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ENHANCE RESISTANCE TO *ALTERNARIA ALTERNATA* CAUSING POTATO BROWN LEAF SPOT DISEASE BY USING SOME PLANT DEFENSE INDUCERS

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Abstract: Host resistance is an efficient and effective component in integrated management of plant diseases. The aim of this study was to test whether Acibenzolar-S-methyl (ASM), Chitosan, Heads-up and Acetyl Salicylic Acid (ASA), known to induce resistance against various diseases, can help protect potato crop against brown leaf spot. The effect of these inducers, on two potato cultivars, Goldrush and FL1879 against Alternaria alternata, causal agent of brown leaf spot at two different field sites were evaluated. To determine the effects of the application of inducers on disease resistance, the foliage of the potato cultivars was sprayed with appropriate concentrations of ASA, chitosan, and ASM. Heads-up was also applied as a pre-plant treatment on potato tubers. The results obtained from the both field experiments indicated the highest yield performance was achieved in plots treated with ASM, followed by Heads-up and chitosan treatments. However, no significant difference in terms of tuber yield production has been noted between ASA treated potato foliage, and the untreated control plants. Results of experiments with detached leaves showed that there was a significant difference regarding disease index reduction between plots which been treated with defense inducers and untreated, inoculated plots. It was clear that on both potato cultivars, application of chitosan and ASM encouraged enhancement of the disease resistance compared to the ASA and Heads-up treatments. In the laboratory experiment, disease progress was recorded on leaves from three different heights of the crop canopy. The results indicated that disease severity was low in the apex, moderate in the middle and high in the lower parts of the crop, in both potato cultivars. These results suggest that chitosan and ASM may offer alternative methods for controlling brown leaf spot of potato.

Key words: Canopy Position, Systemic Acquired Resistance (SAR), Acibenzolar-S-methyl, Heads-up, chitosan

INTRODUCTION

The cultivated potato, *Solanum tuberosum* L., is the most consumed vegetable in the world. It is also the most economically important vegetable with a total farm value of $2.564 billion in the USA *Alternaria alternata* (Fries) Keissler, is one of the prevalent pathogens causing potato brown leaf spot in North America as well as other parts of the world (Thomma 2003). Brown leaf spot disease has been known as one of the destructive and common diseases of the cultivated potato in areas with heavy dew, frequent rainfall, and high relative humidity (Nash and Gardner 1988). The disease can also occur over a wide range of climatic conditions. Brown leaf spot depends mainly on the frequency of wet foliage from rainfall, fog, dew, or irrigation, on the nutritional status of foliage, and on cultivar susceptibility (Stevenson *et al*. 2001). This disease progressively weakens the plant and increases its susceptibility to infection. Brown leaf spot reduces the photosynthetic leaf area and increases the imbalance between nutrient demand in the tubers and nutrient supply from the leaves, subsequently leading to reduced yields (Simmons 2000). Losses due to the disease are typically around 20 percent; however, there have been cases of 70–80% losses, where the disease has been left uncontrolled (Wharton and Kirk 2008). These losses can be increased when the disease is combined with other diseases like early blight, black-leg and Verticillium wilt (Jansky *et al*. 2008).

Unlike early blight, brown leaf spot can occur any time during the season. According to previous publications (Simmons 2000; Jansky *et al*. 2008), early in the growing season, the disease develops first on fully expanded leaves near the soil surface and progresses slowly on juvenile tissues. Although the conditions affecting infection and disease severity are known, little information is available on lesion production on infected tissues. It is not clear whether the availability of free moisture in the lower part of the crop canopy or the leaf age is responsible for the infection reaching the primarily expanded leaves.

No major gene resistance towards brown leaf spot is known. Genetic sources for partial resistance have been identified within wild species of potato. The resulting lines from crosses of potato with these wild species still do not have satisfying crop qualities. Therefore, the disease con-

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trol is mainly conducted with protective fungicides (Stevenson *et al*. 2001). These fungicides do not always prevent the infestation of the plant and severe losses can still occur. Additionally, although yield loss may be minimized with fungicides, growers are interested in reducing chemical inputs for both environmental and economic reasons.

An alternative to the usual chemical plant protection methods could be to employ systemic acquired resistance (SAR) effects. Resistance to disease can be induced systematically in a number of plant species by biological and chemical means (Ryals *et al*. 1994; Spletzer and Enyedi 1999). One of the potential management approaches is the use of SAR to activate host defense mechanisms, which would not involve the application of toxic compounds to plants (Durrant and Dong 2004). Exogenous application of salicylic acid (SA) or structural analogues of SA such as benzo (1,2,3) thiadiazole-carbothioic-acid-S-methylester (BTH) and 2,6-dichloroiso-nicotinic acid (INA) that appear to act similarly to salicylic acid, can induce SAR (Hammerschmidt 1999). The most studied systemic inducer is Acibenzolar-S-methyl (ASM). It is marketed by Syngenta under the name Bion in Europe and Actigard in United States. This compound stimulates the salicylic acid defense pathway and activates SAR in many crop plants against fungi, oomycetes, bacteria, viruses, nematodes and insects (Tally *et al*. 1999).

The exogenous application of Acetyl Salicylic Acid (ASA) and Acibenzolar-S-methyl (ASM) to tobacco (White 1979; Andreu *et al*. 2006), and ASA to tomato plants (Ward *et al*. 1991) resulted in disease resistance which is correlated with pathogen-related (PR) gene expression. Another potential approach involves the use of natural bioactive substances, such as Chitosan and Heads-up. Chitosan inhibits fungal growth and also activates defense mechanisms of plants (Kendra *et al*. 1989; El Ghaouth *et al*. 1991). These two processes can induce a multitude of biological developments in plant tissues, including the stimulation of chitinases (El Ghaouth *et al*. 1991), accumulation of phytoalexins (KENDRA et al. 1989), and increased lignifications (El Ghaouth *et al*. 1992). Heads-up is an extract of *Chenopodium quinoa* containing saponins (approximately equimolar amounts of triterpene bidesmosidic glycosides of oleanolic acid, hederagenin, and phytolaccagenic acid; 49.65%). Although the product is not directly fungicidal, it is thought that the mode of action may be an induction of the systemic acquired resistance response in plants. Heads-up, has recently been introduced as a preplant seed and pre-transplant seedling treatments for the prevention of fungal, bacterial and viral diseases of plants (www.sar-headsup.com).

There is little information on the use and effectiveness of applying these putative biochemical defense inducers to control potato brown leaf spot. Thus, the objectives of this investigation were to test foliar treatment with ASM, Chitosan and ASA as well as a pre-planting treatment with Heads-up, for their ability to effectively induce resistance against *A. alternata*. For the first time, we investigated the direct effects of these compounds on the development of brown leaf spot as well as the effects of the compounds on potato leaf canopy position. Finally, we examined the possible consequences of these treatments on potato tuber yield *cvs.* Goldrush and FL1879.

MATERIALS AND METHODS

Fungal cultures and inoculation

A virulent single spore isolate of *A. alternata,* which produces blighted symptoms in potato foliage tissue, was used. Conidia of the isolate were maintained at 4°C in the dark on filter paper. Axenic cultures of the isolate were produced by placing a 1 mm^2 section of filter paper containing conidia of the stored culture on Potato Dextrose Agar (PDA). For inoculums production, cultures of *A. alternata* were grown on Potato Carrot Agar (PCA) in the dark at 18°C for 14 days prior to the date of inoculation. Conidia were harvested by flooding the surface of the Petri dish with sterile distilled-deionized water (5 ml) and gently scraping the surface of the media with an L-shaped glass rod to dislodge the conidia. The conidial suspension was stirred with a magnetic stirrer for 1 h and strained through 4 layers of cheesecloth to remove mycelial fragments. The concentration was then adjusted to $1x10^5$ conidia/ml using a hemocytometer.

Foliar and tuber treatments

Potato cultivars Goldrush (suitable for processing French fries) and FL1879; chip processing cultivar, were used in all the experiments. Whole tubers were harvested in October from certified seed crops grown in northern Michigan in 2006 and 2007. Tubers free from symptoms of brown leaf spot (and other diseases) were selected for the experiments. The tubers were stored in the dark at 3°C and 95% RH until the spring of the following year. Tubers were removed from storage, warmed from 4°C, in 2°C increments, every two days, up to 12°C over a period of eight days. These tubers were maintained at 12°C for a further two days in the dark in a controlled environment chamber with forced air ventilation at 5,950 l/min until cutting and treatment. Tubers were cut longitudinally in half with a sterile knife ensuring that viable sprouts were present on both halves.

Treatment applied to seed pieces were: 1) not treated, 2) treated with Heads-up (active substances: extract of *Ch. quinoa* containing saponins; approximately equimolar amounts of triterpene bidesmosidic glycosides of oleanolic acid, hederagenin, and phytolaccagenic acid; 49.65%; Plant Protectants Inc., Kamsack, Saskachewan, Canada) at the manufacturers recommended rate (1 g/l). An application was made to germinated seed potatoes. It was obvious that the potatoes were germinated seed potatoes because of the sprouting activity coming from the potato eyes. Potato tubers were sprayed until run-off with Heads-up solution, using a hand held sprayer prior to planting. Chemical compounds were applied as foliar treatments. Acetyl Salicylic Acid (ASA) from Sigma Chemical Company (St Louis, MO, USA) was tested at rate of 400 mg/l of water. Chitosan, from crab shell was obtained from the Sigma Chemical Company (St Louis, MO, USA). The degree of deacetylation of chitosan was 85% and the molecular weight was $2x10^5$ Daltons. For experimental use, the solution of chitosan (1 mg/ml) was continuously stirred in 0.5% (v/v) acetic acid until it was dissolved. When dissolved, the pH value of the chitosan

solution was adjusted to 5.6 using molar NaOH solution; 0.05% (w/v). Tween-80 as a surfactant, was added to improve the wetting properties of the solution. Acibenzolar-S-methyl (active ingredient: 1,2,3-benzothiadiazole-7-thiocarboxylic acid-S-methyl-ester 50.0%, Actigard® 50WG, Syngenta, Basel, Switzerland) was applied at a concentration of 100 mg l^{-1} of water. All foliage application was done 50 days after emergence. Leaves were sprayed with a hand-held sprayer till runoff. Chemicals were freshly dissolved in water with 0.01% v/v Tween 20 about 1 h before spraying. Water was used as a control treatment. All other chemicals used for buffers were obtained from Sigma Chemical Company (St Louis, MO, USA) unless otherwise specified.

Field experiments

The field experiment was conducted in two different Michigan State University (MSU) field experimental sites, in 2008. The treated cut seed pieces were planted at the MSU Montcalm Potato Research Farm, Edmore, MI , USA as well as in the MSU Muck Farm. At the Muck Farm, the soil type was Houghton Muck – level, deep, organic soil with moderate permeability and neutral pH. Whereas the soil type in Montcalm Farm was sandy-loam. The tomato seed pieces were planted in single-rows in 9 m plots (ca. 22 cm between seed pieces, to give an intended population of 40 plants at 86 cm row spacing) replicated four times in a randomized complete block design. Fertilizer was formulated according to the results of the soil tests and drilled into plots before planting. Additional nitrogen (final N 31 kg/ha) was applied to the growing crop with irrigation 45 days after planting (DAP). To control potato late blight, the fungicide Bravo WS 6SC® (active substance: chlorothalonil) was applied at 1.75 l/ha on a seven-day interval cycle (eight applications) to all treatments, starting when the canopy was about 50% closed. A permanent irrigation system was established prior to the commencement of fungicide sprays. The fields were maintained at about 80% soil moisture capacity throughout the season by frequent (minimum 5 day) irrigations delivering about 1.5 cm H_2O/ha per irrigation. Weeds were controlled by hilling and with the herbicides metolachlor (Dual 8E®) at 2.3 l/ha 10 DAP, and sethoxydim (Poast®) at 1.8 l/ha 40 DAP. Insects were controlled with the insecticides imidacloprid (Admire 2F®) at 1.4 l/ha at planting, carbaryl (Sevin 80S®) at 1.4 kg/ha 31 and 55 DAP, endosulfan (Thiodan 3 EC®) at 2.7 l/ha 65 and 87 DAP, and permethrin (Pounce 3.2EC®) at 0.56 l/ha 48 DAP.

In both experimental fields, data were collected on canopy closure, plant stand and yield. Disease development in treated and control plants naturally occurred in the field, and was recorded forty-five (at the Montcalm Site) and fifty-five (Muck Farm) days after application of treatments by visual assessment of the leaf area showing brown leaf spot. Disease severity was recorded by estimating the percentage of the brown leaf spot symptoms.

Evaluation of foliage protection within the canopy position

Ten days after foliar application of chemical inducers (or water treatment as a control), the percentage of foliage protection against *A. alternata* was evaluated by using the detached leaf method as follows. Five leaves per each of the three different levels of plant canopy (Top, Middle and Lower part) were detached from the foliage of ten plants per replication and treatment. At the laboratory, the detached leaves were artificially inoculated by placing a 50 µl droplet of conidial suspension ($1x10^5$ conidia/ml) on the centre of each leaflet. The inoculated leaves were placed in boxes with humid filter paper and incubated in a growth chamber in darkness at 18°C for 24 h. After this period, leaf incubation continued in a growth chamber at 21°C and 16 h day length provided by fluorescent tube with a radiation intensity of 12 $W/m)^2$ at plant level. Disease development in treated and control leaves was recorded daily from 3 to 7 days after inoculation by visual assessment of the leaf area showing brown leaf spot on inoculated leaflet area. Disease severity was recorded by estimating the lesions on a scale from 1 to 7, where: 1 = no lesions, 2 = a few circles, 3 = up to 5%, 4 = 6–10%, 5 = 11–25%, 6 = 26–50%, 7 = 51–100% of leaf area with brown leaf spot symptoms.

Statistical analysis

Rules for analyzing: Displaying data were based on main effect analysis using multi-variable ANOVA in JMP version (5.0.1). Depending on the outcome, data were grouped by variety, treatments (field experiments) and for canopy position. If there was no significant difference between *cvs*; nor significant difference between treatments, then data showed the difference among varieties only. If there were significant difference between *cvs*. then data were applied into cultivar groups.

RESULTS

Disease suppression in field experiment

Field experiments were carried out in the 2008 growing season in two different sites: Muck Farm and the Montcalm Research Station. In both experiments, two potato cultivars Goldrush and FL1879 were planted. Five treatments were compared: chitosan, ASM, Heads-up, ASA and water spray (the control treatment). Potato brown leaf spot was more severe on *cv.* Goldrush than *cv.* FL1879 as shown by a higher average disease severity index (58% *versus* 39% – the Muck Farm Site). In terms of disease suppression, all of the treatments were significantly effective compared to the untreated, inoculated control (Table 1). The results obtained from field experiments at the Muck Farm site did show that there was more disease development there, than at the Montcalm site. However, it was clear that at both sites, application of ASM was the most encouraging of all of the treatments (chitosan, Heads-up and ASA), for enhancing disease resistance (Tables 1, 2). The other three treatments also significantly reduced disease symptoms compared to the control plots, however, there were no significant differences among them. Similar results were obtained on two different potato cultivars (Goldrush and FL1879) in both field experimental sites (Tables 1, 2, 3).

Application of ASM has indicated higher tuber yield production on the potato cultivar Goldrush, at both field

Table 1. Effects of application of defense inducers on potato tuber production and brown leaf spot disease on potato cultivars (Goldrush and FL1879) in Muck Farm field experiment. Plants were naturally inoculated with *A. alternata* in the field

Field Site	Cultivar	Treatments	Mean Tuber [yield t/ha]	*Disease Index [%]
Muck Farm	Goldrush	ASM	39.3 a	41.25 cde
		Heads-up	34.8 ab	60.0 abc
		chitosan	34.4 ab	56.25 abcd
		ASA	31.1 ab	61.25 ab
		untreated	26.8 b	71.25 a
	FL1879	ASM	38.4 a	23.75 e
		Heads-up	37.3 ab	38.75 de
		chitosan	35.5 ab	42.5 bcde
		ASA	34.3 ab	38.75 de
		untreated	26.4 b	51.25 bcd

*disease development was measured fifty-five days post-treatment. Values in columns marked with the same letter are not significantly different at p = 0.01; Acibenzolar-S-methyl (ASM), chitosan, Acetyl Salicylic Acid (ASA)

Table 2. Effects of application of defense inducers on potato tuber production and brown spot disease index on potato cultivars (Goldrush and FL1879) in Montcalm field experiment. Plants were naturally inoculated with *A. alternata* in the field

Field Site	Cultivar	Treatments	Mean Tuber [yield t/ha]	*Disease Index [%]
Montcalm	Goldrush	ASM	17.3 b	45.0 cd
		Heads-up	16.8 bc	55.0 bc
		chitosan	15.7 bcd	40.0 de
		untreated	14.1 cde	72.5 a
		ASA	14.1 cde	60.0 ab
	FL1879	Heads-up	19.7 a	18.75 fg
		chitosan	17.5 ab	11.25 g
		ASM	16.3 bc	15.0 fg
		untreated	13.5 de	27.5 ef
		ASA	12.5 e	25.0 f

*disease development was measured forty-five days post-treatment. Values in columns marked with the same letter are not significantly different at p = 0.01; Acibenzolar-S-methyl (ASM), chitosan, Acetyl Salicylic Acid (ASA)

Table 3. Summary of the analysis of variance of the main effects of foliage and tuber treatment application[a] in two cultivars[b] of potato recorded forty-five and fifty-five days after treatments at the Montcalm and Muck Farm sites

Field Site	Source	DF	F Ratio	Prob > F
Montcalm	variety	1	438.806	< 0.0001
	treatment	4	27.056	< 0.0001
	variety* treatment	4	2.9664	0.0354
Muck Farm	variety	1	27.3798	< 0.0001
	treatment	4	9.1190	< 0.0001
	variety* treatment	4	1.3264	0.2829

[a] treatment applications: Acibenzolar-S-methyl (ASM), chitosan, Acetyl Salicylic Acid (ASA), Heads-up and water (untreated);
[b] potato cultivars: Goldrush and FL1879; *interaction between two treatments

experiment sites. But, the most potato yield production at the Montcalm site on cv. FL1879 was achieved by applying Heads-up followed by chitosan and ASM (Table 2). Application of Heads-up had no direct effect on reduction of brown leaf spot disease index. However, the field data has indicated that the potato yield in plots treated with ASM and Heads-up were much higher than for the other treatments (Tables 1, 2). Response to ASM treatment included significant suppression of brown leaf spot in three of the four trials.

Disease suppression in the detached leaves experiment

In terms of disease suppression, all of the treatments were effective compared to the untreated, inoculated control (Tables 4, 5). The results obtained from the detached leaves experiments of the Muck Farm site showed no significant difference regarding disease index reduction among defense inducers (Table 5). However, it was clear that in both potato cultivars, application of the chitosan and ASM were more encouraging to enhancement of disease resistance compared to the ASA and Heads-up treatments (Tables 4, 6). The same result was obtained from the laboratory experiment on detached leaves, harvested in the Muck Farm field trial. In this case also, application of chitosan was the most effective in reducing development and progress of brown leaf spot disease on both cultivars Goldrush and FL1879, compared to other treatments (Tables 5, 6).

Consistently, these two defense inducers have indicated higher tuber yield production in both potato cultivars at Muck Farm and also at Montcalm Farm on potato cv. Goldrush. But at the Montcalm site on *cv*. FL1879, the most potato yield production has been achieved by applying Heads-up followed by chitosan and ASM (Tables 1, 2, 3).

Canopy position

This experiment was designed to test the hypothesis that disease progress is affected by the leaf age or availability of free moisture in the lower part of the crop canopy. In both experimental sites, prior to treatment, no

Table 4. Effect of application of defense inducers on potato brown spot disease index on potato cultivars (Goldrush and FL1879) at the Montcalm field experimental Station. Pre-treated, detached potato leaves from different canopy positions were artificially inoculated *in vitro* with 50 µl droplets containing $5x10^6$ spore/ml of *A. alternata* spore suspensions

Montcalm	Cultivars	Treatment	Canopy Position	*Mean of Disease Index
1	FL1879	ASM	lower	4.57 bcd
2			middle	2.38 hijkl
3			upper	0.36 n
4		ASA	lower	4.30 bcdefg
5			middle	2.77 fghij
6			upper	0.50 mn
7		chitosan	lower	3.43 defghi
8			middle	2.15 ijklm
9			upper	0.25 n
10		Heads-up	lower	4.43 bcdef
11			middle	3.12 defghi
12			upper	0.43 n
13		untreated	lower	8.51 a
14			middle	4.41 bcdefg
15			upper	0.61 mn
16	Goldrush	ASM	lower	5.42 bc
17			middle	2.83 efghij
18			upper	1.08 klmn
19		ASA	lower	5.19 bc
20			middle	3.35 defghi
21			upper	1.40 jklmn
22		chitosan	lower	3.87 cdefgh
23			middle	2.73 ghijk
24			upper	1.07 klmn
25		Heads-up	lower	5.76 b
26			middle	3.89 cdefgh
27			upper	0.97 lmn
28		untreated	lower	9.45 a
29			middle	4.53 bcde
30			upper	1.40 jklmn

*means followed by the same letter are not significantly different at $p < 0.001$ as determined by Tukey's test

Table 5. Effects of application of defense inducers on potato brown spot disease index on potato cultivars (Goldrush and FL1879) at the Muck farm field Station. Pre-treated detached potato leaves from different canopy positions were artificially inoculated *in vitro* with 50 µl droplets containing 5x10^6 spore/ml of *A. alternata* spore suspensions

Muck Farm	Cultivars	Treatment	Canopy Position	*Mean of Disease Index
1	FL1879	ASM	lower	4.7 bcdefgh
2			middle	2.4 ijklmn
3			upper	1.0 n
4		ASA	lower	4.9 bcdefg
6			upper	1.4 mn
7		chitosan	lower	3.6 efghijk
8			middle	2.5 ijklmn
9			upper	1.0 n
10		Heads-up	lower	4.8 bcdefgh
11			middle	3.3 fghijkl
12			upper	1.1 n
13		untreated	lower	8.9 a
14			middle	5.0 bcdef
15			upper	2.5 ijklmn
16	Goldrush	ASM	lower	5.7 bcd
17			middle	3.1 hijklm
18			upper	1.6 lmn
19		ASA	lower	5.8 bc
20			middle	3.7 efghij
21			upper	1.9 jklmn
22		chitosan	lower	4.2 cdefghi
23			middle	3.2 ghijkl
24			upper	1.7 lmn
25		Heads-up	lower	6.3 b
26			middle	4.0 defghi
27			upper	1.9 klmn
28		untreated	lower	9.7 a
29			middle	5.1 bcde
30			upper	2.5 ijklmn

*means followed by the same letter are not significantly different at $p < 0.001$ as determined by Tukey's student range test

Table 6. Summary of the analysis of variance of the main effects of foliage and tuber treatment application[a] in two cultivars[b] of potato on disease development on detached leaves harvested from the Montcalm and Muck Farm sites

Field Site	Source	DF	F Ratio	Prob > F
Muck Farm	variety	1	28.4303	< 0.0001
	canopy position	2	385.7096	< 0.0001
	treatment product	4	67.6046	< 0.0001
	variety* canopy position	2	1.2201	0.2953
	variety* treatment product	4	0.9029	0.4612
	canopy position* treatment product	8	11.9356	< 0.0001
	variety* canopy position* treatment product	8	0.2257	0.9864
Montcalm	variety	1	38.0995	< 0.0001
	canopy position	2	553.2428	< 0.0001
	treatment product	4	56.5253	< 0.0001
	variety* canopy position	2	0.9549	0.3849
	variety* treatment product	4	0.2014	0.9377
	canopy position* treatment product	8	19.9329	< 0.0001
	variety* canopy position* treatment product	8	0.337	0.9519

[a] treatment applications: Acibenzolar-S-methyl (ASM), chitosan, Acetyl Salicylic Acid (ASA), Heads-up and water (untreated);
[b] potato cultivars: Goldrush and FL1879; *interaction between two treatments

disease symptoms were present on either the emerging or the last fully emerged leaves.

The results from the detached leaves experiment indicated that leaf position has a significant effect on the lesion growth rate of *A. alternata* on leaves from upper part of the canopy, irrespective of the cultivar (Tables 4, 5). The pattern of the disease progress was similar in both cultivars, Goldrush and FL1879; however, disease incidence was significantly greater on untreated plots. Results obtained from two separate detached leaves experiments are presented in tables 4, 5. Disease progress was recorded on leaves from three parts of the canopy. Disease severity was low at the apex, moderate in the middle, and high in the lower part of the canopy in both cultivars.

DISCUSSION

Although plants are constantly exposed to various micro-organisms, disease rarely develops from these contacts. In other words, resistance is the rule in plants, and susceptibility to infection is a rare exception (Hammerschmidt 1999).

In this study, we performed field and laboratory experiments to characterize the potential of four resistance inducers in pathosystems involving the potato and economically important fungal pathogen *A. alternata*. These experiments demonstrated that the use of defense activators can enhance resistance to brown leaf spot in potato. The effect of the inducers on treated plants was measured by monitoring plant growth and disease suppression after inoculation naturally in the field/artificially in laboratory; with the plant pathogenic fungus *A. alternata*. Both ASM and chitosan, reduced disease symptoms, and the effect was enhanced not only in the field trials but also in detached leaves experiments (Tables 1–6). This systemic resistance effect also has been reported in other plant pathosystems, against leaf disease (Gorlach *et al*. 1996; Ishii *et al*. 1999: Bokshi *et al*. 2003). In this study, brown leaf spot disease was effectively reduced by both ASM and chitosan, but not by ASA treatments. Previously Bokshi *et al*. (2003) and Friedrich *et al*. (1996) reported similar effects with the application of benzothiadiazole on *Alternaria* spp. on potato and tobacco, respectively. More recent work done by Hadi and Balali (2010) indicated comparable results, as they reported the number of potato tubers increased with the application of 2 mM salicylic acid to plants that had been infected with *Rhizoctonia solani*.

In our study, the results showed that these two commercially available resistance inducers are able to decrease *A. alternata* disease infection when given as a foliage spray. Despite the good performance of Heads-up in terms of tuber yield production, it seems that this compound was not promising for brown leaf spot disease suppression. The encouraging effect on yield performance might be due to the ability of Heads-up to significantly reduce other disease effects. Our results suggest that both ASM and chitosan treatments against potato brown leaf spot disease may be associated with the activation of some novel defense pathways. Investigation on the interplay between plants and this necrotrophic pathogen show that the relative roles of both plant and pathogen in these interactions are complex and poorly understood. This also has been reported by Liu *et al*. (2011).

Leaf position proved to be significant and had a large effect on the lesion growth rate of *A. alternate*. Leaves from the upper part of the canopy were far more resistant than the lower leaves (Tables 4, 5), irrespective of cultivar and growing conditions (climate-controlled or field location). We have used the detached-leaflet method of disease screening which does not need the extensive space or facility requirements of field or greenhouse screening. Furthermore, this method provides for uniform inoculation of experimental objects with no disease escape. However, there are mixed reports on the reliability of this screening technique and its correlation with field or greenhouse disease data (Goth 1997; Vleeshouwers *et al*. 1999; Visker *et al*. 2003). Our experience with the detached-leaflet screening method indicated that this technique provides a reasonable assessment of brown leaf spot resistance, and could be a reliable screening system. This is in agreement with assessments by Visker *et al*. (2003). They have found that leaf position is the most significant factor in potato resistance to late blight disease. In our study, we found that older leaves in the lower part of the canopy seemed to be more susceptible to brown leaf spot disease than younger leaves in the upper part. This was true, aside from the fact that there are more favorable microclimatic conditions for disease development in the lower parts of the crop canopy.

In general; the results are encouraging, if not consistent. With a greater understanding of the SAR mechanism and the conditions related to the products efficacy, such defense activators may become effective tools for agricultural crop production. The present work suggests that use of ASM and Chitosan to induce host resistance could be considered as a management tool for reducing the impact of *A. alternata* causing brown leaf spot disease on potato.

Although, application of Heads-up had no direct effect on the reduction of brown leaf spot disease index, the field data indicated the potato yield in plots treated with Heads-up was much more than the yield under other treatments. At least at one site of the field experiment (Montcalm Farm), applying Heads up led to achieving the most potato yield production. It may be concluded from the results, that plant defense inducers that are able to induce broad disease resistance, offer an additional option for farmers to complement genetic disease resistance and the use of fungicides.

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