Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems

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Deliverable 8.6

Assessment of the environmental impact of potato late blight resistant potato in an IPM strategy

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Contents

Abstract

For the purpose of AMIGA WP8, a novel, more durable, Integrated Pest Management (IPM) strategy (termed IPM2.0) for the control of potato late blight disease was developed and evaluated under field conditions in the Netherlands (2013 and 2014) and in Ireland (2013, 2014, 2015). IPM2.0 builds on the principles of IPM and deployment of resistant potato cultivars as the primary line of defence against infection by *Phytophthora infestans*. It also includes pathogen population monitoring for virulence to the resistance genes (R genes) deployed and an "only when necessary, low input fungicide spray strategy" to mitigate the effects of pathogen evolution. For the purposes of AMIGA, we used a cisgenically modified, potato cultivar Desiree based, resistant potato clone named A15-031. Comparators included the original, conventional, but susceptible potato cultivar Desiree and the conventional but highly resistant potato cultivar Sarpo mira. This report describes AMIGA deliverable 8.6: "Assessment of the environmental impact of potato late blight resistant potato in an IPM strategy".

Overall, the IPM2.0 control strategy developed here and described above reduced the average fungicide input, in a total of five field trials in two different countries, on resistant potato cultivars or clones by 80 - 90%. The environmental effects, as measured using the "environmental yardstick", were reduced proportionally.

Introduction

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

Host resistance and subsequent cultivation of potato late blight resistant potato cultivars is the most (cost) effective and environmentally friendly way to control potato late blight (Schepers et al., 2009). Currently however, the cultivation of resistant cultivars in the EU and elsewhere is restricted to almost non-existent due to the market's overwhelming demand for a limited number of commercially successful but highly late blight susceptible cultivars. In addition, potato breeding is complex and time 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 gene (R gene) mediated host resistance (e.g. Black et al., 1953, Fry 2008; Haas et al. 2009; McDonald and Linde 2002), especially if resistance is based on a single R gene.

In 2009, the origin of this adaptive capability was shown to reside in the *P. infestans* genome in combination with its high reproductive capacity (Haas & Kamoun et al., 2009). As a result, adaptation is "the Phytophthora infestans way of life", which results in R genes being overcome (e.g. Black et al., 1953, Fry 2008; Haas et al. 2009; McDonald and Linde 2002), resistance to active ingredients of fungicides (e.g. Dowley and O'Sullivan 1981) and increased 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), Cooke et al. (2012) and Fry et al. (2013).

In response, from 2006 – 2015 the Wageningen University and Research Centre DuRPh project (www.durph.wur.nl) 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 through cisgenic modification (Jacobsen and Schouten, 2007) in combination with adequate resistance management (Haverkort et al., 2008; 2015). One output from this initiative and the preceding conventional "Umbrellaplan Phytophthora" was the development of a novel, more durable and low fungicide input control strategy for potato late blight. This strategy was developed to overcome problems of insufficient disease control on resistant cultivars due to the high adaptive ability of *P. infestans*. It is based on the principles of Integrated Pest Management (IPM, e.g. described in EU directive 2009/128/EC) and incorporates (cisgenically modified) resistant potato clones. The following components are included in the complete control strategy (Haverkort et al 2015):

a. Introduction of resistance, preferably based on R gene stacks.
b. Monitoring the local *P. infestans* population for (emerging) virulence to the R genes contained in the R gene stack.
c. Adoption of a preventative, zero tolerance "we do not spray unless" strategy. In this scenario the 'do not spray' strategy is only reverted when:
    a. Virulence to all R genes contained in a potato clone or cultivar are present in the local *P. infestans* population.
    b. A validated decision support system (DSS) predicts an infection event in the immediate future.
    c. The remaining fungicide protection is insufficient.
d. Application of reduced dose rates of protectant fungicides on resistant cultivars
e. Replacement of the overcome R gene cassette with a new, functional R gene cassette to restart the cycle.

Within the EU AMIGA project this strategy was put to the test with respect to components a – d in the Netherlands (2013 and 2014) and Ireland (2013, 2014 and 2015). In this regard, we studied the effect of this strategy on disease progress, fungicide input, subsequent environmental impact and practical applicability for a conventional susceptible potato cultivar (Desiree), a GM resistant version of this same cultivar (A15-31) and a conventionally bred resistant cultivar (Sarpo Mira), which is typically favoured by organic growers in the UK.

The time frame necessary to test component 5: “replacement of R gene cassettes” was outside the AMIGA project duration.

For the purpose of AMIGA DL 8.6, the results of all five field trials will be discussed with respect to the environmental impact of cultivation of resistant potato using an IPM2.0 strategy for potato late blight. The added value of host resistance and cisgenic modification, within an IPM context, to improved sustainability of potato late blight control in agricultural ecosystems is discussed in AMIGA Deliverable 8.3. The selective effect of growing (GM) resistant potato cultivars on *P. infestans* populations is discussed in AMIGA Deliverable 8.4. A modelling approach on durability of resistance and potato cultivation is presented in DL 8.5.

Materials and Methods

Environmental effects of potato late blight control

Field trials, plant material, potato late blight control strategies and results, including the necessary fungicide inputs, have been described in detail in DL8.3. Environmental effects for the purpose of AMIGA deliverable DL8.6 were calculated using the environmental yardstick for pesticides accessible through www.milieumeetlat.nl (Reus and Leendertse, 2000). For this purpose, effects of organic matter (3-6%), season (spring, March – August) and drift (1%) were standardized and assumed to be fixed at the values/settings given in brackets.

Environmental effects for the various field trials (appendix II) were calculated as the sum of the effects of single applications. Environmental effects of single applications of fungicides were calculated using the tools available at www.milieumeetlat.nl and are given in appendix I.

Results

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

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.

Fungicide input

Fungicides applications were carried out based on the relevant control strategy. Weekly applications were applied according to a fixed, calendar based, 7 day interval spray schedule, irrespective of the weather and according to common practice in most of the EU. For the IPM2.0 strategy, protectant fungicides were applied just prior to predicted infection events. Strongly reduced dose rates of protectant fungicides (25% of the recommended dose rate) were applied on the highly resistant Sarpo mira and A15-031. Occasionally, curative fungicide applications were carried out within two days after a non-treated infection event occurred. Eradicant fungicide applications were necessary when active infections were found 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 cured. The unsprayed control was not sprayed with fungicides at all. The fungicide treatments applied are summarized in Figure 2. Details can be found in Appendix II. The resulting levels of disease control are given in Figure 4.

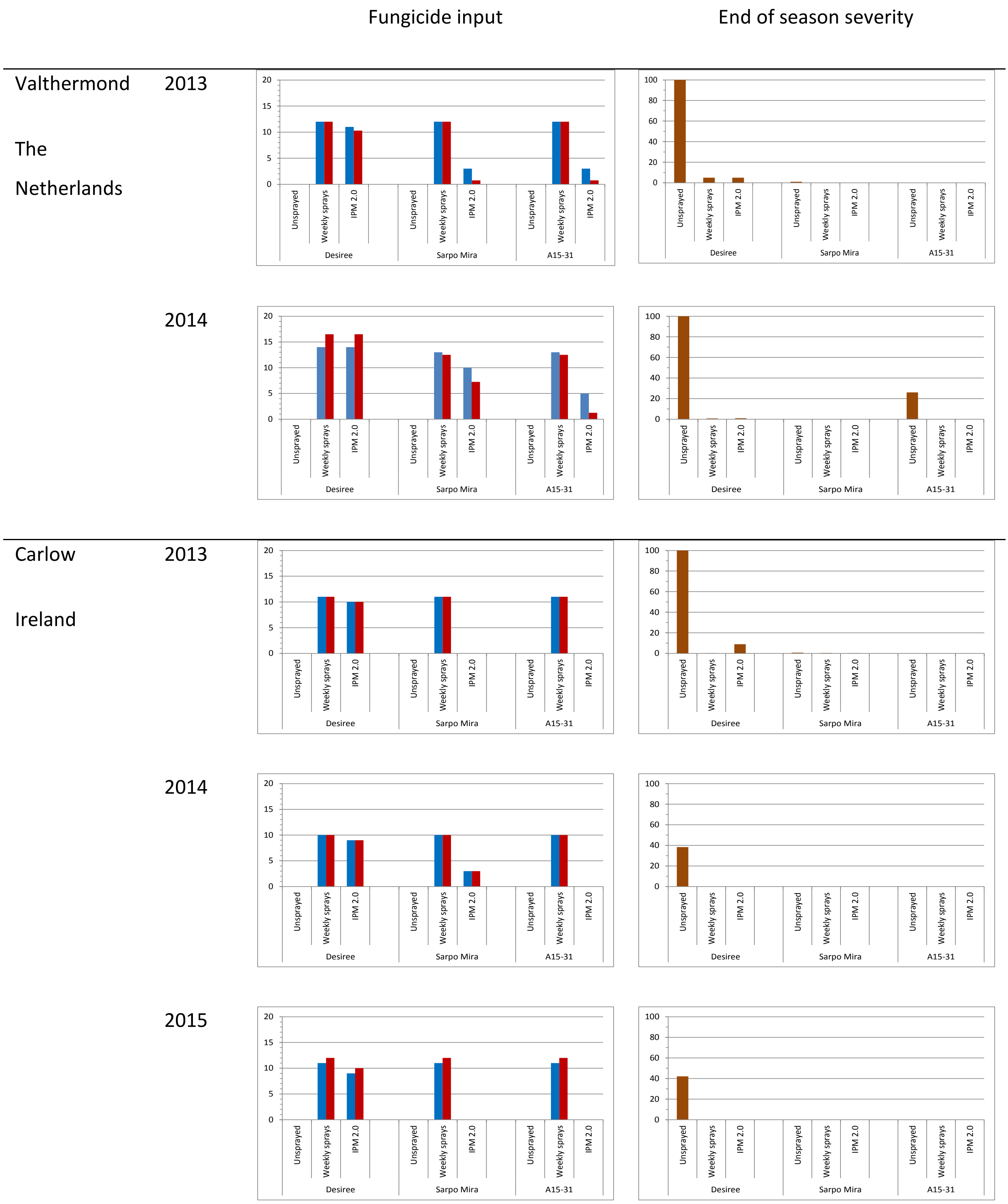

Figure 2. Fungicide input (left column) and end of season severity (right column, ■: % destroyed foliage) for all five AMIGA GM-IPM trials in three years and two locations. Fungicide input represents the number of spray applications (■ blue) and the number of “full dose rate equivalents” applied (■ red).

Environmental effects

The environmental effects, relative to current common practice (weekly spray applications on a susceptible cultivar), are given in Figure 3. The reduction of environmental effects by the IPM2.0 control strategy depends on the level of host resistance: a reduction of 10 - 20% on susceptible cultivar Desiree, an approximate 80% reduction on the highly resistant cultivar Sarpo mira and more than 90% reduction on the highly resistant potato genotype A15-03. The level of reduction is therefore highly dependent on the virulence to the R genes "grown" contained in the local *P. infestans* population but the value of host resistance to reducing the environmental impact of potato late blight control and potato cultivation is paramount. Details on the sprays applied and the calculation of environmental impact can be found in Appendix II.

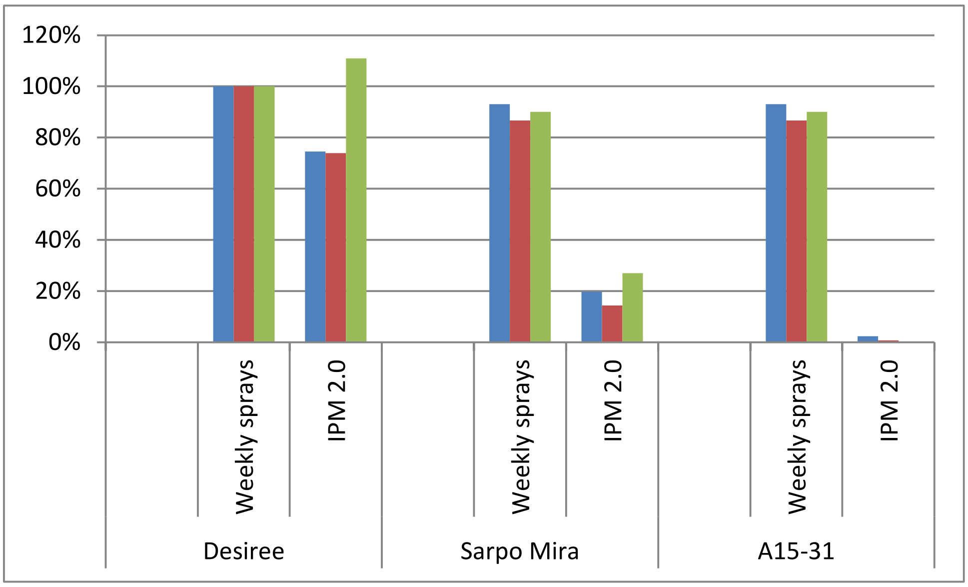

Figure 3. Average environmental effects of the AMIGA "IPM 2.0 control strategy", relative to current practice (weekly spray schedule), for aquatic life (■), soil life (■) and ground water (■). Data include the results of all three field trials in Ireland and both field trials in the Netherlands. The reduction of environmental effects by the IPM2.0 control strategy depends on the level of host resistance: a reduction of 10 - 20% for susceptible cultivar Desiree, an approximate 80% reduction for the highly resistant cultivar Sarpo mira and an average reduction over 90% for the highly resistant potato genotype A15-031.

Disease Management

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

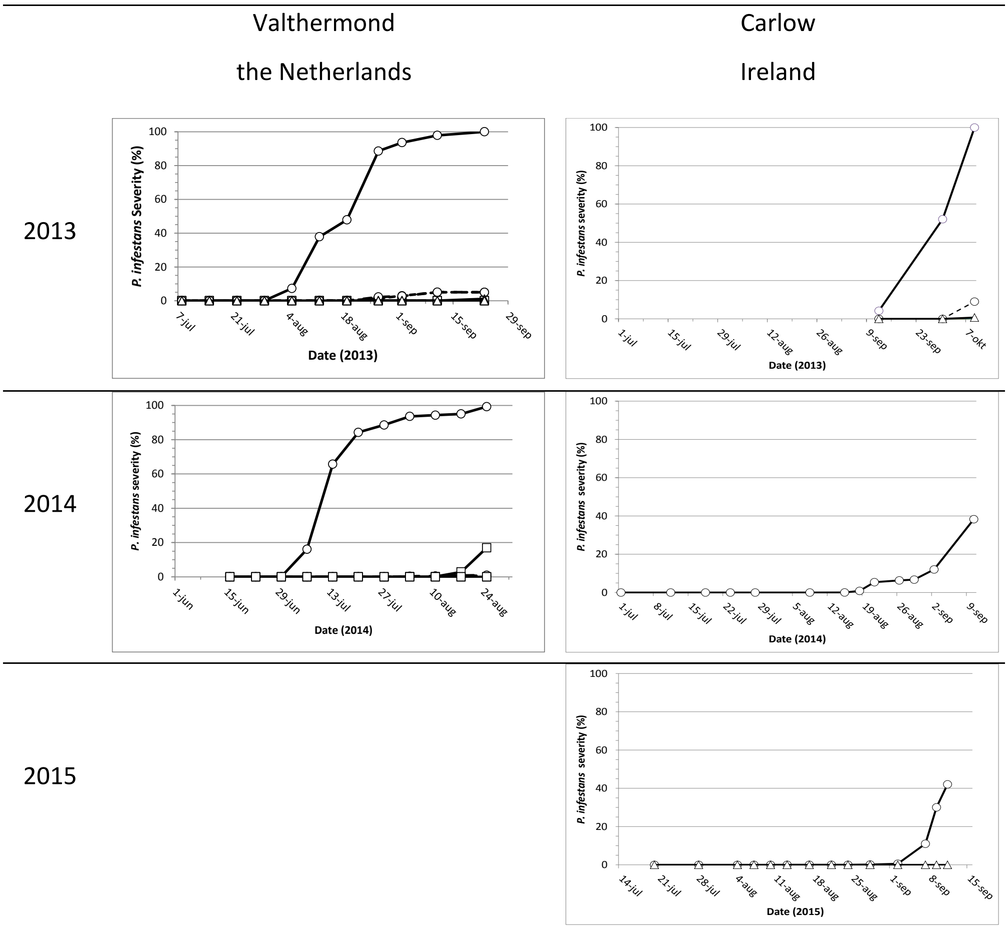

Figure 4. Epidemic progress on all nine potato genotypes x management strategy combinations for both locations and both years. ○: Desiree. □: A15-031. △: Sarpo mira. _____: Unsprayed Control. _ _ _ _ : Weekly spay schedule (Common practice). ----: Next level/IPM2.0 control strategy.

The necessary fungicide input and the environmental effects (Figure 3 and 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 II) were triggered by a primary preventive spray advice that could not be carried out due to e.g. excessive rain.

Discussion

The objectives of WP8 were to:

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

Here we report on AMIGA deliverable 8.6: “Assessment of the environmental impact of resistant potato in an IPM strategy”.

From AMIGA deliverable 8.3 we know that host resistance to potato late blight was found to be an extremely valuable addition/component to an IPM potato late blight control strategy. Host resistance 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 **Error! Bookmark not defined.** onwards).

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 be equal to the control efficacy of the current common practice “a weekly spray schedule”(Figure 2 and Figure 3). The fungicide input necessary was however markedly reduced. On the susceptible potato cultivar Desiree, the fungicide input could be reduced due to optimized dynamic spray timing but only marginally. On the resistant cultivar Sarpo mira and the resistant Desiree based potato clone A15-031 the fungicide input and the environmental effects could be reduced by 80 – 90%. Yields were similar between the potato cultivars/cones involved apart from unsprayed Desiree where yield was reduced due to late blight destroying the foliage.

With respect to AMIGA deliverable 8.6, environmental effects were greatly reduced when host resistance was incorporated into the IPM2.0 late blight control strategy (Figure 3 and Appendix II).

On (more) resistant potato genotypes it is therefore possible to:

1. Not spray fungicides when virulence to the R gene(s) contained is absent from the local pathogen population.
2. 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.
3. 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 **Appendix I. Environmental effects** of single applications of fungicides used to control potato late blight calculated May 2016 using

2 www.milieumeetlat.nl (Reus and Leendertse, 2000).

Fungicide			Environmental effects		
(Commercial name)	**Dose rate (l/ha or kg/ha)**	**Active ingredient (kg/ha)**	**Aquatic organisms**	**Soil organisms**	**Groundwater**
Revus	0.6	0.15	6	4	0
Revus	0.15	0.037	2	1	0
Shirlan	0.4	0.2	276	24	1
Valbon	1.6	1.14	42	80	3
Valbon	2	1.425	52	100	4
Curzate M	2.5	1.813	75	145	5
Proxanil	2	0.9	10	28	0
Infinito	1.2	0.705	8	4	1
Infinito	1.6	0.94	11	5	2
Ranman Top	0.5	0.08	13	2	0
Ranman Top	0.125	0.02	3	1	0

Appendix II. Summary of sprays applied and environmental effects.

Valthermond 2013			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	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

Valthermond 2014			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	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					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	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			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	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			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