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#### 1 Abstract

For the purpose of AMIGA WP8, a novel, more durable, Integrated Pest Management (IPM) 2 strategy (termed IPM2.0) for the control of potato late blight disease was developed and 3 evaluated under field conditions in the Netherlands (2013 and 2014) and in Ireland (2013, 4 2014, 2015). IPM2.0 builds on the principles of IPM and deployment of resistant potato 5 cultivars as the primary line of defence against infection. It also includes pathogen 6 population monitoring for virulence to the resistance genes (R genes) deployed and a "only 7 when necessary, low input fungicide spray strategy" to mitigate the effects of selection 8 pressure. For the purposes of AMIGA, we used a cisgenically modified, potato cultivar 9 Desiree based, resistant potato clone named A15-031. Comparators included the original but 10 susceptible potato cultivar Desiree and the conventional but highly resistant potato cultivar 11 Sarpo mira. This report describes AMIGA deliverables 8.3: "Development and assessment of 12 IPM strategies for the cultivation of GM potato" and 8.6: "Assessment of the environmental 13 impact of resistant potato in an IPM strategy". 14

The IPM2.0 control strategy builds on host resistance as the primary defence against potato 15 late blight. Pathogen population monitoring is deployed to gain insight into pathogen 16 evolution towards virulence on the R genes deployed. Fungicides are not used as long as 17 virulence is not found. When virulence to the R gene(s) deployed is found in the local 18 pathogen population, a low input fungicide spray strategy is used to mitigate the effects of 19 selection pressure and protect the R gene(s) so that they can be used for much longer 20 periods of time. Overall, the IPM2.0 control strategy developed here reduced the average 21 fungicide input, in a total of five field trials in two different countries, on resistant potato 22 cultivars or clones by 80 - 90%. The environmental effects, described in AMIGA DL 8.6, as 23 measured using the "environmental yardstick", were reduced proportionally. 24

#### 1 Introduction

Potato late blight, one of the world's most devastating plant diseases in potato and tomato, 2 is caused by the oomycete Phytophthora infestans. In the past, potato late blight was 3 responsible for e.g. the Great Irish - and Continental Famine leading to mass starvation, 4 disease, and emigration for Ireland and contributing to the revolutions of 1848 on the 5 European Continent as described by Zadoks (2008). Currently potato late blight remains the 6 most important disease in potato cultivation and is traditionally controlled by highly 7 frequent (calendar based) fungicide applications (Cooke et al., 2011) supported by 8 preventive cultural measures such as crop rotation, the use of healthy seeds and the timely 9 destruction of primary sources of inoculum. In spite of these measures potato late blight 10 remains responsible for an estimated annual economic loss of M€ 1000 on the 6 Mha of 11 potato grown in the EU (Haverkort et al., 2008). 12

Host resistance and subsequent cultivation of potato late blight resistant potato cultivars is 13 the most (cost) effective and environmentally friendly way to control potato late blight 14 (Schepers et al., 2009). Currently however, the cultivation of resistant cultivars is restricted 15 due to the market's overwhelming demand for a limited number of commercially successful 16 but highly late blight susceptible cultivars. In addition, potato breeding is complex and time 17 18 consuming (Rietman et al., 2012) due to the tetraploid nature of the crop. The issue is further complicated by the highly adaptive potential of *P. infestans* to overcome resistance 19 gene (R gene) mediated host resistance (e.g. Black et al., 1953, Fry 2008; Haas et al. 2009; 20 McDonald and Linde 2002), especially if resistance is based on a single R gene. 21

In 2009, the origin of this adaptive capability was shown to reside in the P. infestans genome 22 in combination with its high reproductive capacity (Haas & Kamoun et al., 2009). As a result, 23 adaptation is "the Phytophthora infestans way of life", which results in R genes being 24 overcome (e.g. Black et al., 1953, Fry 2008; Haas et al. 2009; McDonald and Linde 2002), 25 resistance to active ingredients of fungicides (e.g. Dowley and O'Sullivan 1981) and increased 26 27 aggressiveness (e.g. Flier and Turkensteen, 1999). The net result from this are the all too regular dramatic and sudden population changes as described by e.g. Drenth et al. (1993), 28 Cooke et al. (2012) and Fry et al. (2013). 29

1 In response, from 2006 – 2015 the Wageningen University and Research Centre DuRPh 2 project (<u>www.durph.wur.nl</u>) aimed to develop a proof-of-concept that existing potato varieties could be made durably resistant to late blight when provided with stacked R genes 3 through cisgenic modification (Jacobsen and Schouten, 2007) in combination with adequate 4 resistance management (Haverkort et al., 2008; 2015). One output from this initiative and 5 the preceding conventional "Umbrellaplan Phytophthora" was the development of a novel, 6 more durable and low fungicide input control strategy for potato late blight. This strategy 7 was developed to overcome problems of insufficient disease control on resistant cultivars 8 due to the high adaptive ability of *P. infestans*. It is based on the principles of Integrated Pest 9 Management (IPM, e.g. described in EU directive 2009/128/EC) and incorporates 10 (cisgenically modified) resistant potato clones. The following components are included in the 11 complete control strategy (Haverkort et al 2015): 12 a. Introduction of resistance, preferably based on R gene stacks. 13 b. Monitoring the local *P. infestans* population for (emerging) virulence to the R genes 14 contained in the R gene stack. 15 c. Adoption of a preventative, zero tolerance "we do not spray unless" strategy. In this 16 scenario the 'do not spray' strategy is only reverted when: 17 a. Virulence to all R genes contained in a potato clone or cultivar are present in 18 the local *P. infestans* population. 19 b. A validated decision support system (DSS) predicts an infection event in the 20 immediate future. 21 22 c. The remaining fungicide protection is insufficient. d. Application of reduced dose rates of protectant fungicides on resistant cultivars 23 e. Replacement of the overcome R gene cassette with a new, functional R gene cassette 24 25 to restart the cycle. Within the EU AMIGA project this strategy was put to the test with respect to components 26 a – d in the Netherlands (2013 and 2014) and Ireland (2013, 2014 and 2015). In this regard, 27 we studied the effect of this strategy on disease progress, fungicide input (and subsequent 28 environmental impact) and practical applicability for a conventional susceptible potato 29 cultivar (Desiree), a GM resistant version of this same cultivar (A15-31) and a conventionally 30

31 bred resistant cultivar (Sarpo Mira), which is typically favoured by organic growers in the UK.

- 1 The time frame necessary to test component 5: "replacement of R gene cassettes" was
- 2 outside the AMIGA project duration.
- 3 The results will be discussed with respect to the added value of host resistance and cisgenic
- 4 modification, within an IPM context, to improved sustainability of potato late blight control
- 5 in agricultural ecosystems for AMIGA Deliverables 8.3 and 8.6. The selective effect of
- 6 growing (GM) resistant potato cultivars on *P. infestans* populations will be discussed in
- 7 AMIGA Deliverable 8.4.

# 1 Materials and Methods

# 2 Potato late blight control strategies

In a series of field trials in Ireland and the Netherlands, potato late blight was controlled 3 using one of three treatment options: unsprayed control, weekly spray schedule (common 4 practice in the Netherlands and Ireland) and a next level IPM control strategy. All other weed 5 6 and disease problems were controlled when strictly necessary using conventional control measures with a minimum chemical input. Insecticides were not used. The unsprayed 7 control treatment was not sprayed with fungicides. The weekly spray schedule was sprayed 8 with fungicides every week. The next level control strategy (IPM2.0) builds on three basic 9 components and was only sprayed when strictly necessary. 10

Host resistance: Potato genotypes may contain a range of single or multiple narrow
 and/or broad spectrum R genes. For this IPM strategy to function, it is essential to
 know the R gene content of the potato genotypes or cultivars grown. Pathogen
 population monitoring serves to track pathogen evolution towards virulence to the R
 genes "grown".

P. infestans population monitoring : Under this IPM control strategy potato
 genotypes only receive additional fungicide protection when virulence to all R genes
 contained in the potato genotype is present in the local *P. infestans* population. For
 susceptible potato genotypes, this is the case from the beginning of the growing
 season onwards. For more resistant, R gene containing, potato genotypes virulence
 may or may not occur during the growing season. The field trial itself plus the
 monitoring plots were monitored for this purpose.

3. Fungicides to supplement the protection provided by R gene(s) when necessary: 23 Fungicides were applied to protect R gene(s) and supplement the protection 24 provided by the R gene(s) to the required level when necessary. A zero tolerance, 25 preventive spray strategy was implemented in a simple decision support system 26 (DSS) described by Lucca et al., (in Prep/submitted). Past and future infection events 27 were identified based on measured - and forecasted hourly weather data provided in 28 Ireland by Met Eireann and in the Netherlands by Dacom b.v. and the Meteorology 29 and Air Quality Group of Wageningen University. Ideally and preferably, significantly 30 reduced dose rates of protectant fungicides were applied just prior to predicted 31

1	infection events when virulent <i>P. infestans</i> was present and the remaining fungicide
2	protection was insufficient. When necessary, curative fungicides were applied up to 2
3	days after infection and eradicant fungicide combinations were applied on untreated
4	older latent- and active infections. The choice of fungicide is thus guided by the
5	biology of the pathogen and the fungicide characteristics given in e.g. the Euroblight
6	fungicide table ( <u>www.Euroblight.Net</u> ). Fungicide degradation was calculated using
7	the algorithms described for SIMCAST (Fry et al., 1983; Grunwald et al., 2000;)
8	Overall, the strategy can be summarized as "we do not spray unless":
9	a. Virulence to all R genes of a potato clone or cultivar is present in the local <i>P. infestans</i>
10	population AND
11	b. A validated decision support system (DSS) predicts an infection event in the
12	immediate future AND
13	c. The remaining fungicide protection is insufficient.
14	
15	It is important to note that similar to e.g. Clayton and Shattock (1995) and Nærstad et al
16	(2007), the default dose rate for protectant fungicides was reduced with increasing levels of
17	host resistance. Susceptible cultivar Desiree received 100% of the recommended dose rate
18	whereas the highly resistant A15-031 and Sarpo mira, when sprayed at all, received 25%.
19	Finally, the distance weighted infection pressure (DWIP, Skelsey et al., 2009) was calculated
20	and used to delay fungicide applications on highly resistant potato genotypes A15-031 and
21	Sarpo mira when the 33% threshold was not reached.
22	
23	All in all, for the next level (IPM2.0) control strategy, susceptible Desiree was sprayed from
24	the first predicted infection event after emergence. Resistant potato genotypes A15-031 and
25	Sarpo mira were sprayed, with reduced dose rates, from the first infection period after
26	virulence was found in the local P. infestans population taking into account the distance
27	weighted infection pressure DWIP.
28	Field trials were carried out in the Netherlands (Valthermond [GPS coordinates 52.873828°,
29	6.942644], 2013 and 2014) and in Ireland (Oak Park, Carlow [GPS coordinates; 52.8560667
30	-6.9121167, 2013, 2014 and 2015). Since part of the plant material was genetically modified,

1 these trials were carried out under permit IM10-006 for the Netherlands and in Ireland the

2 trials were licensed by the Environmental Protection Agency as per Notification No.

3 B/IE/12/01.

Valthermond is located in the north east of the Netherlands, the major Dutch production
area for starch potatoes. Both IPM experiments were carried out at experimental farm "t
Kompas" on reclaimed peat soil (90.1% sand, 9.9% organic matter, pH = 5.1). The Oak Park
campus in Carlow is situated in the south east region of Ireland. The soils at Oak Park are a
mix of light textured gravelly soils derived from limestone gravels and heavy textured soils
derived from limestone till commonly known as boulder clay.

10

## 11 Plant Material

Two potato cultivars and one potato clone were used in the field trials: the conventionally
bred and highly susceptible cultivar Desiree, the highly resistant and conventionally bred
cultivar Sarpo mira and the highly resistant "Desiree based", cis-genetically modified clone
A15-031 (described in detail in Haesaert at al., 2015 and Haverkort et al 2016). Previously
(Jacobsen and Schouten, 2007), A15-031 was generated through cisgenic modification of the
Desiree cultivar with the transfer of the *Rpi-Vnt1.1* potato late blight resistance gene (Pel et
al., 2009) originally obtained from *Solanum venturii*.

Sarpo mira is reported to contain the *R3a*, *R3b*, *R4*, *Rpi-Smira1* and *Rpi-Smira2* potato late
blight resistance genes (Rietman et al., 2012).

Seed tubers for the IPM field trials in the Netherlands, were produced on a sandy loam soil in Lelystad, the Netherlands in 2012 and 2013, the year prior to the IPM experiment. In vitro plantlets of potato cultivar Desiree and clone A15-031 were planted to produce the necessary seed potatoes. Seed crops were subjected to a conventional cropping system with respect to fertilization, weed-, pest- and disease control. Sarpo mira seed potatoes were obtained commercially.

27 For Ireland, seed tubers (Desiree and A15-031) were generated under glasshouse conditions

using plantlets that originated from *in vitro* cultures as a measure to offset potato virus

29 transmission. Sarpo Mira seed potatoes were obtained from commercial sources.

1 Monitoring plots were located outside the area under permit in both Ireland and the

2 Netherlands. These plots therefore only contained conventional potato cultivars and clones:

3 Desiree and Sarpo mira plus RH06-975-8, a vnt365-1 derived, Rpi-Vnt1.3 containing

4 conventional potato breeding clone. *Rpi-Vnt1.1* and *Rpi-Vnt1.3* are reported to share the

5 same resistance spectrum (Pel et al., 2009).

6

7 Field trials

8 IPM field trials were laid out as randomized block experiments (Appendix I) including the 3

9 potato genotypes mentioned above, 3 potato late blight control strategies (unsprayed

10 control, weekly spray schedule and the "IPM2.0 strategy" and 7 replicates.

11 For Valthermond, each of the 63 plots measured 6m x 6m. They were separated/surrounded

12 by 6m of grass on all sides. Planting dates at Valthermond were: 10 June 2013 (100%

emergence on 29 June 2013) and 21 May 2014 (100% emergence on 5 June 2014). Crops

were desiccated on 25 September 2013 and 10 September 2014 resulting in an 88 and a 93

15 day growing season in 2013 and 2014 respectively.

16 In Ireland, the plots sizes were 3m x 3m, with each separated by 3m of grass. Planting was

17 completed by 18 May 2013, 4 June 2014 and 4 June 2015. Post desiccation, crops were

harvested on 23 October 2013, 22 October 2014 and 12 October 2015.

19

20 Monitoring plots

Monitoring plots served the purpose of monitoring the local *P. infestans* population for
virulence against the R genes deployed in the field trial. Both field trials in Valthermond were
surrounded by ten monitoring plots distributed over the entire, 102 ha, farm. For Oak Park,
11 plots were deployed in 2014 and 2015 across the campus to ensure that they were
positioned between the IPM sites and the prevailing south westerly winds, out to distances
of 1200m from the trial sites. Monitoring was not possible in 2013 due to the unavailability
of seed of the conventional RH06-975-8.

Each monitoring plot contained 6 (NL) or 3 (IE) plants of Desiree, Sarpo mira and the

29 conventional Vnt1.3 containing clone RH06-975-8 plants. RH06-975-8 was used as a

Page | 10

1 conventional substitute for the Cisgenic Rpi-Vnt1.1 containing clone A15-031. Monitoring 2 plots were not sprayed with crop protection products but at times (e.g. Ireland 2014) received additional irrigation. From emergence onwards, these plots were monitored for 3 infection on a weekly basis. When infection was found on one of the potato clones, virulence 4 for the R gene(s) contained in the clone was assumed to be present. Monitoring plots were 5 planted on 8 July 2013 and 26 June 2014 in Valthermond and in Oak Park on 27 June 2014 6 and 30 June 2015. In addition to the monitoring plots, the much larger, untreated plots in 7 the field trials themselves were also taken into account as 'early warning sites' for emerging 8 *P. infestans* virulence's. 9

10

## 11 Disease progress

For the field trials, the percentage destroyed foliage per plot (severity) was visually assessed at a weekly interval (with the exception of Ireland 2013). For the monitoring plots, the number of lesions per clone was counted or estimated (for very large numbers) at a weekly interval.

16

# 17 Yield analysis

In Ireland, drills in each plot were lifted by machine and tubers then hand-picked from the
 soil surface. Each plot was harvested three times to ensure all tubers were collected. On
 completion yield was calculated as kg/m<sup>2</sup>.

## 1 **Results**

In general, weather conditions were conducive for potato late blight for at least part of the 2 growing season across the years examined at both locations (Appendix II). Overall, more 3 infection periods were recorded in the Valthermond than in Carlow. For the Netherlands, 4 2013 was an average late blight year whereas 2014 was an extreme late blight year with 5 frequent infection periods from beginning to end. For Ireland, the 2013 late blight season 6 was impeded in July by uncharacteristically above average temperatures and no rainfall. 7 However, a severe blight epidemic prevailed through August and September 2013. For 2014 8 and 2015, blight epidemics started ~mid-July and continued through to early September. 9 Since at least seven untreated and susceptible Desiree plots were present in each trial, 10 disease pressure developed to extreme levels during the epidemics. 11

12



**Figure 1.** Top: Valthermond, the Netherlands, 2014. Trials included 3 potato genotypes, 3 control strategies and 7 replicates in a randomized block design with 63 plots. Plots were separated by 6m of bare soil or grass. Bottom: Carlow, Ireland, 2014: two sites, each containing 3 potato genotypes, 3 control strategies and 6 replicates in a randomized block design with 54 plots. Plots were separated by 3m of grass.

#### 1 Monitoring plots

First observations of potato late blight in the monitoring plots surrounding the trials and in 2 the trials themselves are given in Table 1. The results show that the first infections were 3 generally found in the field trial itself rather than in the monitoring plots. It is likely this is 4 caused by the size difference between the monitoring plots (for Valthermond: 10 x 6 plants 5 per potato genotype) and the field trial plots (for Valthermond: 21 x 144 plants per potato 6 genotype). The next level / IPM2.0 control strategy used this information to modify the spray 7 strategy on resistant potato genotypes: from non-spraying to spraying reduced dose rates 8 prior to predicted infection events when virulence was found. 9

Table 1. First observations of Potato Late Blight in the monitoring plots and the trials in 2013 and 2014 for Ireland and the
 Netherlands.

		20	13 (NL) / 20	014 (IE)	2014 (NL) / 2015 (IE)				
Location	Potato Genotype	Monitoring plots	Trial	First spray application IPM2.0 plots	Monitoring plots	Trial	First spray application IPM2.0 plots		
Valthermond	Desiree	20-aug	22-jul	2-jul	25-jul	23-jun	11-jun		
	Sarpo Mira	17-sep	27-aug	30-aug	29-aug	24-jun	24-jun		
	A15-031	-	23-sep	30-aug	5-sep	4-aug	5-aug		
Carlow	Desiree	18 - aug	11 - jul	30 - jun	22 - aug	10 - aug	2 -jul		
	Sarpo Mira	21 - aug	21 - aug	21 - aug	-	7 - sept	-		
	A15-031	-	-	-	-	-	-		

12

## 13 Fungicide input and environmental effects of potato late light control

Fungicides applications were carried out based on the relevant control strategy. For the 14 IPM2.0 strategy, protectant fungicides were applied just prior to predicted infection events. 15 Strongly reduced dose rates of protectant fungicides (25% of the recommended dose rate) 16 were applied on the highly resistant Sarpo mira and A15-031. Occasionally, curative 17 fungicide applications were carried out within two days after a non-treated infection event 18 occurred. Eradicant fungicide applications were necessary when active infections were found 19 20 in the trials. Eradicant fungicide applications are combinations of two fungicides at 100% dose rate, a strong curative plus a strong anti-sporulant, repeated until the infection is 21 cured. The fungicide treatments applied are summarized in Figure 2. 22

23 Environmental effects for the purpose of AMIGA deliverable DL8.6 were calculated using the

- 1 environmental yardstick for pesticides accessible through <u>www.milieumeetlat.nl</u> (Reus and
- 2 Leendertse, 2000). The results are described in AMIGA DL8.6.



**Figure 2.** Fungicide input (left column) and end of season severity (right column, ■: % destroyed

- 4 foliage) for all five AMIGA GM-IPM trials in three years and two locations. Fungicide input
- 5 represents the number of spray applications (**■** blue) and the number of "full dose rate
- 6 equivalents" applied (■ red).

## 1 Disease Management

Potato late blight epidemic progress in the trials in the Netherlands and Ireland is given in 2 Figure 3. Overall, potato late blight was well controlled by the weekly spray strategy and the 3 IPM2.0 strategy. The unsprayed control treatment always resulted in a heavily infected or 4 destroyed crop, earlier or later in the season depending on weather conditions. Unsprayed 5 but Sarpo mira plots was also infected in Ireland and the Netherlands although infection 6 levels were much lower than for Desiree (Figure 3). Unsprayed A15-031 received low level 7 infections in the Netherlands where virulence to Vnt1 was present in the local P. infestans 8 population. This immediately illustrates the necessity to apply a low input spray strategy, as 9 developed and demonstrated here, on resistant potato cultivars when virulence to the R 10 gene(s) contained is present in the local pathogen population. 11

12



13 **Figure 3.** Epidemic progress on all nine potato genotypes x management strategy combinations for both locations and both

14 years. O: Desiree. □: A15-031. △: Sarpo mira. \_\_\_\_: Unsprayed Control. \_ \_ \_ \_ : Weekly spay schedule (Common

15 practice). ----: Next level/IPM2.0 control strategy.

The necessary fungicide input and the environmental effects (Figure 2) for these two strategies were however markedly different, especially on the highly resistant potato genotypes where the fungicide input and the environmental effects were reduced by 80 – 90+ %. The few curative and eradicant treatments that were necessary (Appendix III) were triggered by a primary preventive spray advice that could not be carried out due to e.g. excessive rain.

7

#### 8 Yield

Mean yields (tubers, fresh weight) from the three years of study at Carlow Ireland are 9 presented in Figure 4. Average yields across treatments was ~ 15 kg/m<sup>2</sup> except for the 10 untreated Desiree plots where yield is just above 12 kg/m<sup>2</sup>. What is significant here is the 11 differential (P<0.05) between the untreated Desiree and the untreated A15-031 (and Sarpo 12 Mira) and then separately the fact that there was no significant difference (P>0.05) between 13 14 the yields returned from the IPM2.0 strategies versus the weekly spray regimes. It is important to highlight that the yields obtained here were excessive compared to yields 15 typically returned through standard agricultural practise. This effect was probably due to the 16 small size plots and the corresponding edge effects associated with this design. 17 Notwithstanding that point, the disparity between treatments indicates the potential of 18 IPM2.0 to return equivalent yields to a standard commercially-relevant full spray 19 programme. 20



Figure 4. Average tuber yield (kg/m<sup>2</sup> fresh weight) over three years in Carlow Ireland for the
 various potato genotypes and control strategies.

# 1 **Discussion**

2 The objectives of WP8 were to:

- 1. Identify Integrated Pest Management (IPM) components positively or negatively 3 influenced by adoption of GM-crops with resistance to potato late blight. 4 2. Integrate new disease control options into the selected GM-crop case studies. 5 3. Assess the environmental impacts of different management options for selected GM-6 crops in representative 'receiving environments' and test key Genotype-Environment 7 interactions important in IPM, 8 4. Facilitate the adoption of IPM-practices which optimize the environmental and 9 economic benefits of using GM-crops, under EU Directives requiring IPM strategies 10 for all member states by 2014. 11 5. Propose IPM-based risk mitigation measures by combining IPM tools that 12 a. Reduce selection pressure on individual components of the GM cropping 13 system (e.g., R genes), 14 b. Reduce pesticide use in terms of active ingredients and 15
- c. Optimise the role of eco-services, particularly biocontrol agents and
   pollinators.

Here we report on AMIGA deliverables 8.3 and 8.6: General purpose, AMIGA deliverables 8.3: "Development and assessment of IPM strategies for the cultivation of GM potato" and 8.6: "Assessment of the environmental impact of resistant potato in an IPM strategy" addressing topics 1, 2, 3, 5 and 6 of the list above.

With respect to AMIGA deliverable 8.3, host resistance to potato late blight was found to be an extremely valuable addition/component to an IPM potato late blight control strategy. It was incorporated into a novel, more durable, control strategy building primarily on host resistance, monitoring of the pathogen population for virulence development and a low input spray strategy to mitigate the effects of selection pressure on the pathogen population and protect the R genes (described from Page 7 onwards). The number of potato late blight R genes available is limited which make them an extremely valuable commodity.

The control efficacy of the novel, IPM2.0 control strategy was tested in a series of five large scale field experiments in Ireland and the Netherlands in 2013, 2014 and 2015 and found to 1 be equal to the control efficacy of the current common practice "a weekly spray schedule" (Figure 2). The fungicide input necessary was however markedly reduced. On the 2 susceptible potato cultivar Desiree, the fungicide input could be reduced due to optimized 3 dynamic spray timing but only marginally. On the resistant cultivar Sarpo mira and the 4 resistant Desiree based potato clone A15-031 the fungicide input and the environmental 5 effects could be reduced by 80 - 90%. Yields were similar between the potato 6 cultivars/cones involved apart from unsprayed Desiree where yield was reduced due to late 7 blight destroying the foliage. 8

On (more) potato late blight resistant potato genotypes the following additional options are
 available with respect to late blight control::

Not spray fungicides when virulence to the R gene(s) contained is absent from the local
 pathogen population.

Start much later in the growing season with preventive fungicide applications when
 virulence to the R gene(s) is locally generated during epidemic build up.

Apply strongly reduced dose rates (25% of the recommended dose rate) on resistant
 potato cultivars when preventive sprays are necessary.

Pathogen population monitoring and reliable advice on spray timing are key to successful
implementation of the IPM2.0 control strategy developed and tested here. In addition it is
likely that a similar control strategy can be developed for other fungal foliar pathogens when
host resistance is available.

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#### 1 **References**

- Black W., Mastenbroek C., Mills W.R., Peterson L.C. 1953. A proposal for an international
   nomenclature of races of Phytophthora infestans and of genes controlling immunity in
   Solanum demissum derivates. Euphytica 2: 173–240
- Cooke D.E.L., Cano L.M., Raffaele S., Bain R.A., Cooke L.R., et al. (2012) Genome Analyses of
   an Aggressive and Invasive Lineage of the Irish Potato Famine. Pathogen. PLoS Pathog
   8(10): e1002940. doi:10.1371/journal.ppat.1002940
- Cooke, L. R., Schepers, H. T. A. M., Hermansen, A., Bain, R. A., Bradshaw, N. J., Ritchie, F., D.
  S. Shaw, S., Evenhuis, A., Kessel, G.J.T., Wander, J. G. N., Andersson, B., Hansen, J. G.,
  Hannukkala, A., Nærstad, R. and Nielsen, B. J. 2011. Epidemiology and integrated
  control of potato late blight in Europe. Potato Research, 54: 183-222.
- Clayton, R.C. and Shattock, R.C. 1995. Reduced fungicide inputs to control Phytophthora
   infestans in potato cultivars with high levels of polygenic resistance. Potato Research
   38: 399 405.
- Dowley, L.J. and O'Sullivan, E. 1981. Metalaxyl-resistant strains of Phytophthora infestans.
   Potato Research 24: 417–421
- Flier, W.G. and Turkensteen, L.J. 1999. Foliar aggressiveness of Phytophthora infestans in
   three potato growing regions in the Netherlands. European Journal of Plant Pathology
   105: 381–388, 1999.
- Fry, W. E., M. T. McGrath, A. Seaman, T. A. Zitter, A. McLeod, G. Danies, I. M. Small, K.
   Myers, K. Everts, A. J. Gevens, B. K. Gugino, S. B. Johnson, H. Judelson, J. Ristaino, P.
   Roberts, G. Secor, K. Seebold, K. Snover-Clift, A. Wyenandt, N. J. Grünwald and C. D.
   Smart 2013. "The 2009 Late Blight Pandemic in the Eastern United States Causes and
   Results." Plant Disease 97(3): 296-306.
- Fry, W. E. 2008. Phytophthora infestans: The plant (and R gene) destroyer. Mol. Plant Pathol.
  9:385-402.
- Fry W.E., Apple A.E., Bruhn J.A., 1983. Evaluation of potato late blight forecasts modified to
   incorporate host resistance and fungicide weathering. Phytopathology 73, 1054 1059.

Grunwald N.J., Rubio-Covarrubias O.A., Fry W.E. 2000. Potato late-blight management in the Toluca Valley: forecasts and resistant cultivars.Plant Disease 84: 410 - 416.

Haas B.J., Kamoun S., Zody M.C., Jiang R.H., Handsaker R.E., Cano L.M., Grabherr M., Kodira 3 CD, Raffaele S, Torto-Alalibo T, Bozkurt TO, Ah-Fong AM, Alvarado L, Anderson VL, 4 Armstrong MR, Avrova A, Baxter L, Beynon J, Boevink PC, Bollmann SR, Bos JI, Bulone 5 V, Cai G, Cakir C, Carrington JC, Chawner M, Conti L, Costanzo S, Ewan R, Fahlgren N, 6 Fischbach MA, Fugelstad J, Gilroy EM, Gnerre S, Green PJ, Grenville-Briggs LJ, Griffith J, 7 Grünwald NJ, Horn K, Horner NR, Hu CH, Huitema E, Jeong DH, Jones AM, Jones JD, 8 Jones RW, Karlsson EK, Kunjeti SG, Lamour K, Liu Z, Ma L, Maclean D, Chibucos MC, 9 10 McDonald H, McWalters J, Meijer HJ, Morgan W, Morris PF, Munro CA, O'Neill K, Ospina-Giraldo M, Pinzón A, Pritchard L, Ramsahoye B, Ren Q, Restrepo S, Roy S, 11 Sadanandom A, Savidor A, Schornack S, Schwartz DC, Schumann UD, Schwessinger B, 12 Seyer L, Sharpe T, Silvar C, Song J, Studholme DJ, Sykes S, Thines M, van de 13 Vondervoort PJ, Phuntumart V, Wawra S, Weide R, Win J, Young C, Zhou S, Fry W, 14 Meyers BC, van West P, Ristaino J, Govers F, Birch PR, Whisson SC, Judelson HS, 15 Nusbaum C. 2009. Genome sequence and analysis of the Irish potato famine pathogen 16 17 Phytophthora infestans. Nature 461, No. 7262: 393-398.

Haesaert G., Vossen J.H., Custers R., De Loose M., Haverkort A.J., Heremans B., Hutten R.,
 Kessel, G., Landschoot S., Van Droogenbroeck B., Visser R.G.F., Gheysen G. 2015.
 Transformation of the potato variety Desiree with single or multiple resistance genes
 increases resistance to late blight under field conditions. Crop Protection, Volume 77:
 163–175.

Haverkort A.J., Boonekamp P.M., Hutten R., Jacobsen E., Lotz L.A.P., Kessel G.J.T., van der
 Vossen A.E.G. and Visser R.G.F. 2016. Durable Late Blight Resistance in Potato Through
 Dynamic Varieties Obtained by Cisgenesis: Scientific and Societal Advances in the
 DuRPh Project. Potato Research 59 (1): 35-66.

Haverkort A.J., Boonekamp P.M., Hutten R., Jacobsen E., Lotz L.A.P., Kessel G.J.T., Visser
 R.G.F., van der Vossen A.E.G. 2008. Societal costs of late blight in potato and prospects
 of durable resistance through cisgenic modification. Potato Research 51: 47-57.

1	Jacobsen, E., Schouten, H.J., 2007. Cisgenesis strongly improves introgression breeding and
2	induced translocation breeding of plants. Trends In Biotechnology 25: 219-223.
3	Lucca, M.F., Caldiz, D.O., Schepers, H.T.A.M., de Lasa, C. and Kessel, G.J.T. The Potential of
4	Decision Support Systems to control Potato Late Blight in Argentina. In Prep.
5	McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential,
6	and durable resistance. Annu. Rev. Phytopathol. 40:349-379.
7	Nærstad, R., Hermansen, A. and Bjor, T. 2007. Exploiting host resistance to reduce the use of
8	fungicides to control potato late blight. Plant Pathology 56: 156–166.
9	Pel, M.A., Foster, S.J., Park, T.H., Rietman, H., van Arkel, G., Jones, J.D., Van Eck, H.J.,
10	Jacobsen, E., Visser, R.G.F., Van Der Vossen, E.A.G. 2009. Mapping and cloning of late
11	blight resistance genes from Solanum venturii using an interspecific candidate gene
12	approach. Molecular Plant-Microbe Interactions 22(5): 601-615.
13	Rietman, H., Bijsterbosch, G., Cano, L.M., Lee, H.R., Vossen, J.H. Jacobsen, E., Visser, R.G.F.
14	Kamoun, S. and Vleeshouwers V.G.A.A. 2012. Qualitative and quantitative late blight
15	resistance in the potato cultivar Sarpo Mira is determined by the perception of five
16	distinct RXLR effectors. Mol. Plant Microbe Interact., 25: 910–909.
17	Reus, J. A., and Leendertse, P. C. 2000. The environmental yardstick for pesticides: a practical
18	indicator used in the Netherlands. Crop Protection, 19(8), 637-641. Environmental
19	Yardstick for Pesticides. Online: http://www.milieumeetlat.nl/en/home.html
20	Schepers H.T.A.M., Evenhuis, A. and Spits, H.G. 2009. Strategies to Control Late Blight in
21	Potatoes in Europe. Acta Horticulturae, 834, ISHS 2009: p. 79-82.
22	Skelsey, P., Rossing, W. A. H., Kessel, G. J. T., and van der Werf, W. 2009. Scenario approach
23	for assessing the utility of dispersal information in decision support for aerially spread
24	plant pathogens, applied to Phytophthora infestans. Phytopathology 99: 887-895.
25	Zadoks J.C. 2008. The potato murrain on the European continent and the revolutions of
26	1848. Potato Research 51: 5–45. doi: 10.1007/s11540-008-9091-4.

# 1 Appendix I. Statistical Trial setup 2013 for Valthermond and Oak Park respectively.

2 Trials included 3 potato genotypes, 3 control strategies and 7 replicates in a randomized block design.

SarpoMira	A15-31	Desiree	Desiree	A15-31
IPM2.0	WeeklySchedule	NoControl	IPM2.0	IPM2.0
Desiree	SarpoMira	A15-31	SarpoMira	SarpoMira
WeeklySchedule	WeeklySchedule	NoControl	NoControl	NoControl
Desiree	Desiree	A15-31	SarpoMira	Desiree
IPM2.0	WeeklySchedule	NoControl	IPM2.0	NoControl
SarpoMira	Desiree	A15-31	SarpoMira	A15-31
NoControl	WeeklySchedule	IPM2.0	WeeklySchedule	WeeklySchedule
A15-31	A15-31	SarpoMira	Desiree	A15-31
WeeklySchedule	IPM2.0	WeeklySchedule	NoControl	NoControl
Desiree	SarpoMira	A15-31	Desiree	SarpoMira
IPM2.0	IPM2.0	WeeklySchedule	NoControl	WeeklySchedule
Desiree	A15-31	SarpoMira	SarpoMira	Desiree
WeeklySchedule	IPM2.0	IPM2.0	NoControl	IPM2.0
A15-31	Desiree	A15-31	Desiree	A15-31
IPM2.0	NoControl	WeeklySchedule	WeeklySchedule	NoControl
SarpoMira	A15-31	Desiree	SarpoMira	SarpoMira
IPM2.0	NoControl	IPM2.0	NoControl	WeeklySchedule
Desiree	SarpoMira	SarpoMira	A15-31	A15-31
IPM2.0	IPM2.0	WeeklySchedule	IPM2.0	NoControl
Desiree	Desiree	A15-31	SarpoMira	Desiree
NoControl	WeeklySchedule	WeeklySchedule	NoControl	NoControl
SarpoMira	SarpoMira	A15-31	A15-31	A15-31
NoControl	IPM2.0	IPM2.0	WeeklySchedule	NoControl
		Desiree WeeklySchedule	Desiree IPM2.0	SarpoMira WeeklySchedule

Trials included 3 potato genotypes, 3 control strategies and 6 replicates per site in a randomized block design. Two sites were planted
 providing up to 12 replicates per treatment.

	Block 1		Block 2		Block 3		Block 4		Block 5		Block 6
Plot											
	Desiree		Desiree		Desiree		A15-031		Sarpo Mira		Desiree
63	IPM 1.0	72	IPM 2.0	81	IPM 1.0	90	IPM 1.0	99	IPM 2.0	108	No Treatment
	A15-031		Sarpo Mira		A15-031		A15-031		A15-031	407	Desiree
62	IPM 1.0	/1	No Treatment	80	IPM 1.0	89	IPM 2.0	98	No Treatment	107	IPM 1.0
64	Desiree	70	Desiree	70	A15-031		Sarpo Mira	07	Desiree	400	A15-031
61	No Treatment	70	No Treatment	79	No Treatment	88	IPM 1.0	97	IPM 2.0	106	IPM 2.0
	Sarpo Mira		A15-031	70	Sarpo Mira	07	Desiree		A15-031	105	A15-031
60	IPM 1.0	69	No Treatment	78	IPM 2.0	87	No Treatment	96	IPM 1.0	105	IPM 1.0
50	A15-031	69	Sarpo Mira	77	Sarpo Mira	96	Desiree	05	Desiree	404	Sarpo Mira
59	No Treatment	00	IPM 1.0	"	No Treatment	00	IPM 1.0	90	IPM 1.0	104	IPM 1.0
E 0	A15-031	67	Sarpo Mira	76	Sarpo Mira	95	A15-031	04	Sarpo Mira	402	A15-031
50	IPM 2.0	67	IPM 2.0	70	IPM 1.0	00	No Treatment	94	IPM 1.0	103	No Treatment
57	Sarpo Mira	66	Desiree	75	A15-031	04	Desiree	02	A15-031	102	Sarpo Mira
57	IPM 2.0	00	IPM 1.0	15	IPM 2.0	04	IPM 2.0	93	IPM 2.0	102	IPM 2.0
56	Desiree	65	A15-031	74	Desiree	02	Sarpo Mira	02	Sarpo Mira	101	Desiree
20	IPM 2.0	60	IPM 1.0	74	No Treatment	03	No Treatment	92	No Treatment	101	IPM 2.0
55	Sarpo Mira	64	A15-031	70	Desiree	82	Sarpo Mira	04	Desiree	100	Sarpo Mira
55	No Treatment	64	IPM 2.0	13	IPM 2.0	82	IPM 2.0	91	No Treatment	100	No Treatment

1 Appendix 2a. Calculated infection periods 2013.







- 1 Appendix 2b. Calculated infection periods 2014.
- 2 Valthermond NL (top) and Carlow IE (bottom).







# 1 Appendix III. Summary of sprays applied and environmental effects.

Valthermone	d 2013		Spray summary						Environmental effects		
		End of		Cumulative							
Potato	Late Blight	Season	Total nr of	relative dose	# Preventive	# Curative	# Eradicative	Aquatic	Soil	Ground	
Genotype	Control Strategy	severity (%)	Sprays	rates applied	Sprays	Sprays	Sprays	life	life	water	
Desiree	Unsprayed	100.00	0	0.0	0	0	0	0	0	0	
	Weekly sprays	5.01	12	12.0	12	0	0	238	398	12	
	IPM 2.0	5.02	11	10.3	11	0	0	197	244	11	
Sarpo Mira	Unsprayed	1.09	0	0.0	0	0	0	0	0	0	
	Weekly sprays	0.00	12	12.0	12	0	0	238	398	12	
	IPM 2.0	0.00	3	0.8	3	0	0	9	0	0	
A15-31	Unsprayed	0.01	0	0.0	0	0	0	0	0	0	
	Weekly sprays	0.00	12	12.0	12	0	0	238	398	12	
	IPM 2.0	0.00	3	0.8	3	0	0	9	0	0	

2

Valthermone	d 2014			Sp	ray summary			Environ	Environmental effects			
		End of										
Potato	Late Blight	Season	Total nr of	Cumulative relative	# Preventive	# Curative	# Eradicative	Aquatic	Soil	Ground		
Genotype	Control Strategy	severity (%)	Sprays	dose rates applied	Sprays	Sprays	Sprays	life	life	water		
Desiree	Unsprayed	100.00	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.71	14	16.5	10	1	3	179	125	4		
	IPM 2.0	1.00	14	16.5	10	1	3	179	125	4		
Sarpo Mira	Unsprayed	0.02	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	13	12.5	13	0	0	117	42	2		
	IPM 2.0	0.00	10	7.3	7	1	2	132	80	3		
A15-31	Unsprayed	26.11	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	13	12.5	13	0	0	117	42	2		
	IPM 2.0	0.01	5	1.3	5	0	0	15	5	0		

Carlow 2013			Spray summary					Enviror	Environmental effects			
				Cumulative								
Potato	Late Blight	End of Season	Total nr of	relative dose	# Preventive	# Curative	# Eradicative	Aquatic	Soil	Ground		
Genotype	Control Strategy	severity (%)	Sprays	rates applied	Sprays	Sprays	Sprays	life	life	water		
Desiree	Unsprayed	100.00	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.21	11	11.0	11	0	0	1686	164	6		
	IPM 2.0	9.00	10	10.0	6	4	0	350	64	9		
Sarpo Mira	Unsprayed	0.73	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.46	11	11.0	11	0	0	1686	164	6		
	IPM 2.0	0.21	0	0.0	0	0	0	0	0	0		
A15-31	Unsprayed	0.00	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	11	11.0	11	0	0	1686	164	6		
	IPM 2.0	0.00	0	0.0	0	0	0	0	0	0		

Carlow 2014	1				Spray summary			Enviror	Environmental effects			
				Cumulative								
Potato	Late Blight	End of Season	Total nr of	relative dose	# Preventive	# Curative	# Eradicative	Aquatic	Soil	Ground		
Genotype	Control Strategy	severity (%)	Sprays	rates applied	Sprays	Sprays	Sprays	life	life	water		
Desiree	Unsprayed	38.33	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	10	10.0	8	2	0	156	190	10		
	IPM 2.0	0.03	9	9.0	6	3	0	148	189	12		
Sarpo Mira	Unsprayed	0.00	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	10	10.0	8	2	0	156	190	10		
	IPM 2.0	0.00	3	3.0				33	15	6		
A15-31	Unsprayed	0.00	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	10	10.0	8	2	0	156	190	10		
	IPM 2.0	0.00	0	0.0	0	0	0	0	0	0		

Carlow 2015	5		Spray summary						Environmental effects			
Potato Genotype	Late Blight Control Strategy	End of Season severity (%)	Total nr of Sprays	Cumulative relative dose rates applied	# Preventive Sprays	# Curative Sprays	# Eradicative Sprays	Aquatic life	Soil life	Ground water		
Desiree	Unsprayed	42.08	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.01	11	12.0	7	3	1	220	260	14		
	IPM 2.0	0.01	9	10.0	4	4	1	163	181	13		
Sarpo Mira	Unsprayed	0.00	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	11	12.0	7	3	1	220	260	14		
	IPM 2.0	0.00	0	0.0	0	0	0	0	0	0		
A15-31	Unsprayed	0.00	0	0.0	0	0	0	0	0	0		
	Weekly sprays	0.00	11	12.0	7	3	1	220	260	14		
	IPM 2.0	0.00	0	0.0	0	0	0	0	0	0		