Postharvest Biology and Technology 178 (2021) 111540

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Assessment of pre- and post-harvest anti-sprouting treatments to replace CIPC for potato storage

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ARTICLE INFO

Keywords:
Potato
Sprouting
Maleic hydrazide
Post-harvest treatments
Genotypes

ABSTRACT

To avoid losses from sprouting during potato storage, the anti-sprouting agent chlorpropham [CIPC] has been widely used over the past few decades. However, the European Union recently decided not to authorize the renewal of CIPC, prompting the value chain to find alternative treatments. We assessed for three years the potential of pre- and post-harvest anti-sprouting treatments to replace CIPC using four potato-processing varieties. Pre-harvest application of maleic hydrazide [MH] and post-harvest applications of 3-decen-2-one, 1,4-dimethylnapthalene [1,4-DMN] and CIPC were performed following supplier's recommendations. In addition, we evaluated the potential of 3-decen-2-one and 1,4-DMN to prolong the efficacy of pre-harvest MH treatment anti-sprouting activity during storage. All molecules significantly reduced sprouting after seven months of storage compared with the untreated control group. MH, 3-decen-2-one, 1,4-DMN and CIPC displayed respectively 86.9 %; 77.9 %, 73.6 % and 99.8 % of efficacy to control sprout weight and 79.4 %; 73.4 %, 68.4 % and 96.9 % of efficacy to control sprout length. Our results suggest that using 3-decen-2-one and 1,4-DMN in combination with MH do not bring additional benefit to control sprouting. Because differences in dormancies could be observed between varieties, we also showed that the efficacy of post-harvest treatments is genotype-dependent, while MH pre-harvest treatment is effective equally for all varieties. Applications of CIPC and MH led to detectable residues in tubers, while no residue of 1,4-DMN has been detected in tubers treated with this molecule (< LOQ). We concluded that treatments with MH, 1,4-DMN and 3-decen-2-one are valuable alternatives to CIPC to control sprouting of processing potatoes.

1. Introduction

Potatoes (*Solanum tuberosum* L.) are an economically important crop. According to FAO, potato was the fourth largest food crop worlwide with 377×10^6 t produced in 2016, after rice (741×10^6 t), wheat (749×10^6 t), and maize (1.06×10^9 t) (FAO, 2018).

During potato storage, losses occur mainly due to water loss, disease, and sprouting (Magdalena and Dariusz, 2018). The evolution of potatoes' physiological age coincides with an increase in sprouting (Delaplace et al., 2008), which alters potato quality in different ways. Sprouting modifies potatoes' physical properties by reducing turgidity, inducing shrinkage, and accelerating weight loss (Alexandre et al., 2015; Sonnewald and Sonnewald, 2014; Teper-Bamnolker et al., 2010). Premature sprouting also leads to a reduction in nutritional and processing qualities, thereby eliciting economic losses (Alexandre et al., 2015; Sorce et al., 1997; Suttle et al., 2016). Moreover, potato sprouting can result in the production of toxic compounds in the potato flesh, such as solanine and chaconine (Koffi et al., 2017). To prevent the

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https://doi.org/10.1016/j.postharvbio.2021.111540
Received 24 August 2020; Received in revised form 12 January 2021; Accepted 24 March 2021
Available online 14 April 2021

aforementioned problems, it is important to delay potato sprouting during the storage period.

As soon as tuber formation begins, the young tuber is in a dormant condition, during which it imports sucrose from photosynthetic organs. During the dormancy period, the tuber cannot sprout, even under favorable conditions (Delaplace, 2007; Reust, 1982). Potato sprouting appears during storage when the dormancy period is broken progressively (Coleman, 1987; Daniels-Lake and Prange, 2007). Several parameters can be controlled to delay potato sprouting during long-term storage, including the use of varieties with long dormancy periods, low temperature storage, and the application of sprouting inhibitors. Despite the availability of varieties with good performance under long-term storage, it is not always possible for growers and retailers to use them because they do not necessarily comply with potato value chain requirements. Cold-induced sweetening (CIS), which occurs in most processing varieties, also limits the possibility of using cold-temperature storage to mitigate sprouting. CIS leads to dark color, alteration of potato quality, and an increase in acrylamide content after frying, which may pose risks to human health (Paul et al., 2016a; Wiberley-Bradford and Bethke, 2017). In this context, the potato value chain has relied heavily on the use of chemicals such as chlorpropham [CIPC], which was released commercially in 1951 and so far has been viewed as the most effective potato-sprouting suppressant (Paul et al., 2016c). CIPC is applied in post-harvest treatments and acts as an anti-sprouting molecule by inhibiting mitosis in potato cells (Campbell et al., 2010; Kleinkopf et al., 2003; Nurit et al., 1989; Wiltshire and Cobb, 1996). Studies have demonstrated that single or multiple applications with 18–36 g of CIPC per tonne of potatoes allow for potato storage without sprouting for five to 12 months at temperatures between 8 and 12 °C (Corsini et al., 1979; Mahajan et al., 2008; Paul et al., 2016b).

Maleic hydrazide [MH] is a systemic plant growth regulator first reported by Schoene and Hoffmann in 1949 (Schoene and Hoffmann, 1949). MH-based products are applied on the field during vegetative growth (Kennedy and Smith, 1951; Paterson et al., 1952) and are transported from leaves to growing progeny tubers, where they build up (Dias and Duncan, 1999; Hoffman and Parups, 1964; McKenzie, 1989; Venezian et al., 2017). MH's mode of action is not fully characterized. It has been suggested that it disrupts mitosis and/or interacts with the metabolism of hormones such as auxin and gibberellin (Hoffman and Parups, 1964; Venezian et al., 2017). Treating potatoes with MH-based products allows for delaying initial sprouting and inhibiting sprout growth for six to eight months without affecting sugar content (Caldiz et al., 2001; Yada et al., 1991).

1,4-dimethylnapthalene [1,4-DMN] is a product from the naphthalene group of chemicals found naturally in potatoes and has been found to control potato sprouting (Campbell et al., 2010; Campbell et al., 2012; Kleinkopf et al., 2003; Lewis et al., 1997). Lewis et al. (1997) showed that one application of DMN molecules (isomer mixture) at 100 mg kg^{-1} (expressed on a fresh weight basis) was sufficient to suppress sprout growth for six months during storage of the Russet Burbank variety. So far, 1,4-DMN's action mechanisms to control sprouting are not completely understood, but recent studies suggest that 1,4-DMN readily could inhibit plastid development at the initial stages, whereas this molecule also may lead to lasting transcriptional changes (Campbell and D'Annibale, 2016).

The α,β-unsaturated aliphatic aldehydes and ketone compounds have been described as having the ability to cause necrosis in potato sprouts during storage. It also has been reported that among these compounds, 3-decen-2-one, an α,β-aliphatic unsaturated ketone molecule, has been shown to control sprouting (Knowles and Knowles, 2012, 2015). The 3-decen-2-one treatment usually is vaporized on potatoes when their dormancy breaks, leading to necrosis in sprout tissue within 24−36 h (Immaraju, 2021, Personal Communication). It also induces a transient increase in tuber respiration rate, rapid desiccation of sprouts, and an overall reduction in the tissue's ability to modulate oxidative stress (Knowles and Knowles, 2012, 2015). It is important to note that 3-decen-2-one vapor is active only when the sprouts' fast-growing meristematic tissues are exposed to the product. This destruction and desiccation of external sprout tissue also elicit internal cell structure breakdown, leading to a "burnt out" appearance (Immaraju, 2021, Personal Communication).

Because of its high efficacy and cost-affordability, CIPC so far has remained the preferred anti-sprouting treatment in the potato value chain. However, due to the presence of data gaps in the application file for the renewal of the CIPC registration, and due to the raise of concerns for the consumer regarding a potential risk of this active substance and the metabolite 3-chloroaniline, the European Union recently decided not to authorize the renewal of this molecule (European Commission, 2019a; European Food Safety Authority (EFSA) et al., 2017).

This non-renewal indicates an urgent need for safe alternative treatments and procedures to reduce potato sprouting during storage. The aforementioned molecules appear to be promising and are already or coming onto the market as anti-sprouting products.

Contrary to CIPC, 1,4-DMN and 3-decen-2-one molecules require more than one or two treatments to elicit sprouting control during an entire storage season. These molecules also necessitate stricter monitoring of potatoes during storage. For instance, as mentioned earlier, to be effective, the 3-decen-2-one molecule needs to be applied on fast-growing meristematic tissues, i.e., the potatoes' sprouting state needs to be monitored carefully. Furthermore, sprouting control for processing varieties needs to be followed closely, as they usually are stored between 7 and 10 °C to avoid CIS, i.e., temperatures more conducive to sprouting, compared with potatoes headed for fresh markets, which usually are stored between 5 and 6 °C (Bishop et al., 2012).

Therefore, this study's purpose is to propose new suitable strategies for processing potatoes to cope with the CIPC non-renewal while maintaining good quality during storage. To reach this goal, this study focusses on different points. Potato variety's effect on sprouting was assessed to evaluate genetic factors' influence on sprouting. Therefore, two crisp varieties and two French fries varieties were compared. The following molecules' efficacy was evaluated for different genotypes to propose anti-sprouting treatments to replace CIPC that are suitable for processing potatoes, and are easy to use: MH (pre-harvest treatment); 3-decen-2-one; 1,4-DMN; and CIPC (post-harvest treatments). Combinations of pre-and post-harvest treatments also were tested to verify potential benefits from combinations in sprouting control. Finally, residues in treated and untreated potatoes were assessed at the end of the storage period to evaluate potential health concerns from treated potatoes and potential cross-contamination.

The main limitation of previous studies undertaken to assess anti-sprouting products' efficacy is that each product usually is tested in a different storage chamber. This implies that storage conditions are not exactly the same among the tested products, inducing a risk of unexpected bias in the results. In our study, we solved this technical problem by having a separated experimental chamber for each post-harvest product tested to avoid cross-contamination, while all the experimental chambers were located in the same cold storage chamber, i.e., all the storage conditions were equal for all tested products. Furthermore, all the tubers used for the experiments were produced in the same location the previous year, guaranteeing homogeneity in the physiological age of the tubers tested in the experiment. To our knowledge, this is the first experiment to evaluate the efficacy of 3-decen-2-one, 1,4-DMN, and CIPC molecules alone, and in combination with the MH pre-harvest treatment, coping with the aforementioned experimental precautions.

2. Materials and methods

2.1. Plant material, growing conditions, and field treatments

Field trials have been conducted by Agroscope, a center for agricultural research in Switzerland. Two crisp varieties (Lady Claire and

Verdi) and two French fries varieties (Markies and Fontane) were planted in April and harvested at the end of August or beginning of September over three consecutive years: 2015; 2016; and 2017 (planting dates: 13 April 2015, 11 April 2016 and 18 April 2017; harvest dates: 3 September 2015, 30 August 2016 and 29 August 2017, respectively). The following fertilizers were used: 120 kg ha^{-1} of N; 85 kg ha^{-1} of P_2O_5; 450 kg ha^{-1} of K_2O; and 25 kg ha^{-1} of Mg.

After planting, an herbicide treatment was performed for weed control. Haulm destruction was implemented in two phases. A first treatment was performed using the Reglone® (active ingredient: 200 g L^{-1} Diquat) in accordance with the supplier recommendations (Syngenta, 2018a), combined with mechanical destruction (using an Envi-MaxX machine from Rema Environmental Machinery B.V. [NL]). The dates of treatments are the following: 3 August 2015, 9 August 2016 and 7 August 2017. A second treatment was applied using the product Spotlight® Plus (active ingredient: 60 g L^{-1} Carfentrazone-ethyl) in accordance with the supplier recommendations (Syngenta, 2018b). The dates of treatments are the following: 10 August 2015, 12 August 2016 and 14 August 2017. During the period from planting to haulm killing, water deficit was monitored and calculated according to pluviometry and evapotranspiration, and corrected with a crop coefficient. In case of drought (a water deficit above 40 mm), the potatoes were irrigated with at least 30 L m^{-2}. Altogether, the potatoes were irrigated with 180, 65, and 138 L m^{-2} in 2015, 2016, and 2017, respectively. The potatoes were treated to protect them from potato blight (*Phytophtora infestans*) approximately once a week after emergence until haulm killing, with different products following recommendations from the PhytoPRE decision support system (PhytoPRE+, 2000). The plot was separated in two blocks and one of the two blocks was treated with Fazor®, which contains 60 % of maleic hydrazide [MH]. Five kilograms of Fazor® in 400 L of water were applied per hectare with a Birchmeier® backpack sprayers equipped with a pump driven by a combustion engine. This treatment has been performed when 80 % of the potato tuber size reached 25 mm (dates of treatments: 26 June 2015, 23 June 2016 and 26 June 2017) in accordance with the supplier recommendations (Leu+Gygax AG, 2018).

2.2. Harvest and grading

At harvest, the potatoes were stored at about 15 °C for two weeks in the dark to promote healing. Potato tubers then were weighed and calibrated to assess the tuber size. The tuber size used for the post-harvest trials was between 42.5 and 70 mm in diameter.

2.3. Experimental design

The potatoes were stored in small experimental chambers (0.8m × 1.2m x 2.0 m), with a total capacity of approximatively 200 kg of potatoes per experimental chamber (Fig. 1).

Each experimental chamber contained a tray on which two piles of stacked plastic crates were placed: one pile for potatoes treated with MH on the field and one pile for potatoes untreated on the field. Each pile is composed of four varieties disposed in four distinct plastic crates (experimental unit [EU]) (0.6 m × 0.4 m x 0.18 m), and each crate was filled with 100 tubers of a given variety. There were eight EUs per experimental chamber (4 varieties x 2 field treatments). Each experimental chamber was covered with an airtight plastic sheet inside a plastic structure and hermetically sealed on the tray using magnet bands. Anti-sprouting molecules were tested individually in each experimental chamber placed in the same storage chamber, which allows for having the same temperature for all experimental chambers. The storage chamber's temperature was 12 °C for one week, and then the temperature was brought to 8 °C with a decrease of 1 °C per week and kept at 8 °C for the remaining duration of the storage period. At the end of the storage period, a reconditioning was applied with an increase of 1 °C per week to reach 15 °C by the end of May.

Fig. 1. Picture of one experimental chamber (0.8m × 1.2m x 2.0 m) with a total capacity of 200 kg of potatoes.

Each chamber was equipped with fans, an air extractor, and CO_2 sensors (CozIR®-A CO_2 Sensor) connected to a microcomputer (Raspberry Pi 3, B Model) to control temperature, humidity, and CO_2 parameters. During storage, potatoes were stored in a controlled atmosphere with the following characteristics: 80 % RH; continuous ventilation; and air renewal to keep CO_2 concentration in the air below 0.124 mol m^{-3} (= 3000 ppm). The air extracted from the experimental chambers was expelled outside the storage chamber to avoid air contamination among chambers.

The following active molecules were tested and applied in post-harvest treatments: 3-decen-2-one (SmartBlock® - global registration owner: AMVAC Chemical Corporation); 1,4-Dimethylnaphthalene [1,4DMN or DMN] (1,4SIGHT® / DORMIR® - European registration owner: DormFresh Ltd); and chlorpropham [CIPC] (Neo-Stop Starter® - Global registration owner: UPL Benelux). These molecules were tested on potatoes treated or untreated on the field with MH. An experimental chamber containing the untreated control was also added to the experimental design. Anti-sprouting molecules were applied following commercial recommendations (Table 1).

After each treatment, an air circulation (without renewal) was applied for 24 h to allow the proper distribution of the product. After that period, the air was automatically renewed when the CO_2 concentration exceeded 0.124 mol m^{-3} (= 3000 ppm). The CIPC post-harvest treatment was applied using a MAFEX® Ultra-Low Volume (ULV) Fine Spray Unit for application of liquid products. 1,4-DMN and 3-decen-2-one post-harvest treatments were applied by hot fogging in each chamber using an electric fogger (Burgess® 982 Electric Professional Fogger, Model 16,982,150). To allow the fogging, the products were heated at a temperature ranging from 232 to 274 °C (The Fountainhead Group company, 2021).

This experimental design followed a split-split plot design comprising four anti-sprouting molecule levels (three molecules and an untreated control). Within each chamber are two distinct field treatment (FT) groups (treated or untreated on the field), and within each FT group

Table 1
Dosage and frequency of treatments for the tested molecules and the device used for treatments.

	Concentration of the active ingredient	Treatment quantity (mL t^{-1})	Frequency and total number of treatments during each entire season of storage	Dates of first treatments for each season of storage	Number of treatments before sprouting assessment	Dates of the last treatments for each season of storage	Application device
SmartBlock®	98 % pure 3-decen-2-one (AMVAC Chemical Corporation)	100	When all varieties had sprouts > 3 mm, 4 treatments	16 November 2015; 8 November 2016; 20 November 2017	3	15 May 2016; 27 April 2017; 1 May 2018	Burgess® 982 Electric Professional Thermal Fogger
1,4SIGHT®	98 % pure 1,4-DMN (DormFresh Ltd)	20	Every 6 weeks, 6 treatments	28 October 2015; 18 October 2016; 20 October 2017	4	23 May 2016; 15 May 2017; 24 May 2018	Burgess® 982 Electric Professional Thermal Fogger
Neo-Stop Starter®	300 g L^{-1} Chlorpropham (UPL Benelux)	60	One treatment	27 October 2015; 18 October 2016; 10 October 2017	1	27 October 2015; 18 October 2016; 10 October 2017	MAFEX® ULV Fine Spray Unit for application of liquid products

are four EUs (four varieties), each containing 100 individual tubers. This design was repeated during three years and the year is considered as a random factor.

2.4. Observations

2.4.1. Tuber size at harvest

The total yield at harvest (weight of potatoes) was recorded, as well as potatoes' tuber sizes for different varieties and field treatment groups. The following tuber sizes were considered: tubers smaller than 42.5 mm in diameter (small tubers) and larger than 42.5 mm (large tubers) (N = two years).

2.4.2. Sprouting during storage

For the three years of trial, sprouting was assessed after seven months of storage (end of March or early April), by sampling 25 tubers for each treatment and variety. This assessment was done by measuring the following parameters on sprouts with a minimum size of 1 mm: weight of sprouts from the 25 tubers and average length of the longest sprout of each tuber.

2.4.3. Sugar content

Sugar analysis was conducted using the ion chromatography method with conductivity detector (Zweifel Pomy-Chips AG, 2018) to assess the effect from products on potatoes' sugar content after seven months of storage (end of March or early April). Sucrose, fructose, and glucose levels were also measured for each sample (results are expressed on a fresh potato weight basis). The sum of glucose and fructose is viewed as the "reducing sugars" in potatoes. This observation was performed for two consecutive years (2017 and 2018) on two varieties (Verdi and Lady Claire).

2.4.4. Residues

Residues analysis were performed during two consecutive storage seasons (2016–2017 and 2017–2018) at the end of the storage period and after the reconditioning from 8 to 15 °C for all the tested molecules, except 3-decen-2-one. At least one month after the last treatment (Table 1), potatoes of the variety Fontane were washed with tap water for 30 s and sampled for residue analysis (dates of sampling: 15 June 2017 and 25 June 2018). The period between the last treatment and the sampling for residues analysis varies among products. Sampling for residue analysis were performed one month after the last treatment with 1,4-DMN, 8–8.5 months after the last treatment with CIPC and 12 months after the field treatment with MH. One kilogram of tubers was sampled and kept with the skin and another kilogram was peeled before analysis. Each sample was then cut into pieces and blended (Moulinex® - Ovatio 3 Duo Press) and disposed in plastic bags (Domédia kitchen, six liters zip lock bags). Then, the samples were kept in the freezer (- 80 °C) until the analysis.

A method based on the Dutch mini-Luke ("NL") extraction method was used for the extraction of the sample (Balleix, 2014; EURL-FV, 2014). Then, the Gas Chromatography - Mass Spectrometry - Triple Quad (GC–MS-TQ) method was used to detect the molecules 1,4-DMN, CIPC and 3-chloroaniline (limit of quantification [LOQ] = 0.01 mg kg^{-1}). The liquid chromatography-tandem mass spectrometry (LC–MS/MS) method was used to analyze the MH (LOQ = 0.1 mg kg^{-1}), according to the European Union guidance documents for pesticide residues [SANTE/11,945/2015 for samples of the year 2017 and SANTE/11,813/2017 for samples of the year 2018] (Balleix, 2014; EURL, 2015; European Commission, 2017).

2.5. Statistical analysis

R software, Version 3.6.3 (R Core Team, 2019), was used for the statistical analysis. A linear mixed model was used to analyze the measured variables: sprout weight and length; sugars in potatoes; total yield weight; and small and large tubers' weight. Due to the wide variability of the data and to fulfill parametric model assumptions, it was decided to transform the average sprout length and weight variables with (log +1) to ensure variance homogeneity and response variable normality when necessary. The year is viewed as a random factor. Significance tests were performed using chi-square tests provided by the "car" R package, Version 3.0–7 (Fox and Weisberg, 2019). To analyze the effect from significant variables, the marginal post hoc Tukey's test (emmeans method) was used as a multiple comparison test to identify mean differences within factors and interactions. To perform the aforementioned analysis, we used different R packages ("lme4," "emmeans," "Matrix," and "nlme") (Bates and Maechler, 2019; Bates et al., 2015; Lenth, 2020; Pinheiro et al., 2019). For data summary and graphics, we used different R packages ("ggplot2", "plyr", "Rmisc", "lattice," and "cowplot") (Hope, 2013; Sarkar, 2008; Wickham, 2011, 2016; Wilke, 2019).

3. Results and discussion

3.1. Varieties have differences in dormancy length

After seven months of storage, we observed a significant effect from potato variety on dormancy in terms of sprout weight (Table 2).

The Fontane variety had the highest sprout development, at 22.4 g, followed by Lady Claire, at 16.6 g; Markies, with 10.5 g; and Verdi, with 7.4 g. Sprout weight is significantly different for the Fontane ($p = 0.019$; Tukey's test) and Lady Claire ($p = 0.043$; Tukey's test) varieties compared with the Verdi variety (Fig. 2). It should be noted that no significant difference in sprout length was observed between the four varieties (Table 2).

Dormancy differences between varieties are a phenomenon that is well-characterized in the literature. Daniels-Lake and Prange (2007) and Magdalena and Dariusz (2018) reported that the dormancy period's length is mainly variety-dependent and modulated by other parameters, such as storage temperature and weather conditions during growing season. However, in our study, this result must be treated with caution

Table 2
ANOVA *P-values (Pr[>chi-sq])* for the measured parameters in response to the different factors and their interactions after seven months of storage (* = statistically significant).

Factors	Weight of sprouts	Length of sprouts	Reducing sugars	Sucrose
Product	<0.001***	<0.001***	0.874	0.039*
Field treatment	<0.001***	<0.001***	0.859	0.432
Variety	0.005**	0.111	0.192	0.050
Product x Field treatment	<0.001***	<0.001***	0.214	0.012*
Product x Variety	0.0496*	0.092	0.419	0.543
Field treatment x Variety	0.963	0.995	0.408	0.553

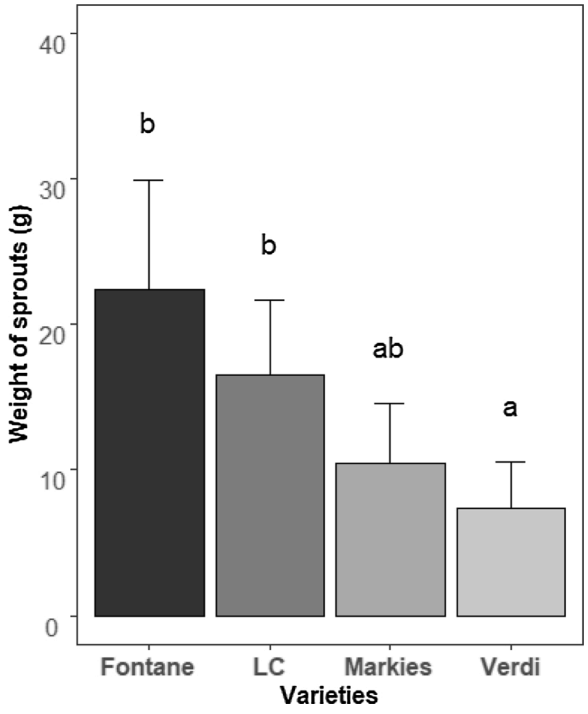

Fig. 2. Average sprout weight for each variety; over four products, two field treatments, and three years (n = 24) after seven months of storage (80 % RH); error bars represent standard error of the mean; (LC = lady Claire). Groups sharing the same letter are not significantly different (Tukey's test, confidence level of 95 %).

because there is little interaction (p = 0.0496) between the variety and product factors for sprout weight (Table 2). This interaction is described in section 3.5.

Among the two varieties tested, we observed that sucrose content was not significantly different (average of 2.3 g kg^{-1} for Verdi and 2.2 g kg^{-1} for Lady Claire), and reducing sugar content also was nearly equal for both varieties (average of 0.3 g kg^{-1} for Verdi and 0.2 g kg^{-1} for Lady Claire) (Table 2). Verdi and Lady Claire are crisp varieties known to be less susceptible to sweetening, explaining why sugar content is low for both.

At harvest time, total yield and tuber size balances were different between varieties (Table 3).

Tukey's test did not allow for distinguishing between varieties in terms of total yield and small tuber yield (p > 0.05); however, we observed a higher yield of large tubers for the Fontane variety (average of 50.69 kg) compared with the Lady Claire variety (average of 35.58 kg) (p = 0.028; Tukey's test) (Fig. 3).

Table 3
ANOVA *P-values (Pr[>chi-sq])* of the linear mixed model for the measured parameters in response to the different variables and their interactions at harvest time (* = statistically significant).

Variables	Yield	Small tuber size	Large tuber size
Variety	0.005**	0.008**	<0.001***
Field treatment	0.462	0.759	0.539
Field treatment x Variety	0.273	0.603	0.451

3.2. All post-harvest treatment products are effective, but CIPC remains the most effective one

3.2.1. Effect from post-harvest products on sprouting

Our results revealed a significant effect from post-harvest products on sprouting measurements (sprout length and weight). We also observed interactions between post-harvest and MH field treatments (Table 2). These interactions will be examined in section 3.4. As there is a low interaction (p = 0.0496) between the variety and product factors for sprout weight (Table 2), the effect of post-harvest products for each variety is detailed in section 3.6.

Our results suggest that all tested molecules (used without prior MH field treatment) effectively control sprouting for up to seven months of storage, as both sprout length and weight were lower in treated potatoes compared with the untreated control group (Table 4).

The sprouts' weight and length were higher for the untreated control group (an average of 64.0 g and 44.8 mm) than for potatoes treated with CIPC (an average of 0.1 g and 1.4 mm), with the 3-decen-2-one (average of 14.1 g and 11.9 mm), and with 1,4-DMN (average of 16.9 g and 14.2 mm) (Fig. 4 A and B).

As expected and already indicated in extant literature (Corsini et al., 1979; Mahajan et al., 2008; Paul et al., 2016b), our results confirmed that CIPC's efficacy is high (96.90 % efficacy for sprout length and 99.80 % for sprout weight compared with the untreated control group). The 3-decen-2-one and 1,4-DMN efficacies were similar: both molecules

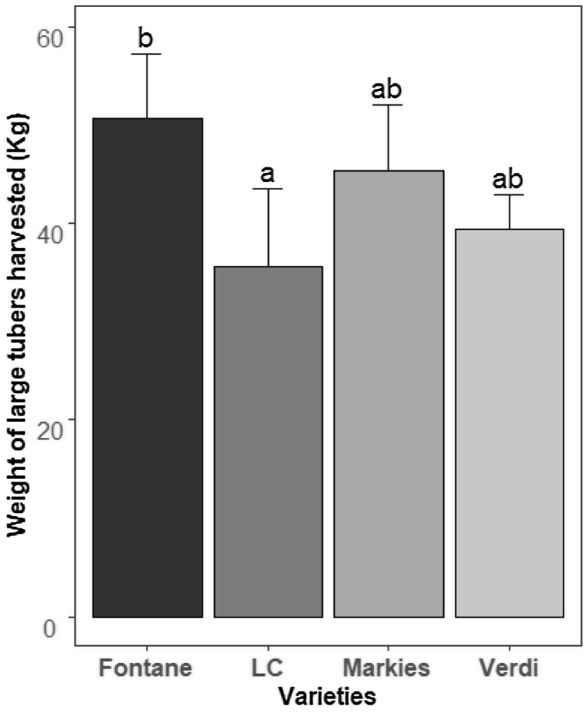

Fig. 3. Average yield's weight for large tubers at harvest time for each variety over two field treatments and two years (n = 4); error bars represent standard error of the mean; (LC = lady Claire). Groups sharing the same letter are not significantly different (Tukey's test, confidence level of 95 %).

Table 4
Tukey's test *P-values (emmeans method)* describing the products' effect on the measured parameters for potatoes treated or not treated with MH after seven months of storage (* = statistically significant) (FT = field treatment; MH = maleic hydrazide).

	Comparison between products	Effect on sprout weight	Effect on sprout length	Effect on sucrose
With FT	(Control + MH) - (1,4-DMN + MH)	0.391	0.419	0.974
	(Control + MH) - (CIPC + MH)	0.045*	0.012*	0.975
	(Control + MH) - (3-decen-2-one + MH)	0.425	0.228	0.710
	(1,4-DMN + MH) -(CIPC + MH)	0.130	0.059	0.845
	(1,4-DMN + MH) - (3-decen-2-one + MH)	0.958	0.882	0.513
	(CIPC + MH) - (3-decen-2-one + MH)	0.739	0.710	0.894
Without FT	Control - 1,4-DMN	0.007**	0.023*	0.114
	Control - CIPC	<0.001***	<0.001***	0.070
	Control - 3-decen-2-one	0.042*	0.036*	0.157
	1,4-DMN - CIPC	0.002**	0.001**	0.925
	1,4-DMN - 3-decen-2-one	0.721	0.921	0.981
	CIPC - 3-decen-2-one	0.220	0.132	0.775

controlled sprouting at 73.38 % and 68.37 % efficacy for sprout length and 77.94 % and 73.60 % efficacy for sprout weight, respectively, compared with the untreated control group.

Our results correspond with those of Lewis et al. (1997), who tested DMN's efficacy to control sprouting and showed that one application of DMN molecules (a mixture of isomers) at 100 mg kg-1 (expressed on a fresh weight basis) was sufficient to suppress sprout growth for six months in storage. Furthermore, similar to results in the literature (Knowles and Knowles, 2012, 2015), in our study, the 3-decen-2-one molecule controlled sprouts by causing desiccation and necrosis of sprouts in 24 h, confirming that 3-decen-2-one acts as a curative product, ensuring flexible, long-term management of potato sprouting during storage.

3.2.2. *Post-harvest products do not affect sugar content*

No significant effect on sucrose content was recorded (Table 4), although lower sucrose content was observed in about 40 %, 30 % and 34 % for potatoes treated with CIPC, 3-decen-2-one, and 1,4-DMN, respectively, compared with untreated potatoes (Fig. 4 C). It is reported in the literature that sprouting increases respiration and water loss of potato tubers and accelerates physiological aging (Pinhero and Yada, 2016). Therefore, treated tubers, which are less sprouted, have a reduced physiological aging. Consequently, the lower sucrose content observed in treated potatoes in our study is probably due to reduced physiological aging for treated potatoes compared with untreated ones. Our results correspond with those of Mehta and Singh (2015), who showed that sucrose concentration increased linearly during storage in both untreated and CIPC-treated potatoes and that the increase is lower in potatoes treated with CIPC. In their study, both reducing sugar and sucrose content remain low compared with the freshly harvested control group. The authors noted that the lower sugar content in CIPC-treated potatoes may be due to lower physiological aging compared with the control group (Mehta and Singh, 2015; Mehta et al., 2012).

We found that the anti-sprouting products tested in this study did not affect the reducing sugar content in potatoes (Table 2). The results are consistent with studies conducted with CIPC by Blenkinsop et al. (2002) and Mehta et al. (2012), who found that CIPC did not significantly affect crisp color quality or reducing sugars content in potatoes.

3.3. *The MH field treatment controls sprouting and sucrose content effectively*

3.3.1. *Effect from MH field treatment on sprouting*

The MH field treatment controlled sprouting very effectively. After seven months of storage, both sprout weight and length appeared to be significantly higher for tubers from untreated plants (average weight of 64.0 g and average length of 44.8 mm) than for tubers from plants treated only with MH (average weight of 8.4 g and average length of 9.2 mm) (Table 5) (Fig. 5 A and B). The MH was highly effective, with 86.94 % efficacy for sprout weight and 79.38 % for sprout length, compared with the untreated control. Similar efficacies also were found in a previous study by Caldiz et al. (2001), who reported that MH treatments delay the initial sprouting date and inhibit sprout growth for up to eight months.

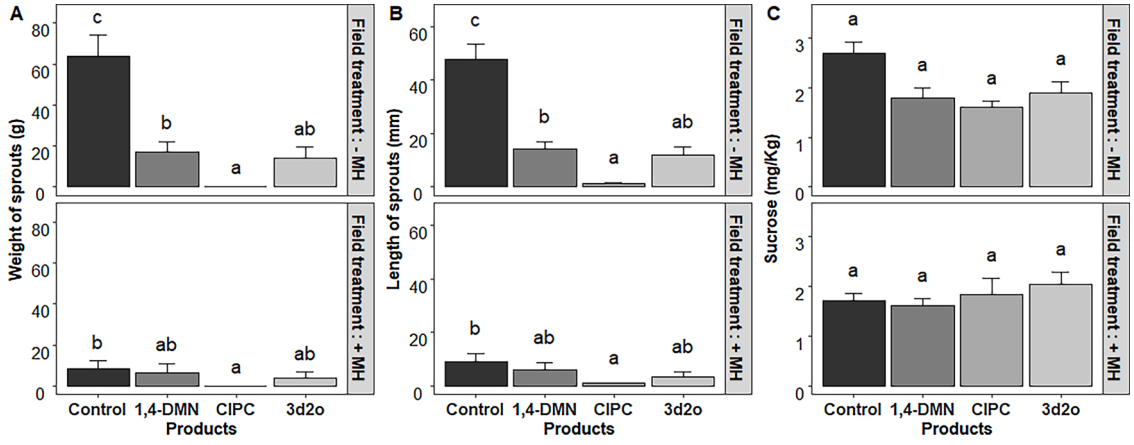

Fig. 4. Average sprout weight (**A**), sprout length (**B**), and sucrose content in potatoes (**C**) for each post-harvest treatment; with (+ MH) or without (- MH) MH field treatment after seven months of storage (80 % RH); over four varieties and three years for sprout weight and length measurements (n = 12) and over two varieties and two years for sucrose measurement (n = 4); error bars represent standard error of the mean; (3d2o = 3-decen-2-one; MH = maleic hydrazide). For a given observation and within each field treatment, groups sharing the same letter are not significantly different (Tukey's test, confidence level of 95 %).

Table 5
Tukey's test *P-values (emmeans method)* describing the effect of the MH field treatment on the measured parameters for each product after seven months of storage (* = statistically significant) (FT = field treatment; MH = maleic hydrazide).

Comparison: molecules used alone or with MH	FT effect on sprout weight	FT effect on sprout length	FT effect on sucrose
(CIPC + MH) - CIPC	0.795	0.658	0.540
(3-decen-2-one + MH) - 3-decen-2-one	0.019*	0.005**	0.679
(1,4-DMN + MH) - 1,4-DMN	0.006**	0.006**	0.640
(Control + MH) - control	<0.001***	<0.001***	0.0498*

3.3.2. *Effect from MH field treatment on sugars*

In our study, sucrose content in tubers harvested from untreated plants (average of 2.68 g kg^{-1}) was significantly higher than in tubers harvested from plants treated only on the field with MH (average of 1.72 g kg^{-1}) (Table 5) (Fig. 5 C). However, the field treatment did not affect the reducing sugar content in potatoes (Table 2). Our results are consistent with results from Sabba et al. (2009), who showed that MH did not lower glucose concentration at harvest or after a storage period of 25 weeks at 9 °C. However, in their study, MH treatment did not impact sucrose concentration, which contradicts our findings, as we observed a decrease in sucrose content for potatoes treated with MH compared with untreated potatoes. The increase in sucrose content during storage of untreated potatoes that we observed could be due to potato aging, as sucrose accumulates in potatoes during long storage periods (Ezekiel et al., 2011; Mehta et al., 2012). This increase can be explained by the formation of invertase inhibitor or the inhibition of the invertase activity at higher temperatures (Mehta and Singh, 2015; Uppal and Verma, 1990), but one of these mechanisms should have been mitigated by MH in our study. Another explanation of the differences observed between our study and Sabba et al. (2009) could be that the metabolism of the invertase is variety-dependent, as different varieties were used in both studies.

3.3.3. *Effect from MH field treatment on yield and tuber size*

In our study, MH field treatment did not affect the yield and size of tubers at harvest (Table 3). Our results correspond with previous research by Yada et al. (1991) and Caldiz et al. (2001) in showing that MH has no effect on yield. However, Ravichandran et al. (2012) reported an increase in the number of tubers in their experimental conditions. Our results showed no effect from MH treatments on tuber size, while Sabba et al. (2009) and Ravichandran et al. (2012) showed that MH field treatment can lead to a decrease in the production of large

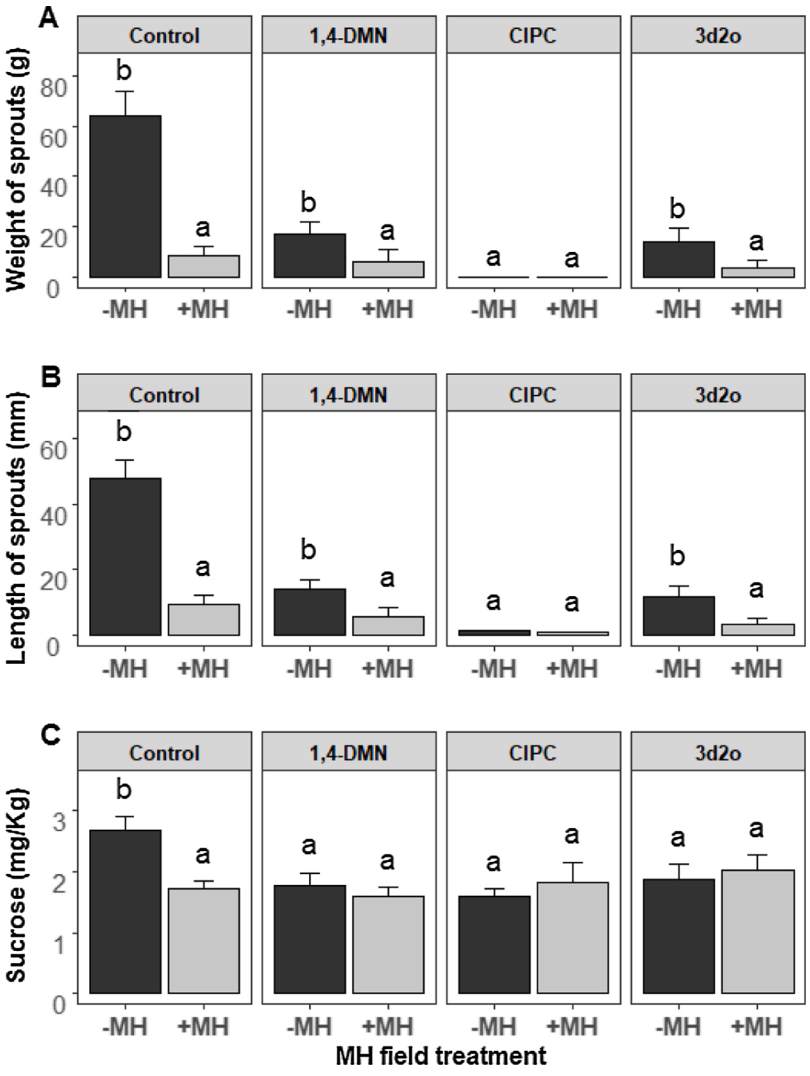

Fig. 5. Average sprout weight (**A**), sprout length (**B**), and sucrose content (**C**) of potatoes treated with maleic hydrazide (+ MH) or not (- MH) in the field and for potatoes treated with different post-harvest treatments after seven months of storage (80 % RH); over four varieties and three years for sprout weight and length measurements (n = 12) and over two varieties and two years for sucrose measurement (n = 4); error bars represent standard error of the mean; (3d2o = 3-decen-2-one; MH = maleic hydrazide). For a given observation and within each post-harvest treatment, groups sharing the same letter are not significantly different (Tukey's test, confidence level of 95 %).

tubers by lowering potato weight.

The discrepancy with our study might be explained by varietal differences in the response to MH treatment, as it has been reported previously that the effect from MH on potato yield is variety-dependent (Sabba et al., 2009).

3.4. Combining post-harvest products and MH field treatment does not come with a systematic added value

3.4.1. Benefit from using combinations of post-harvest treatment and MH field treatment to control sprouting

Our results highlighted that potato-sprouting control was greater in potatoes treated with a combination of CIPC post-harvest treatment and MH field treatment (average sprout weight of 0.0 g and 1.0 mm in length) than for potatoes treated only with MH field treatment (average sprout weight of 8.4 g and 9.2 mm in length) (Table 4) (Fig. 4 A and B). However, we observed that treating potatoes with the CIPC-MH combination does not significantly control sprouting more than treating potatoes only with a CIPC post-harvest treatment (average sprout weight of 0.1 g and 1.4 mm in length) (Fig. 5 A and B) (Table 5). These results show that the CIPC-MH combination does not provide any additional benefit, as the CIPC treatment alone already enables nearly complete inhibition of sprouting.

Sprouting levels in potatoes treated with a combination of MH field treatment and post-harvest molecules 3-decen-2-one and 1,4-DMN (average length of 3.5 mm and 5.9 mm and weight of 3.8 g and 6.3 g, respectively) were not significantly different from sprouting levels in potatoes treated only with MH on the field (average length of 9.23 mm and weight of 8.4 g) (Fig. 4 A and B) (Table 4). However, combinations of the molecules 3-decen-2-one or 1,4-DMN with a MH field treatment significantly control sprouting better than treatments with molecules 3-decen-2-one and 1,4-DMN alone (average sprout length of 11.9 mm and 14.2 mm, and weight of 14.1 g and 16.9 g, respectively) (Fig. 5 A and B) (Table 5).

These results showed that in this case, it was not possible to improve sprouting control significantly in potatoes already treated on the field with MH by performing post-harvest treatment with 1,4-DMN and 3-decen-2-one molecules.

Our results contradict Harper (2019), who tested combinations of post-harvest sprout suppressants and MH field treatment, and concluded that combinations that include MH are effective sprout suppressants, but in their study, they noted that this result could not be categorically established because they used potatoes from different stocks.

3.4.2. No influence from combinations on sugar content in potatoes

A combination of MH field treatment and post-harvest treatments with CIPC, 3-decen-2-one, and 1,4-DMN does not influence the reducing sugars in potatoes (Table 2), as well as sucrose content, compared with pre- and post-harvest treatments used alone (Tables 4 and 5) (Fig. 4 C and Fig. 5 C).

3.5. Variety effect varies according to post-harvest treatment

We observed little interaction ($p = 0.0496$) between the product and variety factors (Table 2) for sprout weight. We performed a separate supplementary Tukey's test analysis of this interaction to check the effect from factor variety on sprout weight for each product.

Sprout weight is not significantly different between varieties in the control group and in potatoes treated with CIPC ($p > 0.05$; Tukey's test), while for potatoes treated with 1,4-DMN, sprout weight is significantly higher for the Lady Claire variety (average of 29.8 g) compared with the Verdi variety (average of 9.4 g) ($p = 0.002$; Tukey's test), with no significant differences observed between the other varieties ($p > 0.05$; Tukey's test) (Fig. 6).

When potatoes are treated with 3-decen-2-one, sprout weight is significantly higher in the Fontane variety (average of 22.6 g) compared with the Markies variety (average of 1.2 g) ($p = 0.017$; Tukey's test), with no significant differences observed between the other varieties ($p > 0.05$; Tukey's test) (Fig. 6). The higher sprout development in the Fontane variety, compared with Markies, for potatoes treated with 3-decen-2-one can be explained because we applied the 3-decen-2-one treatment when sprouts reached a minimum length of 3 mm for all varieties. The Fontane variety has the shortest dormancy period, so the sprouts were bigger than 3 mm at the time of treatment. We think that the product was applied too late for this variety, as it is a product with a curative effect that works better when applied on small sprouts (< 3 mm).

3.6. Effect from products is genotype-dependent

The effect from post-harvest treatments (without previous MH pre-harvest treatment) varies according to variety. Treating potatoes with CIPC, 3-decen-2-one, and 1,4-DMN significantly decreases sprout weight among the Fontane ($p < 0.001$; $p = 0.046$ and $p = 0.005$) and Markies ($p < 0.001$, $p = 0.006$ and $p = 0.036$) varieties compared with the untreated control group, but it should be noted that the effect from 3-decen-2-one on sprout weight for the Fontane variety is low ($p = 0.046$) (Tukey's test, Fig. 7). This low effect is probably due to the fact that the Fontane sprouts were too big at the time of the first treatment with 3-decen-2-one (see 3.5).

For the Lady Claire variety, only the CIPC and 3-decen-2-one treatments decreased sprout weight ($p < 0.001$ and $p = 0.009$), while sprout weight for potatoes treated with 1,4-DMN was not significantly lower

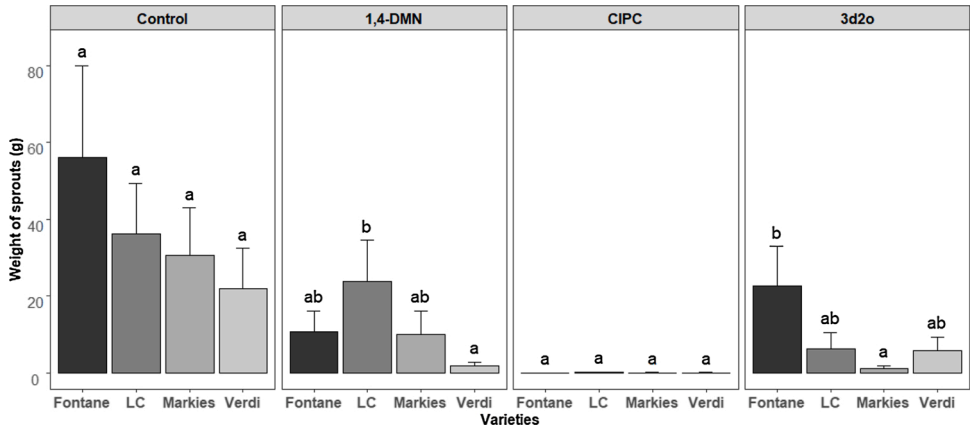

Fig. 6. Average sprout weight for each variety and for potatoes treated with different post-harvest treatments; over two field treatments and three years (n = 6); after seven months of storage (80 % RH); error bars represent standard error of the mean; (LC = lady Claire; 3d2o = 3-decen-2-one). Within each post-harvest treatment, groups sharing the same letter are not significantly different (Tukey's test, confidence level of 95 %).

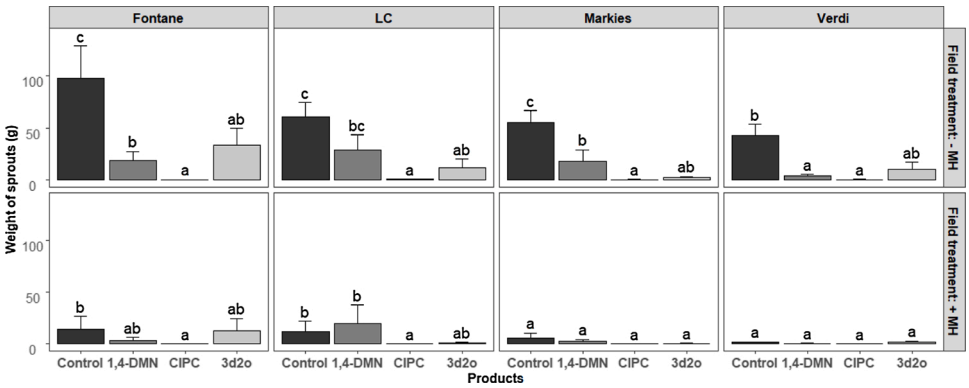

Fig. 7. Average sprout weight for each post-harvest treatment; with (+ MH) or without (- MH) MH field treatment and for different varieties after seven months of storage (80 % RH); over three years (n = 3); error bars represent standard error of the mean; (LC = lady Claire; 3d2o = 3-decen-2-one; MH = maleic hydrazide). Within each variety and each field treatment, groups sharing the same letter are not significantly different (Tukey's test, confidence level of 95 %).

than the control (Tukey's test). Finally, treating Verdi potatoes with CIPC and 1,4-DMN significantly decreased sprout weight compared with the control group ($p < 0.001$ and $p = 0.002$), while 3-decen-2-one did not significantly decrease sprout weight for this variety ($p = 0.112$) compared with the untreated control group, probably due to the data variability and because sprouting in the Verdi variety was relatively low in the control group (Tukey's test) (Fig. 7).

These results show that the effect from post-harvest treatments on sprout weight is genotype-dependent.

When potatoes are treated on the field with MH, among tested post-harvest treatments, only CIPC significantly reduces sprout weight in the Fontane and Lady Claire varieties compared with the control group treated on the field with MH ($p = 0.012$ and $p = 0.031$, Tukey's test).

Post-harvest treatments of the Markies and Verdi varieties already treated with MH on the field did not significantly reduce sprout weight compared with potatoes treated with MH only ($p > 0.05$, Tukey's test) (Fig. 7). Thus, the effect from post-harvest treatments used in combination with an MH pre-harvest treatment is also genotype-dependent.

Therefore, the choice of pre- and post-harvest products to control potato sprouting will necessitate considering choice of variety and adapting and monitoring potato storage accordingly.

3.7. CIPC and MH residues found in treated potatoes

The European Commission established maximum residue levels (MRLs) for the tested molecules in potatoes at 10 mg kg^{-1}, 15 mg kg^{-1}, and 60 mg kg^{-1} for CIPC, 1,4-DMN, and MH, respectively (European Commission, 2019b). 3-decen-2-one is registered as post-harvest treatment on potatoes in the USA and Canada, but not in the European Union. In the USA and Canada, there is an exemption from the requirement of a tolerance (= MRL) for this product (EPA, 2013; Health Canada, 2014).

CIPC residue was detected and was greater on average in potatoes analyzed with skin (22 mg kg^{-1} in 2016–2017 and no residue in 2017–2018 [<LOQ]) than in skinless potatoes (1.4 mg kg^{-1} in 2016–2017 and 1.3 mg kg^{-1} in 2017–2018).

Our results are consistent with those of Ezekiel and Singh (2008), who showed that CIPC residue is higher in the peel than in the flesh. Furthermore, 48 h after CIPC treatment, they found that CIPC residue in the peel was about 4.7 mg kg^{-1}, whereas in peeled potatoes, it was 0.1 mg kg^{-1}. Mahajan et al. (2008) also reported that peeling potatoes lowers residue levels, as they found negligible residue levels in peeled potatoes. Moreover, it was reported that the residue level of CIPC was significantly lower in cooked potatoes (crisps and jacket potato crisps), which was due to the nature of this product, though it is not systemic. Thus, residue remained on the tuber surface, and most of it was removed by peeling the potatoes before processing (Lewis et al., 1996; Mahajan et al., 2008). We observed cross-contamination in our study (2017–2018 trial), as we detected low CIPC residue levels in potatoes that did not receive CIPC treatment (0.012 mg kg^{-1} of CIPC in potatoes treated with the molecule 1,4-DMN and 0.042 mg kg^{-1} of CIPC in untreated potatoes, both analyzed with skin). Furthermore, 3-chloroaniline, the metabolite of CIPC, was not found in the potatoes in our trials (< LOQ).

We found MH residue in potatoes analyzed without skin and in potatoes analyzed with skin. In the potato flesh, 14 and 9.8 mg kg^{-1} of MH residue were found in 2016–2017 and 2017–2018, respectively, while in unpeeled potatoes, no residue was detected in 2016–2017 (<LOQ) and 10 mg kg^{-1} were found in 2017–2018. Previous studies showed similar results. Newsome (1980) found 3.3 ± 0.9 mg kg^{-1} of MH residue in treated potatoes after eight weeks of storage, analyzed with skin. The authors reported that because MH is applied on the field and translocated from the leaves to the potato tubers through the phloem (Dias and Duncan, 1999; Hoffman and Parups, 1964; McKenzie, 1989), residue is expected to be located within the flesh of the potato tuber and distributed evenly throughout the tuber (Lewis et al., 1998; McKenzie, 1989).

Molecules of 1,4-DMN were not found in treated potatoes in 2016–2017 and 2017–2018 (<LOQ).

4. Conclusion

Despite the high efficiency of CIPC, a need exists to develop new sprouting-control strategies in the wake of the European Union's non-renewal of CIPC due to gaps in the renewal application file, and to the raise of concerns for the consumers regarding the CIPC and its major metabolite (European Commission, 2019a; European Food Safety Authority (EFSA) et al., 2017). CIPC residues were detected in our study in potato tubers 8–8.5 months after CIPC treatments. The level of residues detected exceeded the authorized level (MRL = 10 mg kg^{-1}) in one sample of tubers analyzed with the skin. Besides, cross-contamination with CIPC were also found in our study. Such cross-contamination can be due to the high persistence of CIPC in the concrete of potato storage chambers (Douglas et al., 2018) and in devices such as ventilation systems (Martin, 2020).

Our experiments confirmed that MH field treatment is also effective in controlling potato sprouting and, therefore, can be viewed as a good alternative to CIPC. Nevertheless, using MH also resulted in the presence of residues in the potatoes. MH residues may be a problem, as this molecule elicits cytotoxic effects in mammal cells, carcinogenic effects in both mice and rats, and reportedly decreases fertility in rats (Epstein et al., 1967; Ponnampalam et al., 1983; Swietlińska and Zuk, 1978; Yurdakok et al., 2014). Nevertheless, in our trials, maximum MH residue levels were below the authorized level of 60 mg kg^{-1}. However, MH field treatment should be used for potato varieties with a short dormancy period, when drastic sprouting control is needed to avoid

losses during storage. For the other varieties, it is possible to avoid field treatment and schedule post-harvest treatments according to the duration of the variety's dormancy period and the expected storage duration (Visse-Mansiaux et al., 2018). For instance, the Verdi variety displayed a longer dormancy period than the Fontane variety because after seven months of storage, Verdi showed significantly lower sprout development in our study. Thus, the first post-harvest treatment for the Verdi variety could be delayed compared with Fontane. Using varieties with medium to long dormancies could allow for the use of anti-sprouting molecules that are less effective than CIPC, but less persistent in potato tubers, to avoid residue problems.

Our results showed that post-harvest treatments with 1,4-DMN and 3-decen-2-one reduce sprouting effectively during seven months of storage compared to the untreated control, but with lower efficacy compared with CIPC.

However, acceptation level of sprouting is higher for potatoes dedicated to processing compared with potatoes dedicated to the fresh market. In the present study, we concluded that both 1,4-DMN and 3-decen-2-one post-harvest treatments allowed to maintain a good control of sprouting up to seven months for processing potatoes and represent valuable alternative to CIPC. Moreover, no residue of 1,4-DMN has been detected in tubers treated with this molecule in our study (< LOQ). The benefit of the 3-decen-2-one post-harvest treatment is that this molecule allows to burn and dry out sprouts and can be used to save potato stocks that already have sprouted, as the study authors reported that applying 3-decen-2-one on potatoes leads to necrosis in sprouts within 24−36 h of exposure (Knowles and Knowles, 2015). Such necrosis after treatments with 3-decen-2-one also was observed in our experiments. Nevertheless, 3-decen-2-one treatments should be performed on tubers with small sprouts (< 3 mm), as the efficacy of this product drops for tubers with bigger sprouts. For example, we observed this phenomenon with the Fontane variety.

Our results showed that combining MH field treatment with post-harvest treatments does not improve sprout control compared with pre-harvest or post-harvest treatments used alone. For instance, our results suggest that performing a post-harvest treatment with the molecules 3-decen-2-one or 1,4-DMN on potatoes already treated on the field with MH does not improve sprout control. These findings indicate that these combinations are not economically sustainable and that in this case, pre-harvest treatment with MH alone is sufficient to control sprouting.

Other products on the market have been touted as effective to control post-harvest potato sprouting, such as mint essential oil and ethylene gas, which have the advantage of being authorized for organic farming (Martin, 2012), but they also present drawbacks.

Nebulizing of mint essential oil may increase sprouting-control expenses during storage because a large quantity is required during the storage period, and its price is generally higher than the other chemicals available on the market (Curty, 2012; Martin, 2012).

Costs associated with ethylene gas treatments are in the range of those reported for CIPC; nevertheless, this product often is not recommended for the storage of processing potatoes (Martin, 2012) because ethylene gas is reported to increase reducing sugars in potatoes and thus leads to a risk of darkening of potatoes after frying (Daniels-Lake, 2013). Harper and Stroud (2018) reported that ethylene's effect on processing fry color was variety-dependent; therefore, ethylene could be used for some varieties. However, the authors recommend testing each variety's fry-color response to ethylene before using it on a larger scale. Further research in this area would allow for screening current and future processing-potato varieties suitability for ethylene treatment. Prange et al. (2005) reported that the 1-methylcyclopropene (1-MCP) molecule can be used in combination with ethylene treatment to reduce ethylene-induced fry-color darkening.

Finally, cold storage (at 4 °C) to delay sprouting could be an option for some varieties with a higher tolerance to CIS, either through conventional breeding or genetic engineering. For instance, the Lady Claire, Kiebitz, and Verdi varieties reportedly have limited CIS abilities after being stored at 4 °C (Visse-Mansiaux et al., 2019). Such varieties could be stored at low temperatures and used for processing with a lower risk of acrylamide production. Furthermore, anti-sprouting treatments would be requested only for very long storage periods.

Author statement

All authors listed have contributed significantly to the work and agree to be in the author list.

Margot Visse-Mansiaux: Conceptualization, methodology, visualization, investigation, formal analysis, writing-original draft, validation, writing-reviewing and editing.

Maud Tallant: Investigation.

Yves Brostaux: Formal analysis, validation, writing-reviewing and editing.

Pierre Delaplace: Writing-reviewing, validation and editing.

Hervé Vanderschuren: Supervision, project administration, funding acquisition, methodology, investigation, validation, writing-reviewing and editing.

Brice Dupuis: Supervision, conceptualization, methodology, investigation, project administration, funding acquisition, validation, writing-reviewing and editing.

Declarations of Competing Interest

None.

Funding

The authors would like to thank Innosuisse – the Swiss Innovation Agency (grant number 17865.2 PFLS-LS), Fenaco (Curty Fabien, Kohli Christoph, and Hämmerli Markus), Zweifel (Blumenthal Marco and the team) and Swisspatat in Switzerland, as well as the Ministry of Walloon Region (EUREKA grant from the SPW6), Arysta LifeScience (Minvielle Caroline) and UPL Benelux (Bonnet Marc) in Belgium for their collaboration and/or financial support.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The authors would like to thank several colleagues for their support: Kessler Willy (head of the department); Pellet Didier (head of the group); Schwärzel Ruedi & Frei Peter, scientific collaborators; Torche Jean-Marie; Riot Gaétan; Wild Werner; Greppin Ariane, technicians; and everyone else on the team; as well as Wüst Jim; Maurin Julianne; Merabet Abdelkader & Bayrychenko Zhanna, interns (Agroscope, Ch-1260 Nyon, Switzerland); and Prof. Soyeurt Hélène (Gembloux Agro-Bio Tech University of Liège, 5030 Gembloux, Belgium).

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