Storage of processing potato varieties: the post-CIPC era

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Figure 1 | Automated experimental unit manufactured by Agroscope, which allowed potatoes to be treated independently in the same cold room. (Photo: Margot Visse-Mansiaux, Agroscope)

Abstract

For decades, chlorpropham (CIPC) has been the most commonly used product for controlling potato sprouting during storage. The non-renewal of the authorization of this molecule came into effect in January 2020 within the European Union. In Switzerland, its use has been banned since 30 September 2020. Anticipating this situation, Agroscope performed trials over a five-year period, from 2015 to 2020, to find alternatives to CIPC for the storage of commercial potato varieties. The efficacy of five anti-sprouting molecules applied in post-harvest treatments was evaluated for at least two consecutive years: 1,4-dimethylnaphtalene (1,4-DMN), 3-decen-2-one, ethylene alone or in combination with 1-methylcyclopropene (1-MCP), L-carvone, and D-limonene. In addition, the efficacy of maleic hydrazide (MH), which is applied in the field to crops, was also evaluated. The efficacy of the active molecules was evaluated up to five or seven months of storage and compared to an untreated control and to the efficacy of CIPC. Reducing sugars were measured in tubers of the trial that evaluated the efficacy of ethylene alone or in combination with the 1-MCP molecule. The results show that all the tested molecules are effective in controlling sprouting, but with varying degrees of efficacy within the tested molecules and the experimental conditions. Moreover, the efficacy of certain molecules (MH and ethylene) can vary depending on the variety. We also observed that the active molecule 1-MCP inhibited the increase in reducing sugars caused by an ethylene treatment. In general, the molecules tested were not as effective as CIPC. The use of these molecules should be combined with innovative storage strategies in order to meet the dual challenge of keeping stocks sprout-free for several months and preventing an increase in reducing sugars.

Key words: potatoes, sprouting, maleic hydrazide, essential oils, synthetic molecules.

Introduction

Chlorpropham (CIPC) is a highly effective molecule that has been used worldwide for decades to control potato sprouting during several months of storage (Paul *et al.* 2016).

The use of pesticides within the European Union (EU) and Switzerland has increasingly been subject to constraints for safeguarding human health and promoting environmental sustainability. In this context, the use of CIPC is in its final days. The decision not to renew the authorization of this molecule came into effect in the EU in January 2020 (EU Regulation 2019). The use of CIPC stocks is being phased out, and is already prohibited in several European countries such as Belgium (Martin 2020a). In Switzerland, the sale of CIPC was still authorized up to 15 August 2020, and 30 September 2020 was set as the use-by date for the remaining stocks (FOAG 2020). This means that the 2020–2021 storage season will be CIPC-free in the majority of European countries. Switzerland was theoretically still able to treat potatoes with CIPC in September 2020, but this was discouraged to avoid residues in the tubers and storage cells, given the high persistence of the product. Indeed, although the maximum residue limit (MRL) for CIPC currently remains unchanged in the EU (MRL = 10 mg kg^{-1}) (European Commission 2019), it is expected to change rapidly. A vote is scheduled for autumn 2020 that is expected to endorse a temporary MRL of 0.4 mg kg^{-1} which will be implemented in spring or summer 2021. This temporary MRL will be further reduced at a later stage (Martin 2020a). In Switzerland, the Federal Food Safety and Veterinary Office (FSVO) has decided that the current MRL of 30 mg kg^{-1} will remain in force until 1st July 2021, at which point it will change to 10 mg kg^{-1}. As in the EU, Switzerland is expected to subsequently fix a temporary MRL of 0.4 mg kg^{-1}; however, the date of this revision for Switzerland is not yet known (Swisspatat 2020). CIPC is a product that remains in facilities for a long time; it has been shown to persist in the concrete of storage rooms, as well as in their ventilation systems (Douglas *et al.* 2018; Martin 2020b). Consequently, it is important to anticipate the risks of product persistence in storage facilities. The first measure would be to stop using CIPC, and the second would be to clean the storage facilities to remove a maximum of residues from previous years' treatments.

The ban on using CIPC implies a genuine need to find new anti-sprouting solutions. Several molecules that can be applied post-harvest as an alternative to CIPC are already on the market worldwide, such as:

1,4-dimethylnaphtalene (1,4-DMN)

The molecule 1,4-DMN is a hormone that is naturally present in potatoes (Campbell *et al.* 2012), and chemically synthesized to be used as a sprout inhibitor. 1,4-DMN is approved in six European countries, as well as in the United States, Canada, New Zealand, Mexico, and Kenya (Jina 2020). It has just been approved in Switzerland (FOAG 2020). The MRL of the molecule 1,4-DMN in potatoes is fixed at 15 mg kg^{-1} in the EU (European Commission 2019) and in Switzerland (FSVO 2020).

3-decen-2-one

The molecule 3-decen-2-one is a natural biochemical compound (EPA 2013) found in certain mushroom species of the genus *Boletus* and authorized as a food additive in the EU. This molecule is chemically produced and is approved as a sprout inhibitor in the United States, Canada and Israel. It is expected to be registered in the EU in 2022 (Immaraju 2018).

Ethylene

Ethylene is a hormone that is naturally present in numerous fruits and vegetables. This molecule is approved as a sprout inhibitor with different modes of application. The first is via a so-called 'Biofresh Safestore' system, which uses 99.95 % pure ethylene supplied in compressed-gas cylinders. Ethylene is released in the storage room in a controlled manner by a Biofresh Ethylene Management Unit (EMU) (BioFresh 2020). Offered by the company Biofresh (Biofresh Group Ltd.), this system is approved in six European countries, the United States, and Japan (Caisley 2020). The second delivery mode, offered by the firm Restrain® Company Ltd., is via a generator that transforms ethanol into ethylene directly in the storage room. The Restrain® system is also approved in numerous European countries, particularly in Switzerland, where it is marketed by Netagco (Netagco Suisse Sàrl).

L-carvone

Mint essential oil, which primarily consists of the molecule L-carvone, has been approved as a sprout inhibitor in 18 European countries (including Switzerland) as well as in the United States (De Barbeyrac 2020).

D-Limonene

Orange essential oil, of which the main active molecule is D-limonene, was recently approved as a sprout inhibitor in the Netherlands (Bonnet 2020).

Maleic hydrazide (MH)
Maleic hydrazide-based products are applied in the field. These products are available in Switzerland and help slow sprouting during storage (Caldiz *et al.* 2001). This molecule is very old, given that it was first approved in the late 1940s (Schoene et Hoffmann 1949).
This research project aimed to explore all alternative treatment solutions to CIPC for controlling potato sprouting and maintaining the quality of goods during storage. The efficacy of the different aforementioned molecules was tested under experimental conditions for some molecules (200 kg potatoes) and under semi-industrial (five metric tons) and industrial conditions (>300 metric tons) for others. The trials were conducted at Agroscope and/or at our partner Fenaco.

Materials and methods

Anti-sprouting treatment in the field

Maleic hydrazide (MH)
We tested the efficacy of maleic hydrazide (MH) up to seven months of storage to study the efficacy of this molecule over time in controlling sprouting during storage. The efficacy of MH was compared to an untreated control and to the efficacy of CIPC. Sprouting (the weight of the sprouts from 25 tubers) was observed after three and five months of storage at 8 °C as well as at seven months of storage after a gradual increase in temperature of 1 °C per week, ultimately reaching 15 °C after seven months. This process of gradually increasing the temperature is referred to as 'reconditioning' in the rest of this article. Nine varieties (Agria, Bintje, Fontane, Innovator, Lady Claire, Markies, Panda, Pirol, and Verdi) were tested for two seasons of consecutive trials (2016–2017 and 2017–2018). The treated tubers (100 kg per product) and the control tubers (100 kg) were stored in a cold room at 8 °C and 80 % relative humidity.
The molecules were applied according to the suppliers' recommendations (Tab.1).

Anti-sprouting treatments during storage

1,4-DMN and 3-decen-2-one
The efficacy of the 1,4-DMN and 3-decen-2-one molecules was tested in experimental chambers designed by Agroscope containing 200 kg potatoes and allowing control of the CO_2 level. These experimental chambers were placed in a single cold room at 8 °C and 80 % relative humidity, allowing the molecules to be tested under identical temperature and humidity conditions (Fig. 1). The molecules were tested on nine varieties (Agria, Bintje, Fontane, Innovator, Lady Claire, Markies, Panda, Pirol, and Verdi) for two seasons of consecutive trials (2016–2017 and 2017–2018). In order to evaluate the efficacy of the molecules, sprouting (the weight of the sprouts from 25 tubers) was observed after three and five months of storage at 8 °C for the treated tubers as well as for an untreated control. The sprout control efficacy of 1,4-DMN and 3-decen-2-one was compared to that of CIPC and the untreated control.
The molecules were applied according to the suppliers' recommendations (Tab.1).

Ethylene alone or in combination with 1-MCP
We also evaluated the efficacy of ethylene for controlling sprouting (the weight of sprouts from 25 tubers), alone or in combination with the molecule 1-MCP (trade name: SmartFresh™). These tests were conducted under the same experimental conditions as for the molecules 1,4-DMN and 3-decen-2-one, and compared to an untreated control. The efficacy of these molecules was also compared to that of CIPC. The 'control' and 'CIPC' chambers were placed in a different cold room from the 'ethylene' and 'ethylene + 1-MCP' chambers. The ethylene was released into the cold room via the Restrain® system (Restrain® Company Ltd.), which continuously released 10 ppm of ethylene into the atmosphere (after a progressive increase). Given that the ethylene generator was not fitted directly into the experimental chambers and that said chambers did not continuously draw air from the cold room, the ethylene concentration in the experimental chambers was variable (less than or equal to 10 ppm). The 1-MCP was applied at the end of October and then once a month at a concentration of 2 g SmartFresh™ powder diluted in 20 ml distilled water. When mixed with water, 1-MCP produces a gas that volatilizes in the storage room. We followed the dosage recommended in the publication of Prange *et al.* (2005), which uses 1-MCP via the product EthylBlock®. The dosage of 1-MCP has been adapted for the volume of our experimental chambers in order to obtain the same dosage (0.9 $\mu l.L^{-1}$) with the product SmartFresh™ used in our study.
We also observed the impact of these molecules on potato sugar content. Our partner Zweifel (a potato chip manufacturer in Switzerland) conducted the analyses of the reducing sugars (glucose + fructose) on four varieties (Markies, Agria, Verdi, and Lady Claire) after three and five months of storage and for two consecutive years (2015–2016 and 2016–2017).

Table 1 | Information on the application of the molecules tested for Switzerland (*information given for guidance only; other suppliers, dosages, or application methods may exist).

Trade name of products used	Molecules	Modes of action	Product supplier in Switzer-land*	Amount to be applied*	Frequency of treatments*	Date of first application*	Modalities of application in our trials	Witholding period (waiting time before removal of potatoes from storage after treatment)*	Method of application
Fazor®	60% maleic hydrazide	Inhibits cell division (*inter alia*)	Arysta LifeScience Switzerland Sàrl	5 kg/ha	Single treatment	Size greater than 25–30 mm	When the size was > 25 mm (1 treatment)	–	Liquid spraying
Smart-Block®	98 % 3-decen-2-one	Curative: necrosis via destruction of the internal structure of the sprout cells	Not yet approved	100 mL/t	Application when the sprouts reach 3 mm in size (max. 4 treatments)	When the sprouts reach 3 mm	End of November or December according to the trials (4 treat-ments)	Unknown, as not approved in Europe	Hot-fogging
Dormir®	98 % 1,4-DMN	Preventive: prolongs potato dormancy	AGROLINE (Fenaco**)	10 to 20 mL/t)	Every 6 weeks (max. 120 ml over season)	Possible as of entry into storage	Mid-October (treatments every 6 weeks)	30 days (EU)	Hot-fogging
Neo-Stop Starter®	300 g/l Chlorpro-pham	Inhibits cell division	Arysta LifeScience Switzerland Sàrl	60 mL/t	Single treatment for liquid application	At the beginning of storage	Mid-October (1 treatment)	Four weeks after last treatment	Liquid spraying***
Argos®	843.2 g/L D-limonene	Preventive and curative (necrosis)	Not yet approved	100 mL/t	Every 3 weeks	1 month after entry into storage	Variable from mid-October to mid-November, depending on the trials (treatment every 3 weeks)	No withhol-ding period	Hot-fogging (190 °C) ***
Biox-M®	65 to 85 % L-carvone	Preventive and curative (necrosis)	Andermatt Biocontrol SA	90 ml/t (1st treat-ment) then 30 to 45 ml/t	Every 3 weeks (30 ml/t) or 4 weeks (45 ml/t) and a maximum 360 ml/t in total	6 to 20 days after harvest	Variable from mid-October to mid-November, depending on the trials (treatment every 3 weeks)	No withhol-ding period	Hot-fogging (180–190 °C)***
Éthylène	Ethylene	Preventive – slows the growth of the sprouts and their speed of elongation	Netagco Suisse sàrl	Progressive increase, then 10 ppm continuously	Continuously	At the beginning of storage	From the beginning of storage (Restrain® generator)	No withhol-ding period	Restrain® generator

**The product Dormir® was approved in Switzerland in September 2020 and will be marketed by Fenaco's new AGROLINE unit (https://www.agroline.ch/fr).
***Other application methods exist: check with the suppliers.

The molecules were applied according to the suppliers' recommendations (Tab. 1). Since 1-MCP is not authorized for the treatment of potatoes, it is not listed in Table 1.

Essential oils

The efficacy of the essential oils of mint and orange (L-carvone and D-limonene) was evaluated after three and five months of storage over two years of trials on three varieties (Agria, Verdi, and Innovator) and compared with that of an untreated control. The first year of trials was conducted under semi-industrial conditions (five metric tons for the year 2017–2018) and the second year under industrial conditions (> 300 metric tons for the year 2018–2019). The tubers of the different varieties came from the same batch, except for the tubers of the control from the year 2017–2018, which came from a different batch.

The molecules were applied according to the suppliers' recommendations (Tab. 1).

Experimental design and statistical analyses

The software program R, version 3.6.3 (R Core Team 2019) was used to perform the statistical analyses. Experimentation on the field treatments followed a repeated-measures linear mixed model with the fixed factors 'variety' and 'molecule' and the repeated factor 'observation date'. Year was considered a random factor. Post-harvest trials followed a linear mixed model with the fixed factors 'variety' and 'molecule', and 'year' was considered the random factor. For these post-harvest trials, statistical analyses were performed separately for the observations after three and five months of storage. The aforementioned models were constructed using the 'lmer' function of the R package 'lme4' (Bates *et al.* 2015). For each model, the random factor 'year' was removed when it was found to be insignificant. The models were analyzed with the 'Anova' function of the R 'car' package version 3.0-7, which uses the chi-squared significance test for the linear mixed models (Fox and Weisberg 2019) or the F test for the linear models without random effects. Variables were 'log (x + 1)' transformed when necessary to ensure normality and homogeneity of variance. A Tukey test (multiple comparisons of marginal means using the 'emmeans' method) was performed on the factors or interactions with a significant effect using the R 'emmeans' package (Lenth 2020). The significance threshold for all statistical tests was fixed at 5 %.

Results and discussion

The results of the significance tests showing the effect of the factors 'treatment', 'variety' and 'observation period' as well as the effect of the interactions between the different factors are summarized in Table 2.

Anti-sprouting treatment in the field

Maleic hydrazide (MH)

The efficacy of the treatments varied depending on the variety and observation period (Tab. 2); consequently, we studied the effects of the treatments for each observation period and variety (Fig. 2).

The results show that, compared to the control, MH provides good sprout control up to seven months of storage with temperature reconditioning from 8 °C to 15 °C. After three months of storage at 8 °C, the efficacy of MH is similar to that of CIPC for the majority of varieties. After five months of storage at 8 °C, CIPC is more effective than MH for the majority of varieties. After seven months of storage with temperature reconditioning, CIPC is more effective than MH for all the varieties tested.

After three months of storage, the sprout weight was significantly lower for the CIPC and MH treatments than for the untreated control for the varieties 'Bintje', 'Fontane', 'Lady Claire', 'Markies', 'Panda', 'Pirol' and 'Verdi' ($p<0.05$). For the 'Innovator' variety, the two molecules enabled significant sprout suppression compared to the control ($p<0.001$), although CIPC achieved better control than MH ($p<0.001$). For the 'Agria' variety, a significant but weak sprout-suppressant effect was observed for the CIPC-treated tubers ($p=0.046$), and a marginally significant effect was observed for the tubers from plants treated with MH ($p=0.050$) compared to the control (Fig. 2).

Table 2 | *P-values* from the significance test (chi-squared test or F test according to the trials) showing the effects of the different factors and their interactions; statistics over two years of trials; *statistically different; NA = not analyzed.

Factors	Period	P-values for the trials with different treatments:				
		Effect on sprouting (weight of sprouts from 25 Tubers in g)				Effect on the reducing sugars
		MH, CIPC, control	1,4-DMN, 3-decen-2-one, CIPC, control	Ethylene, ethylene + 1-MCP, CIPC, control	L-carvone, D-limonene, control	Ethylene, ethylene + 1-MCP, CIPC, control
Treatment	3 mos.	$p<0.001$*	$p<0.001$*	$p<0.001$*	$p<0.001$*	$p<0.001$*
	5 mos.		$p<0.001$*	$p<0.001$*	$p<0.001$*	NA
Variety	3 mos.	$p<0.001$*	$p<0.001$*	$p<0.001$*	>0.05	$p<0.001$*
	5 mos.		$p<0.01$*	>0.05	>0.05	NA
Treatment × variety	3 mos.	$p<0.001$*	>0.05	$p<0.05$*	>0.05	$p<0.01$*
	5 mos.		>0.05	>0.05	>0.05	NA
Observation period	–	$p<0.001$*	NA	NA	NA	NA
Observation period × treatment	–	$p<0.001$*	NA	NA	NA	NA
Observation period × variety	–	$p>0.05$	NA	NA	NA	NA

The *p-values* presented in the sections below correspond to the p-values from the Tukey tests.

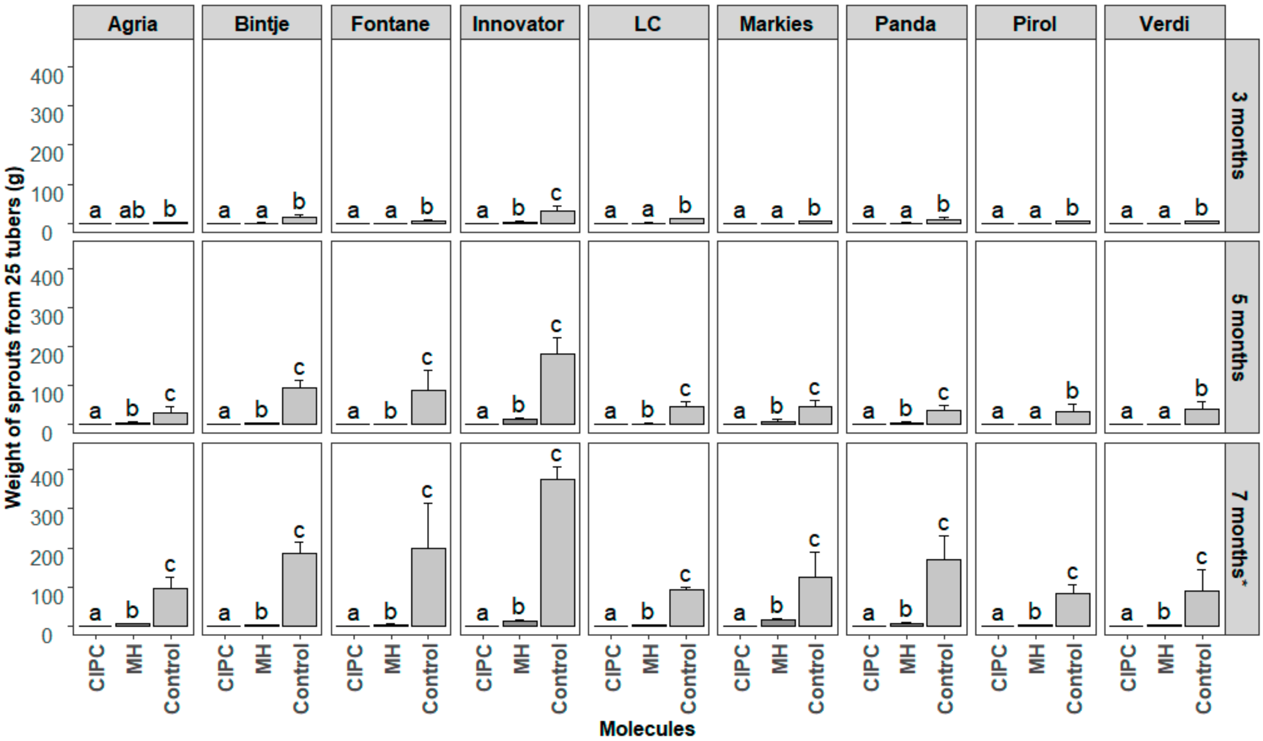

Figure 2 | **Sprout weight of 25 tubers treated with maleic hydrazide (MH) and CIPC and for the tubers of the untreated control after three and five months of storage at 8 °C and after seven months of storage (*seven months of storage with temperature reconditioning starting at 8 °C and ending at 15 °C at seven months) for the nine varieties tested during the two years of trials under controlled experimental conditions (200 kg of potatoes) (mean ± standard error). Means not sharing the same letter are significantly different according to the Tukey test.**

After five months of storage, the sprout weight of the CIPC and MH treatments was significantly lower for the 'Pirol' and 'Verdi' varieties compared to the sprout weight of the untreated control ($p<0.001$). For the seven other varieties, CIPC and MH achieved better sprout suppression than in the untreated control ($p<0.001$), but the sprout weight of the CIPC-treated tubers was systematically lower than that of the tubers from plants treated with MH ($p<0.05$) (Fig. 2).

Finally, after seven months of storage and reconditioning at 15 °C, we observed that the molecules CIPC and MH had a better sprout-suppression effect for the nine varieties than in the control group ($p<0.001$), and that for all varieties, MH was significantly less effective than CIPC ($p<0.05$).

Anti-sprouting treatments during storage

1,4-DMN and 3-decen-2-one

The molecules 1,4-DMN and 3-decen-2-one permit effective sprout control for up to at least five months of storage compared to the untreated control ($p<0.001$). After three months of storage the efficacy of the two molecules is equivalent, and after five months 3-decen-2-one is more effective than 1,4-DMN ($p<0.01$). Nevertheless, the efficacy of both active molecules was lower than that of CIPC for the two observation periods ($p<0.001$; Fig. 3).

The molecule 3-decen-2-one has a curative action, completely necrotizing and desiccating sprouts in just 24 hours (Fig. 4). This explains the lower sprout weight of the potatoes treated with 3-decen-2-one compared with that of the tubers treated with 1,4-DMN after five months of storage. Sprout necrosis and desiccation 24 to 36 hours after application of 3-decen-2-one is reported in the literature (Immaraju 2020; Knowles and Knowles 2015a, b). The advantage of 3-decen-2-one lies in its curative action, which allows it to rescue potato stocks that have already sprouted. This product actually destroys the internal structure of the sprout cells; tissues are completely necrotized and desiccated down to the base of the sprouts. 3-decen-2-one works very well when applied by hot-fogging on small sprouts (<3 mm). It is even more effective when the treatment is done at the first signs of dormancy break (sprouts at the 'white dot' stage), since the product vapors can then penetrate inside the sprouts and kill the developing meristematic tissues (Immaraju 2018, 2020). In our trials, the efficacy

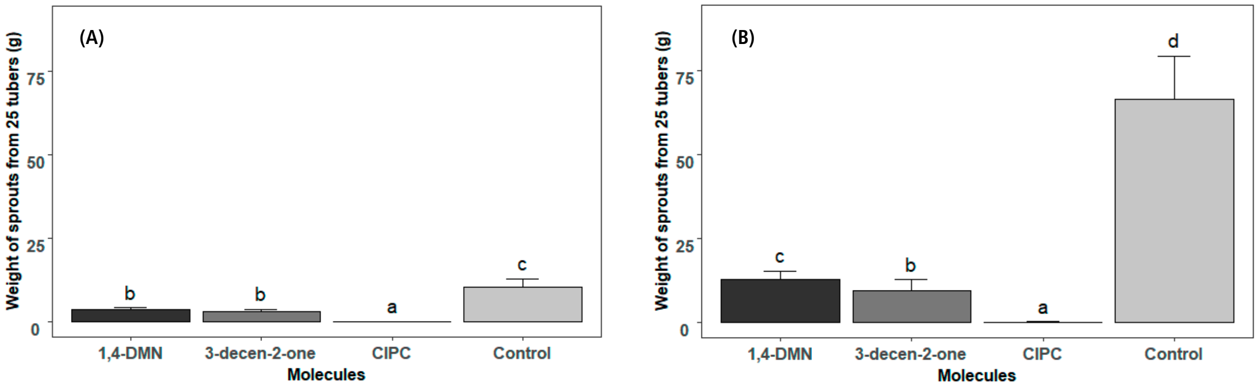

Figure 3 | Efficacy of the molecules 1,4-DMN and 3-decen-2-one after three months (A) and five months (B) of storage over two years of trials under controlled experimental conditions (200 kg of potatoes) and for nine varieties (mean ± standard error). Means not sharing the same letter are significantly different according to the Tukey test.

of this product is probably understated for certain varieties, since several varieties had variable dormancies in the same experimental chamber. Certain varieties that had sprouted more than others should thus have been treated sooner in order to achieve a better curative action. The number of 3-decen-2-one treatments for a complete storage season (seven to eight months) varies depending on the variety and temperature. On average, one to two treatments are necessary for the storage of long-dormancy varieties at low temperatures, and three to four treatments are required for short-dormancy varieties stored at higher temperatures (Immaraju 2018).

Ethylene alone and in combination with 1-MCP

Ethylene alone or in combination with the molecule 1-MCP reduces sprouting for at least up to five months of storage compared to the untreated control ($p < 0.05$; data not presented).

After three and five months of storage, the weight of the sprouts from the tubers treated with CIPC, ethylene, and the combination of ethylene + 1-MCP was significantly lower than the weight of the sprouts from the control ($p < 0.001$; $p < 0.05$ and $p < 0.05$).

Note that the efficacy of ethylene (alone or in combination with 1-MCP) may have been underestimated in our trials, owing to the fact that our experimental chambers did not allow the tubers to be exposed to a constant concentration of ethylene, which is normally recommended for this molecule. This is why we decided not to present the efficacy results obtained with ethylene in this article. However, we did note that the efficacy of ethylene depends on the variety of potato. We observed a less rapid progression of sprouting for the 'Markies' variety than for the three other varieties tested. This difference in efficacy between varieties was also observed in other trials. Flesch and Martin (2019) also observed excellent sprouting control for the 'Markies' variety stored under ethylene, while the sprouting control was less effective for the varieties 'Agria', Fontane' and 'Challenger' and was even less effective for the 'Innovator' variety.

We analyzed the amounts of reducing sugars in the tubers treated with the different molecules and in the untreated tubers of the control after three and five months of storage at 8 °C. Given that the tested tubers had been exposed to a variable concentration less than or equal to the recommended dose (10 ppm), it is possible that the effect of ethylene on the increase in the reducing-sugar content of the treated tubers may have been underestimated in this study. The results are presented in Table 3. Statistical analyses were only performed for the data at three months of storage, for which we had the results for all molecules and all varieties for the two consecutive years of trials (Tab. 4).

As previously shown in the literature (Harper and Stroud 2018), our study showed that the impact of ethylene on sugar levels depends on the variety (Tab. 2). We therefore studied the effect of ethylene on sugars after three months of storage for each of the varieties (Tab. 4). Our results showed that the varieties 'Lady Claire' and 'Verdi' are not susceptible to sweetening under ethylene, while the varieties 'Agria' and 'Markies' are susceptible. Our study showed that 1-MCP helps to limit ethylene-induced potato sweetening. Our results are in line with the study of Prange *et al.* (2005), which showed that 1-MCP can be used to limit ethylene-induced fry-color darkening without inhibiting ethylene's sprout-suppressant effect.

Indeed, after three months of storage, reducing-sugar levels in the varieties 'Lady Claire' and 'Verdi' were low and did not vary much according to the molecules

Figure 4 | Tubers of the 'Verdi' variety treated with the molecule 3-decen-2-one, after five months of storage under experimental conditions. Note the complete necrosis and desiccation of the sprouts. (Photo: Carole Parodi, Agroscope)

tested, while for the varieties 'Agria' and Markies' reducing-sugar levels varied depending on the treatment (Tab. 4). For the 'Agria' variety, reducing sugars were significantly higher in tubers stored in an ethylene-rich atmosphere (mean: 1.97 g kg^{-1}) compared to the reducing sugars measured in the tubers treated with ethylene + 1-MCP (mean: 0.72 g kg^{-1}) and those measured in the control (mean: 0.29 g kg^{-1}). Nevertheless, they were not significantly different from the sugars in the CIPC-treated tubers (mean: 1.18 g kg^{-1}) (Tabs. 3 and 4), because the reducing-sugar concentration was particularly high in the CIPC-treated tubers during the second year of trials (Tab. 3). The reducing sugars for the 'Markies' variety were not significantly different in the tubers treated with CIPC, ethylene + 1-MCP and in the control (means: 0.31, 0.82 and 0.39 g kg^{-1}, respectively) but were significantly higher in the tubers treated with ethylene alone (mean: 1.77 g kg^{-1}) (Tab. 4).

After five months of storage, trends showed that the reducing-sugar levels were still low for the varieties 'Verdi' and 'Lady Claire', regardless of the treatment undergone, while for the varieties 'Agria' and 'Markies', reducing-sugar levels were relatively high in the ethylene-treated tubers (mean: 1.85 g kg^{-1} and 2.27 g kg^{-1}) compared with those of the control (mean: 0.89 g kg^{-1} and 1.15 g kg^{-1}) or with those treated with CIPC (mean: 0.94 g kg^{-1} and 0.52 g kg^{-1}). The decrease in reducing-sugar levels in the tubers treated with ethylene + 1-MCP seemed to be greater after five months of storage than after three. Indeed, after five months of storage, treatment with 1-MCP seems to prevent an increase in reducing-sugar content in tubers of the varieties 'Agria' and 'Markies' (mean: 1.13 g kg^{-1} and 0.54 g kg^{-1}) (Tab. 3). The molecule 1-MCP is not yet authorized in the European Union for use on potatoes. Consequently, although it cannot be used at present to decrease sugar levels in tubers stored under ethylene, authorization of this molecule in the EU for use on potatoes is expected for 2022. A high level of reducing sugars increases the risk of darkening and of production of toxic compounds during frying (Wiberley-Bradford and Bethke 2017). For this reason, our partner Zweifel has fixed a very strict authorized pre-processing limit for reducing sugars (0.4 g kg^{-1}) to avoid any risk of darkening and of the presence of toxic compounds (mainly acrylamide) in the final product. Our results showed that this threshold can be exceeded for tubers stored in an ethylene-enriched atmosphere, including for the least susceptible varieties, such as Lady Claire, and even in the case of storage under ethylene in combination with the active substance 1-MCP (Tab. 3).

This reducing-sugar threshold may vary depending on the final product (potato chips or French fries), the country, and the company. At Frigemo, a French fry manufacturer in Switzerland, the threshold varies depending on the variety. Before processing the potatoes into French fries, a fry test is performed with a visual rating of French-fry color (Swisspatat 2018), after which a correspondence chart allows for the evaluation of the corresponding level of reducing sugars in the French fries (Grob 2003). For example, the 'Markies' variety can be processed into French fries if it does not exceed

the average threshold of 0.76 g kg^{-1} of reducing sugars, while the 'Agria' variety can be processed into French fries if it contains a level less than or equal to 0.95 g kg^{-1} (Schertenleib 2020).
In our study, these thresholds were systematically exceeded for potatoes stored under ethylene and were sometimes also exceeded for potatoes stored under ethylene + 1-MCP (Tab. 3). Consequently, the systematic performance of these fry and/or reducing-sugar tests before processing the potatoes into potato chips or French fries is crucial, to avoid the risk of producing toxic compounds during frying.

Essential Oils

The study showed that the molecules L-carvone and D-limonene also provide good control of sprouting after three and five months of storage compared to the untreated control ($p<0.01$ and $p<0.05$) and with a similar efficacy. Furthermore, no significant difference was observed between the efficacy of the molecules L-carvone and D-limonene ($p>0.05$; Fig. 5).
In our trials, the essential oils caused localized necroses on the tips of the sprouts.

Advantages and drawbacks of the different molecules

The various aforementioned molecules are likely to replace CIPC with more or less efficacy. The drawback of ethylene is its good-to-unsatisfactory efficacy as a sprout inhibitor, depending on the variety (Flesch and Martin 2019) and its negative effect on reducing-sugar content for certain varieties. The drawback of hot-applied essential oils is the high frequency of treatments (every three to four weeks), which require time and additional labor to carry out compared to the other products. The essential oils can also be automatically diffused by evaporation using a device such as the Xedavap®. This type of device should limit labor costs, but we have not tested its efficacy in our trials. Nevertheless, ethylene and essential oils offer certain advantages. Two of these molecules are already approved in Switzerland: the mint essential oil (L-carvone, approved under the name Biox-M®) and ethylene (approved for application on potatoes with the Restrain® generator). These products are compatible with organic farming, and are therefore not subject to an MRL. Ethylene has the advantage of an equivalent price of use to CIPC (Martin 2012; Visse-Mansiaux *et al.* 2017). The other alternatives to CIPC are generally more expensive; for example, the cost of using the mint essential oil is at least two times greater than that of CIPC (Curty 2019; Martin 2012; Visse-Mansiaux *et al.* 2017).
The molecules 1,4-DMN and MH are highly effective and easy to use. However, they are not authorized for use in organic agriculture, and they are subject to an MRL in the final products (MRL = 15 mg kg^{-1} for 1,4-DMN and

Table 3 | **Reducing-sugar levels (g kg-1 of fresh weight) after three and five months of storage in the tubers treated with the molecules CIPC, ethylene, and ethylene + 1-MCP, as well as in the untreated control, for the four varieties tested during two years of trials.**

Molecules	Period	Trial year	Reducing sugars (g kg^{-1}) for the different varieties			
			Agria	Lady Claire	Markies	Verdi
CIPC	3 mos.	2015-2016	0.39	0.06	0.1	0.06
		2016-2017	1.97	0.07	0.51	0.14
	5 mos.	2015-2016	0.74	0.13	0.46	0.13
		2016-2017	1.14	0.29	0.58	0.18
Ethylene	3 mos.	2015-2016	1.77	0.43	1.36	0.18
		2016-2017	2.17	0.41	2.17	0.36
	5 mos.	2015-2016	1.04	NA	2.1	NA
		2016-2017	2.65	0.73	2.43	0.35
Ethylene + 1-MCP	3 mos.	2015-2016	0.66	0.22	0.31	0.09
		2016-2017	0.78	0.29	1.32	0.26
	5 mos.	2015-2016	0.71	0.53	0.69	0.17
		2016-2017	1.54	0.24	0.38	0.51
Control	3 mos.	2015-2016	0.12	0.18	0.08	0.08
		2016-2017	0.45	0.14	0.7	0.12
	5 mos.	2015-2016	0.68	0.08	0.39	0.15
		2016-2017	1.09	0.51	1.91	0.33

Table 4 | *P-values* from the Tukey test comparing the effects of the treatments on the level of reducing sugars (glucose + fructose) after three months of storage for the four varieties tested (statistics for two years of trials: * = statistically different, (*) = cutoff).

Compared treatments	Agria	Lady Claire	Markies	Verdi
CIPC – Ethylene	>0.05	>0.05	<0.01*	>0.05
CIPC – Ethylene + 1-MCP	>0.05	>0.05	>0.05	>0.05
CIPC – Control	0.055(*)	>0.05	>0.05	>0.05
Ethylene – Ethylene + 1-MCP	<0.01*	>0.05	0.034(*)	>0.05
Ethylene – Control	<0.001*	>0.05	<0.01*	>0.05
Ethylene + 1-MCP – Control	>0.05	>0.05	>0.05	>0.05

MRL = 60 mg kg^{-1} for MH in the EU (European Commission 2019) and Switzerland (FSVO 2020). The molecule 3-decen-2-one is also highly effective and easy to use; our results showed that four treatments are sufficient to ensure control of sprouting throughout a storage season. Furthermore, this product has a curative effect, enabling the rescue of potato stocks that have already sprouted. Nevertheless, this molecule has not yet been approved in the EU and Switzerland. Once it has been approved, placing varieties with comparable dormancies in the same cold room will be the preferable approach to optimize product use. Given that the product is applied when the tubers start to sprout (Immaraju 2020), there is no benefit in treating varieties with a long dormancy and thus not yet showing any signs of sprouting at the same time as the tubers of varieties with shorter dormancy that are already showing sprouts.

CIPC can be applied in a single liquid-spray application at the beginning of storage, while the candidate replacement products are either applied several times by fogging, or continuously (by vaporization or gassing) during the storage season (Tab. 1). Thus, suitable storage buildings with powerful ventilation systems for proper distribution of the products are preferable for optimizing the efficacy of these products. The storage facilities must also be sufficiently airtight to prevent product loss, which would lead to reduced efficacy of the product and a direct financial loss. Given that these molecules are less effective than CIPC, wherever possible it would be preferable to favor varieties with medium-to-long dormancies.

CIPC was withdrawn from the market owing to risks of residues in the peel of the tubers (Ezekiel and Singh 2008) sometimes exceeding the authorized MRL of 10 mg kg^{-1} in the EU (European Commission 2019). Nevertheless, this product had the advantage of being partially or totally eliminated during the peeling of the potatoes prior to their industrial processing. Conversely, maleic hydrazide is a systemic product found in the flesh of the tuber, and hence is only partially eliminated during processing; however, MH residues are in general well below the MRL of 60 mg kg^{-1} authorized in the EU.

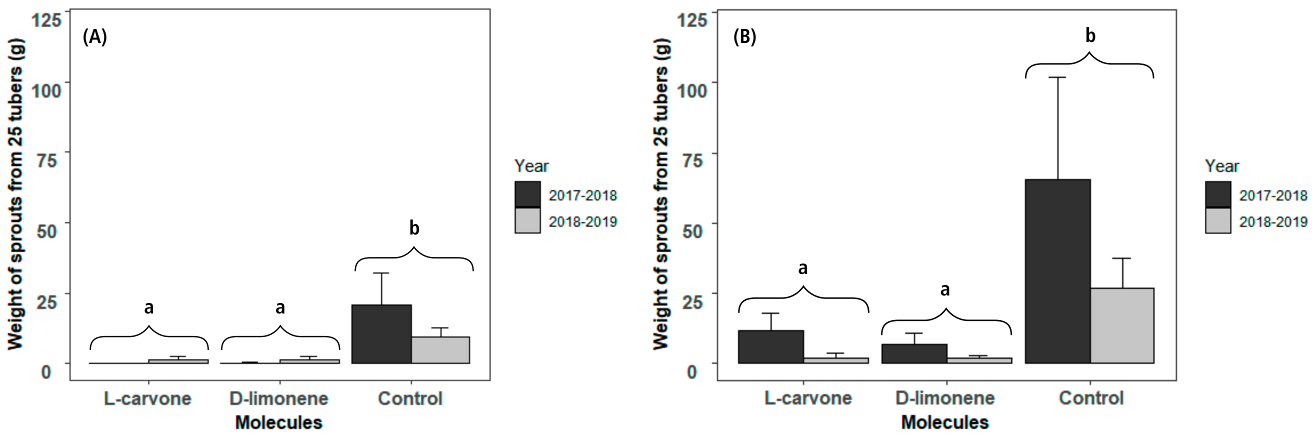

Figure 5 | Efficacy of the essential oils at three months (A) and five months (B) during two years of trials, one year under semi-industrial conditions (five metric tons = year 2017–2018) and one year under industrial conditions (> 300 metric tons = year 2018–2019) for three tested varieties: Agria, Verdi and Innovator (mean ± standard error). In 2017–2018, the tubers from the untreated control were from a different batch. The means not sharing the same letter are significantly different according to the Tukey test.

Compared to CIPC and MH, the molecules 1,4-DMN and 3-decen-2-one have the advantage of leaving very little residue on the tubers, thereby avoiding health and environmental risks. The CIPC, MH, 1,4-DMN, and 3-decen-2-one residue analyses conducted in our trials on treated tubers confirm the information presented above (data not presented).

Conclusions

It is important to combine the use of different alternative molecules to CIPC with new storage strategies in order to prevent loss of goods during potato storage and maintain high-quality stocks for several months without sprouting. Agroscope researchers are developing different innovative solutions for controlling sprouting.

- A sprouting model was developed to predict the dormancy date (and hence the sprouting date) of a given variety during a given season, based on weather parameters during the potato growth period (Visse-Mansiaux et al. 2018). This model will be used as a decision-support system for better potato-storage management based on the expected sprouting date. It will also help reduce or avoid the application of sprout-suppressant products depending on the length of expected dormancy, and thus reduce both treatment costs and the risk of product residues.

- In partnership with Swisspatat, Agroscope is also working on identifying industrial varieties that would not be susceptible to sweetening during storage at low temperatures. This would enable these varieties to be stored at 4 °C or 6 °C to delay tuber sprouting. With such varieties, it would be possible to extend storage and/or reduce or even avoid the application of sprout-suppressant products. This work has already allowed the identification of three varieties with low susceptibility to sweetening for storage at 4 °C: Lady Claire, Verdi, and Kiebitz (Visse-Mansiaux *et al.* 2019).

- Agroscope is also testing the effect of low storage temperature (4 °C) for varieties susceptible to sweetening, followed by reconditioning. We observed that reconditioning allowed a significant reduction of the reducing-sugar levels of certain varieties that are susceptible to sweetening (data not presented). However, sugar levels can vary (even in varieties with a low susceptibility to sweetening at low temperatures) depending on the year and the growing location (data not presented). We therefore recommend the systematic performance of reducing-sugar analyses and/or a fry test before processing potatoes stored at 4 °C with or without reconditioning, in order to limit the risk of darkening and acrylamide production during frying.

In response to the CIPC ban, the various aforementioned strategies must be implemented and combined in order to maintain high-quality storage and guarantee the sustainability of potato storage in Switzerland. ■

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