated into the medium at 100, 50 (corresponding to using the manufacturer's recommended rate on witloof chicory), 25, 5, 1 and 0.2 μg ml^{-1} before autoclaving. Azoxystrobin was tested at 50 (using rate of registration on witloof chicory), 5 and 0.5 μg ml^{-1}, and was added into the medium after autoclaving. Medium without fungicide was also used as control.

2.1.2. Strains of P.cryptogea

Six strains were tested:

UPMC (Université Pierre et Marie Curie) isolated from necrosis on witloof chicory untreated with mefenoxam and regularly used in previous fungicides trials, R3, isolated from necrosis on witloof chicory treated with mefenoxam (J.L. Tanguy, OBS), INRA36, INRA52, INRA206 and INRA207, isolated from several horticultural crops (F. Panabières, INRA).

Mycelial plugs of 6 mm diameter were cut from the margin of a 8–10-days-old culture and placed in the centre of a 5 cm Petri dish, containing 10 ml of test medium (3 replicates for each isolate at each concentration).

2.1.3. Sensitivity assessment

Mycelial growth of strains was determined by measuring colony diameter after 5 days at 20 °C ± 1 °C. After subtraction of plug diameter, values were averaged and the relative growth as percent of control calculated for each rate of fungicide. EC_{50} (effective concentration to inhibit 50% of the radial mycelial growth compared to untreated control) and MIC (minimum concentration provided to inhibit 100% of the mycelial growth compared to untreated control—Andrieu et al., 2001) were estimated. The strains were characterized according to methods used for other *Phytophthora* spp.

- considered as resistant to mefenoxam if colony growth on amended media with 100 μg ml^{-1} was greater than 40% of that on non-amended media (Parra and Ristaino, 2001),
- considered as sensitive if $EC_{50} < 1$ μg ml^{-1} (Taylor et al., 2002),
- considered as highly sensitive if $EC_{50} < 0.5$ μg ml^{-1} (Peters et al., 2003).

2.2. Efficacy trials

Methodology has been described previously (Benigni and Bompeix, 2004). Only the main points are reiterated in this study.

Trials were carried out in blocks with four replicates of 70–80 roots. The hybrid used during all the trials was BEA (INRA/Ctifl), a sensitive cultivar (Benigni, 2002).

2.2.1. Inoculation

A sporangial suspension of strain UPMC was obtained from mycelial disks placed 7–10 d at 18–20 °C in a 9 cm diameter Petri dish containing 20 ml of water (Hoang, 1965). Disk and water were grinded with a mixer and the amount of sporangia adjusted at 5,000 sp/ml. Roots infected with this suspension were first subjected to forcing and then placed among healthy roots at the beginning of the trial (2–3% infected roots compared to healthy roots). The nutrient solution was maintained between 19 and 21 °C, a range frequently used by the growers from September to January. Each watering circuit was independent from the others to avoid mixing of fungicides in nutrient solution.

2.2.2. Fungicide application

When fungicides were applied just before the forcing period (with 5 l m^{-2} of the fungicide solution, which is the necessary volume for the application rate), treatments and inoculation took place on the same day.

When fungicides were applied before the storage, the plants were put in cold room for two months, so the time between treatment and inoculation (DAT) was 60 days. From December 1999 to June 2001, trials were carried out at the *Station Expérimentale de l'Endive* using roots harvested in several plots in northern France (Graincourt, Roye and Souchez). Mefenoxam (Santhal® 480 EC) was compared with mancozeb (Dithane®DG) and fosetyl-Al (Aliette®), fungicides registered in France to control *Phytophthora cryptogea,* and with propamocarb-HCl (Previcur®N) registered against *Pythium* sp. but also allowed to be used against *P.cryptogea*. Untreated roots were used as control. The mefenoxam usage rate for witloof chicory was derived from the rate registered in Belgium with metalaxyl (20 g a.i. 100 l^{-1}). Some studies have shown that in vivo, mefenoxam is 3–10 times more active than the S-enantiomer, also contained in metalaxyl (Zadra et al., 2002). Mefenoxam has similar activity when applied at half the metalaxyl rate against diseases caused by different *Phytophthora* spp. and *Pythium* spp. (Nuninger et al., 1996). The presence of the single active enantiomer in the formulation was taken into account and thus rates of 2.4, 4.8 and 7.2 g a.i. 100 l^{-1} (corresponding in 5, 10 and 15 ml 100 l^{-1} of the formulated compound) were selected in order to limit the risks of finding residues in the chicory hearts.

2.2.3. Disease assessment

At the end of the forcing period, after 21 days, roots were halved longitudinally to record necrosis (Fig. 1). Chicory hearts were harvested in order to assess influence of contamination on the yield.

Fig. 1. Symptoms scale used to evaluate the intensity of the infection. 0: healthy root; 1: slight necrosis; 2: low level of necrosis: <25% root height; 3: high level of necrosis: >25% root height.

2.3. Residue trials

The manufacturer *Syngenta* carried out three residue trials in 2000 and 2001. The rate of active ingredient tested was 9.6 g 100 l^{-1}.

3. Results

3.1. Fungicide sensitivity

3.1.1. Mefenoxam

The strain INRA206 with $EC_{50} < 1\,\mu g\,ml^{-1}$ (Table 1) and $MIC = 5\,\mu g\,ml^{-1}$ can be classified as sensitive. UPMC, INRA36, INRA52 and INRA207 appeared very sensitive, with EC_{50} values $< 0.2\,\mu g\,ml^{-1}$. R3 which exhibited growth on media amended with $100\,\mu g\,ml^{-1}$ greater than 40% of that on non-amended media was classified as resistant.

3.1.2. Azoxystrobin

All strains grew on media amended with $5\,\mu g\,ml^{-1}$ or less, of azoxystrobin. The growth of three strains was inhibited at $50\,\mu g\,ml^{-1}$: UPMC, INRA207 and R3 (Table 1). INRA36 and INRA206 can be characterized as moderately sensitive ($EC_{50} = 5\,\mu g\,ml^{-1}$, and mycelial growth relatively unrestricted by $50\,\mu g\,ml^{-1}$: 30%–40% of that on non-amended medium). INRA52 appeared more sensitive ($EC_{50} = 5\,\mu g\,ml^{-1}$) than INRA36 and INRA206, but less ($MIC > 50\,\mu g\,ml^{-1}$) than UPMC, INRA207 and R3.

3.2. Efficacy

In 1999 and 2000, results obtained by applying fungicides when planting roots showed mefenoxam to be highly effective at 2.4 and 4.8 g 100 l^{-1}. The rate of infected roots never exceeded 10% even though more than 40% of the untreated roots were infected (Table 2).

Table 1
Sensitivity to mefenoxam and azoxystrobin of *P.cryptogea* strains

Strain	mefenoxam		azoxystrobin	
	EC_{50} ($\mu g\,ml^{-1}$)[a]	MIC ($\mu g\,ml^{-1}$)[b]	EC_{50} ($\mu g\,ml^{-1}$)	MIC ($\mu g\,ml^{-1}$)
UPMC	<0.2	0.2	<5	⩽50
INRA36	<0.2	5	5	>50
INRA52	<0.2	1	<5	>50
INRA206	0.2–1	5	5	>50
INRA207	<0.2	<0.2	<0.5	⩽50
R3	>100	>100	<5	⩽50

[a]Effective concentration inhibiting 50% of radial mycelial growth compared to untreated control.
[b]Minimum concentration inhibiting 100% of the mycelial growth compared to untreated control.

Table 2
Percentage of infected roots at the end of the forcing period in each trial

Treatment	Before forcing period					Before storage	
Date	12/1999	01/2000	04/2000	05/2000	06/2001	12/2000	12/2000
Plot DAT	Roye 0 d	Graincourt	Graincourt	Graincourt	Graincourt	Roye 63 d	Souchez 64 d
mefenoxam 2.4 g 100 l^{-1}	1 b	8 b	–	–	–	32 b	40 b
mefenoxam 4.8 g 100 l^{-1}	0 b	3 b	10 b	0 b	2 d	2 c	10 c
mefenoxam 7.2 g 100 l^{-1}	–	–	–	–	–	2 c	3 c
mancozeb 300 g 100 l^{-1}	–	–	–	–	–	97 a	100 a
fosetyl-Al 300 g 100 l^{-1}	–	21 ab	4 b	2 b	75 c	–	–
propamocarb 180 g 100 l^{-1}	–	–	97 a	–	89 b	–	–
untreated control	71 a	41 a	94 a	87 a	97 a	100 a	99 a

Fungicide concentrations are expressed in active ingredient.
For each column, numbers followed by a different letter are significantly different (5% Newman–Keuls test on $\arcsin\sqrt{p}$; p = percentage of infected roots).
–: no test was carried out.

In January 2000, mefenoxam (less than 8% of the roots with necrosis) provided a better protection than that obtained with fosetyl-Al (21%) but in April and May the two fungicides showed the same efficacy. When mefenoxam was applied on the day of the inoculation, increasing dosage to 4.8 g 100 l^{-1} did not significantly improve root protection. However, at 4.8 g 100 l^{-1}, the efficacy of mefenoxam stayed constant in all trials, whatever the treatment method. Applied before storage by root drenching (60 d before inoculation), mefenoxam at 4.8 g 100 l^{-1} achieved a significant reduction in infection: less than 10% of the plants were infected. Under these conditions of high infection level, nearly all control plants had developed the disease and mancozeb at 300 g 100 l^{-1} did not control the pathogen (97–100% of infected roots). Increasing dosage to 7.2 g 100 l^{-1} of mefenoxam did not significantly improve root protection, but reducing the dose to 2.4 g 100 l^{-1} caused a loss in efficacy: 32% (12/2000—Roye plot) to 40% (Souchez plot) of the roots showed necrosis.

Because of these results only the dosage of 4.8 g 100 l^{-1} was used in 2001. Results obtained confirmed that mefenoxam provides a successful control of *P. cryptogea* infection. Only 2% of the roots treated with mefenoxam were infected, compared with more than 75% with fosetyl-Al and 89–100% with propamocarb-HCl.

When applied before the forcing period mefenoxam significantly and regularly reduced the rate of high level necrosis (Table 3). Fosetyl-Al or propamocarb-HCl also limited the level of the infection, but efficacy was reduced especially when the pathogen was most severe in the untreated control (06/2001).

3.3. Chicory hearts yield

In 2000, infection by *P. cryptogea* caused an important loss in production. The difference in produced biomass between uninfected and infected control roots was 21% in the Roye plot and 75% in the Souchez

plot. Production in the best marketable categories (Extra + Cat.1) was also penalized: −34% in the Roye plot and –97% in the Souchez plot. Under these conditions, mefenoxam treatment resulted in chicory hearts yield at the same level as that of uninfected plants (no significant difference—Table 4).

In 2001 (Graincourt plot), infection by *P.cryptogea* reduced biomass of untreated control roots to 50% and best marketable categories to 70% compared with uninfected roots. Treatment with mefenoxam significantly limited the loss in production: −17% in biomass and −25% in the best categories. Under the same conditions, chicory hearts yield of plants treated with fosetyl-Al or propamocarb-HCl decreased, respectively, to 31% and 45% in biomass, to 30% and 47% in best marketable categories.

3.4. Residues

The results of analysis carried out in good laboratory practice showed residues of mefenoxam in the chicory heart leaves from 0.05 to 0.14 mg kg^{-1}(data: *Syngenta*).

Table 3
Percentage of roots in each necrosis class (fungicides applied before the forcing period)

Date	04/2000			06/2001		
	Slight	Low	High	Slight	Low	High
mefenoxam 4.8 g $100l^{-1}$	9	0 c	1 c	0	0 b	2 d
fosetyl-Al 300 g $100l^{-1}$	3	1 c	0 c	10	16 a	49 c
propamocarb 180 g $100l^{-1}$	7	37 a	54 b	1	3 b	85 b
untreated control	3	12 b	79 a	0	4 b	93 a

Fungicide concentrations are expressed in active ingredient.
For each column, numbers followed by a different letter are significantly different (5% Newman–Keuls test on $\arcsin\sqrt{p}$; p = percentage of infected roots).

4. Discussion

The strain of *P.cryptogea* UPMC, collected from chicory root necrosis and used in fungicide efficacy trials appeared sensitive to azoxystrobin and mefenoxam. In these conditions, mefenoxam, applied at 4.8 g a.i. $100l^{-1}$ before the roots storage or the forcing period, provided a high control of *P.cryptogea* on witloof chicory. In treated plants, the rate of roots with necrosis never exceeded 10% even though 41–100% of untreated control roots were infected. Results showed that mefenoxam protected the roots at least 60 d, which constitutes a major improvement for the growers: the storage phase can be lengthened more than two months with a lower risk of *P.cryptogea* development. On the other hand, if the storage does not exceed two months, the use of mefenoxam when harvesting the roots, enables one to limit the number of chemical treatments: the protection does not need to be completed with another fungicide at planting to control *P.cryptogea* during the forcing period. This method of disease management is not possible if mancozeb or fluazinam are used before the roots storage. However, resistant strains of *P.cryptogea* have quickly appeared (1 year after the registration of the mefenoxam). The factors that have induced this emergence are not determined: use of mefenoxam several times on chicory or multiple applications during the crop rotation cycle (potato, shallot) in fields where chicory roots were cultivated.

The tests carried out with five others strains that had never been in contact with mefenoxam may imply that sensitive strains are predominant in natural population. However, further work is needed (tests on a larger sample of strains, survey of strains on witloof chicory) to confirm these data.

Among the six strains tested, two were characterized as moderately sensitive. Strain R3 highly resistant to mefenoxam appeared sensitive to azoxystrobin. So, management of *P.cryptogea* disease in witloof chicory

Table 4
Chicory heart yield in kg/100 chicory hearts

	Before storage				Before forcing period	
Plot and date	Roye 12/2000		Souchez 12/2000		Graincourt 06/2001	
chicory heart yield (kg/100 roots)	Biomass	Extra + cat.1	Biomass	Extra + cat.1	Biomass	Extra + cat.1
healthy control	15.5 a	9.3 a	19.9 a	14.0 a	15.6 a	8.1 a
mefenoxam 2.4 g $100l^{-1}$	15.4 a	9.4 a	17.7 a	11.4 a	–	–
mefenoxam 4.8 g $100l^{-1}$	14.7 a	9.1 a	19.4 a	14.0 a	13.0 b	6.0 b
mefenoxam 7.2 g $100l^{-1}$	15.1 a	9.0 a	19.1 a	13.3 a	–	–
mancozeb 300 g $100l^{-1}$	10.5 c	4.1 c	8.6 b	3.9 b	–	–
fosetyl-Al 300 g $100l^{-1}$	–	–	–	–	10.7 c	5.7 b
propamocarb 180 g $100l^{-1}$	–	–	–	–	8.6 d	4.3 c
infected control	12.2 b	6.1 b	5.0 c	0.4 c	7.8 d	2.4 d

For each column, numbers followed by a different letter are significantly different (5% Newman–Keuls test).
–: no test was carried out.

can be achieved using these two fungicides if they are alternated. When azoxystrobin has been applied in field, mefenoxam could be used before root storage. In this case, azoxystrobin or fosetyl-Al could complete the protection before the forcing period. *Syngenta* does not recommend two consecutive applications of azoxystrobin or mefenoxam during the season on witloof chicory. This study confirms this choice and advice given to the chicory growers by the *Station Expérimentale de l'Endive* will strongly reinforce this important point of the management of the disease. A stricter position would be to avoid treatment with mefenoxam before root storage if this fungicide had been used during the current year. In this case, growers might employ mancozeb in a situation of low disease pressure before storage, or fluazinam for a short period of storage and mefenoxam only when planting roots before the forcing period.

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