A Three-Way Transcriptomic Interaction Study of a Biocontrol Agent (*Clonostachys rosea*), a Fungal Pathogen (*Helminthosporium solani*), and a Potato Host (*Solanum tuberosum*)

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Helminthosporium solani causes silver scurf, which affects the quality of potato. The biocontrol agent *Clonostachys rosea* greatly limited the severity of silver scurf symptoms and amount of *H. solani* genomic DNA in laboratory experiments. Transcriptomic analysis during interaction showed that *H. solani* gene expression was highly reduced when coinoculated with the biocontrol agent *C. rosea*, whereas gene expression of *C. rosea* was clearly boosted as a response to the pathogen. The most notable upregulated *C. rosea* genes were those encoding proteins involved in cellular response to oxidative stress, proteases, G-protein signaling, and the methyltransferase *LaeA*. The most notable potato response to both fungi was downregulation of defense-related genes and mitogen-activated protein kinase kinases. At a later stage, this shifted, and most potato defense genes were turned on, especially those involved in terpenoid biosynthesis when *H. solani* was present. Some biocontrol-activated defense-related genes in potato were upregulated during early interaction with *C. rosea* alone that were not triggered by *H. solani* alone. Our results indicate that the reductions of silver scurf using *C. rosea* are probably due to a combination of mechanisms, including mycoparasitism, biocontrol-activated stimulation of plant defense mechanisms, microbial competition for nutrients, space, and antibiosis.

Skin blemish diseases in potato (*Solanum tuberosum*) have become increasingly important, because they have an impact on the appearance and quality of washed and prepacked products. The seed- and soilborne fungus *Helminthosporium solani* is the causal agent of silver scurf (Firman and Allen 1995; Jellis and Taylor 1977; Mérida and Loria 1994), and tubers infected with this fungus develop lesions with a silvery appearance due to air pockets under the skin where the fungus has killed the cells. The host range of *H. solani* includes tuber-bearing *Solanum* spp. (Rodríguez et al. 1995). The disease develops initially in the field but fully expands in the warehouse under storage conditions favorable to the spread of the

pathogen (Avis et al. 2010). Silver scurf, originally considered a disease of minor importance, has become a disease with significant economic consequences (Errampalli et al. 2001), particularly because of rejection of ware potato due to reduced skin quality. A recent survey of skin blemish diseases carried out in Norway showed the presence of silver scurf in all lots tested (Nærstad et al. 2012). Control of the disease is difficult due to lack of effective management methods and potato cultivars with adequate resistance to silver scurf.

Biological disease control is an approach that has proven successful in combating some postharvest diseases, and promising results have been obtained with biocontrol treatment of Fusarium dry rot and late blight in potato (Schisler et al. 2000; Slininger et al. 2007, 2010). *Clonostachys rosea* (formerly known as *Gliocladium roseum*) is a soilborne ascomycete known for its mycoparasitic and antagonistic abilities against a wide range of plant pathogens and nematodes (Luongo et al. 2005; Rodríguez et al. 2011; Sutton et al. 1997; Zhang et al. 2008). Both antifungal secondary metabolites and mycoparasitism has been suggested to be the mode of action of *C. rosea* as a biocontrol agent (Rodríguez et al. 2011). Also, indirect effects such as induction of host defense response as a result of root surface colonization have been proposed as biocontrol mechanisms (Dubey et al. 2013; Roberti et al. 2008). Tolerance toward secondary metabolites also seem to be a prominent feature in the biology of *C. rosea* (Karlsson et al. 2015). The *C. rosea* strain IK726 was originally isolated from roots of barley in Denmark (Knudsen et al. 1995) and has subsequently been shown to be a potential biocontrol agent against diverse plant pathogens including *Alternaria* spp., *Botrytis cinera*, *Fusarium culmorum*, and *Pythium tracheiphilum* (Jensen et al. 2000; Koch et al. 2010; Mamarabadi et al. 2008; Møller et al. 2003).

We found that *C. rosea* could limit the severity of silver scurf on potato, and our goal was to study the molecular mechanisms in this interaction. Transcriptomics allowed us to identify differentially expressed transcripts in *C. rosea* that could be involved in biocontrol activity of the pathogen, pathogenicity factors from the pathogen *H. solani* that could be important for disease development, and potato response to the two microorganisms. For the potato host, we were especially interested to find out if any biocontrol-activated resistance was present, as reported by Dubey et al. (2013), that showed that *Trichoderma atroviride* induced systemic defense in *Arabidopsis thaliana* against *B. cinerea*.

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*The e-Xtra logo stands for “electronic extra” and indicates that seven supplementary files are published online.

RESULTS AND DISCUSSION

To identify expressed genes in a three-way interaction of *S. tuberosum*, *H. solani*, and *C. rosea*, potato tubers were inoculated with a mixture of biocontrol agent and pathogen, with biocontrol agent or pathogen separately, or with water (mock control). Samples were collected at 7, 14, and 28 days postinoculation (dpi), and RNA-seq analyses were performed. RNA-seq of material with several species are complex, and many similar genes coming from the three different organisms were found in the transcriptomes, especially genes involved in primary metabolism. There potato sequence reads are over-represented in the material, and only a few were from the microorganisms. Because some of the genes in *C. rosea* and *H. solani* have a high degree of sequence similarity, incorrect RNA-seq expression results may occur. To avoid false positives in the interaction study where both fungi were present, transcripts that gave expression results to a fungus that was not present in the specific sample or to potato control were removed from all samples, including those that contained the specific fungus.

H. solani growth and development is suppressed when together with the biocontrol agent *C. rosea* on potato host.

Tubers inoculated with only *H. solani* were covered with 90% silver scurf after 50 days (Fig. 1), whereas no symptoms of silver scurf were observed upon sampling, 7, 14, and 28 dpi (results not shown). *H. solani* growth was clearly suppressed when inoculated together with *C. rosea*, and the tuber surfaces inoculated with a mixture of *C. rosea* and *H. solani* contained less than 5% of silver scurf after 50 days (Fig. 1). This reduction of *H. solani* was reflected in the gene expression results and amount of *H. solani* DNA detected in the different samples. When together with *C. rosea*, no significant ($P < 0.05$) *H. solani* gene expression was observed at 7 dpi, and a dramatic reduction of active genes was observed at 14 dpi (98%) and 28 dpi (58%) compared with when *H. solani* was the only inoculant (Fig. 2A). These results were also substantiated by the amount of detected *H. solani* DNA that was greatly reduced in the presence of *C. rosea* (Fig. 2B).

Fig. 1. Appearance of Berber potato tubers 50 days after treatment with **A**, water (mock control); **B**, *Clonostachys rosea*; **C**, *C. rosea* and *Helminthosporium solani*; and **D**, *H. solani*. No symptoms were observed on the tubers inoculated with water (A) or the biocontrol agent *C. rosea* (B). Growth of *H. solani* was suppressed when inoculated together with *C. rosea* and less than 5% of the tuber surface exhibited symptoms of silver scurf after 50 days (C). Tubers inoculated with only *H. solani* were covered with 90% silver scurf after 50 days (D).

Gene expression in *H. solani* during infection of potato.

Gene prediction, annotation, and expression results of *H. solani* are found in Supplementary Files S2 and S3. The number of expressed genes detected in *H. solani* during infection increased from 154 at 7 dpi to 6,481 at 28 dpi (Fig. 2A). The highest expressed *H. solani* gene at 7 dpi is *Mmc* (g5187), similar to fungal genes involved in the process of microcycle conidiation (MC) (Table 1). This process is a survival

Fig. 2. **A**, Number of expressed genes detected in *Clonostachys rosea* and *Helminthosporium solani* at 7, 14, and 28 days postinoculation (dpi) on potato tubers. C = *C. rosea* alone, H = *H. solani* alone; and CH = *C. rosea* and *H. solani* together. **B**, Relative DNA concentration of *H. solani* and *C. rosea* normalized against potato cytochrome c oxidase gene $\pm$ standard error.

mechanism for some fungi encountering unfavorable conditions, in which asexual spores germinate by direct formation of conidia without the intervention of mycelial growth. MC is not a common mechanism in plant pathogens on their favorite hosts but is suggested to be a mechanism to survive under stressful conditions and on nontarget hosts (Jung et al. 2014). In many plant pathogens such as *F. graminearum* and *F. verticilloides*, MC is normally suppressed (Jung et al. 2014). MC occurs directly after germination of conidia without or with greatly reduced mycelial growth (Liu et al. 2010), and this suggests that some spores of *H. solani* germinated earlier than 7 dpi. The expression values of g5187 were relatively high at all three time points during infection but decreased slowly over time, suggesting that MC is more common early in the infection phase of potato. *H. solani* develops slowly on potato and the silvery lesions typically enlarge during storage (Errampalli et al. 2001). Because MC is more common in unfavorable conditions, this could suggest that potato tubers are an unfriendly environment during early *H. solani* establishment. MC in *H. solani* could also be triggered by nutrient composition of potato (e.g., the high content of starch). Media with glucose and starch stimulated MC development in *Aspergillus* spp. (Saxena et al. 1992).

Another highly expressed gene in *H. solani* encodes a putative peptide transporter (PTR2; g9044), and this was particularly upregulated at 7 and 14 dpi. Pathogenic fungi experience nutrient deficiency during the colonization of their hosts and different nutrient supply mechanisms may operate at different stages of the fungal lifecycle. Organic nitrogen sources such as oligopeptides, dipeptides, and amino acids are taken up from

the environment by oligopeptide transporters, di- or tripeptide transporters (Hauser et al. 2001), and amino acid transporters (Sophianopoulou and Diallinas 1995). This suggests that *H. solani* suffers from nutrient deficiency at early time points in colonizing potato, and the peptide transporter is less needed at later stages. Peptide transporters could also influence secondary metabolite production and, thus, be involved in pathogenicity (Droce et al. 2017).

The majority of the *H. solani* genes expressed at all time points during infection were related to primary metabolism but several potential pathogenicity factors were present. Approximately 7 to 8% of all *H. solani* genes expressed at all time points are encoding potential hydrolase activity, with approximately 30% of these being glucoside hydrolases. These results support the suggestions by Mattupalli et al. (2014), that hydrolases have an active role in pathogenicity of *H. solani*. The genome of *H. solani* possess a single gene encoding a necrosis- and ethylene-inducing peptide 1 (Nep1) (Mattupalli et al. 2014), and this gene (g8075) was expressed at 14 and 28 dpi in *H. solani* during infection of potato (Table 1). Peptide fragments of Nep1-like proteins, occurring in diverse microorganisms of three different kingdoms of life, were found to trigger immunity in the model plant *Arabidopsis*, indicating that they act as a microbe-associated molecular pattern (Oome et al. 2014). These proteins have a common necrosis-inducing *Phytophthora* protein domain, and have been found to accelerate plant cell death, disease, and pathogen growth in several plant hosts (Gijzen and Nürnberger 2006). This indicates a putative role in pathogenicity but also emphasizes the slow development

Table 1. Putative pathogenicity factor genes of *Helminthosporium solani* expressed at 7, 14, and 28 days postinoculation^a

Gene	Annotation	H7	H14	H28	CH7	CH14	CH28
g5187	<i>Mmc</i> ; microcycle conidiation	13,351 ± 1,689	7,002 ± 721	5,850 ± 385	3,548 ± 3,548 (A)	1,866 ± 1,082 (B)	5,745 ± 286
g6982	Deuterolysin metalloprotease	4,828 ± 1,737	6,871 ± 2,207	1,858 ± 329	0	1,833 ± 723	2,269 ± 239
g3300	Elastinolytic metalloprotease	1,851 ± 725	4,190 ± 553	4,708 ± 790	3,361 ± 3,361 (A)	2,271 ± 399	5,471 ± 184
g9044	PTR2 peptide transporter	1,805 ± 267	1,887 ± 432	338 ± 31	955 ± 955 (A)	413 ± 242 (B)	468 ± 90
g8679	Subtilisin-like serine protease	1,190 ± 363	545 ± 152	602 ± 48	152 ± 152 (A)	497 ± 300 (B)	717 ± 95
g2104	Glycoside hydrolase	856 ± 341	555 ± 143	439 ± 40	161 ± 161 (A)	107 ± 107 (A)	602 ± 83
g8075	Necrosis- and ethylene-inducing peptide 1 (Nep1)-like	0	232 ± 86	219 ± 32	0	0	383 ± 112
g4420	Glutathione s-transferase	840 ± 218	269 ± 114	132 ± 10	0	0	106 ± 50
g8791	Siderophore biosynthesis	797 ± 277	220 ± 88	74 ± 34	0	0	115 ± 68
g6875	Heat-shock protein	648 ± 224	229 ± 38	232 ± 34	0	0	254 ± 51
g10061	Woronin body major protein	643 ± 253	312 ± 94	446 ± 32	0	0	450 ± 51
g3344	Glycoside hydrolase	542 ± 183	372 ± 146	269 ± 30	0	114 ± 114 (A)	222 ± 67
g3508	Barwin domain protein	541 ± 143	467 ± 106	319 ± 47	0	0	372 ± 82
g8281	Endochitinase 1 precursor	490 ± 192	224 ± 54	48 ± 7	0	0	62 ± 27
g8039	Tripeptidyl-peptidase 1 precursor	468 ± 174	220 ± 39	207 ± 29	0	88 ± 88 (A)	243 ± 35
g8622	Peptidase s33 tripeptidyl aminopeptidase	467 ± 187	118 ± 40	49 ± 21	0	0	16 ± 10
g3911	Thioesterase family protein	464 ± 91	661 ± 195	637 ± 31	1,524 ± 1524 (A)	1,524 ± 1524 (A)	739 ± 90
g8070	Methyltransferase	445 ± 149	282 ± 242	77 ± 26	0	469 ± 469 (A)	41 ± 24
g1130	Carbohydrate esterase	443 ± 142	107 ± 22	494 ± 91	919 ± 919 (A)	86 ± 86 (A)	406 ± 60
g5760	Heat-shock protein	412 ± 139	1,748 ± 146	132 ± 5	0	414 ± 272 (B)	197 ± 32
g2782	Heat-shock protein	410 ± 74	1,929 ± 122	39 ± 4	89 ± 89 (A)	826 ± 284	21 ± 15
g8418	Glutamine synthetase	386 ± 137	666 ± 96	301 ± 32	0	0	414 ± 31
g7083	Catalase-peroxidase	379 ± 65	367 ± 46	538 ± 63	0	0	398 ± 52
g9643	Dipeptidyl aminopeptidase	332 ± 41	242 ± 30	376 ± 48	0	0	358 ± 47
g4598	Aminopeptidase	295 ± 118	166 ± 124	45 ± 12	0	80 ± 80 (A)	82 ± 13
g3617	APSES transcription factor StuA	267 ± 105	97 ± 56	111 ± 17	0	0	66 ± 36
g4756	Glycoside hydrolase	201 ± 39	55 ± 24	65 ± 13	0	0	73 ± 19
g5509	Peptidase	170 ± 66	147 ± 58	120 ± 26	0	0	95 ± 34
g7768	Glutamate carboxypeptidase	105 ± 38	72 ± 40	120 ± 12	0	0	110 ± 28
g3689	Glutamate synthase	88 ± 17	84 ± 28	38 ± 2	0	44 ± 44 (A)	61 ± 12
g9412	Polyketide synthase	31 ± 12	59 ± 20	48 ± 8	0	0	42 ± 5
g3526	Nonribosomal peptide synthetase	25 ± 8	9 ± 5	6 ± 1	0	0	6 ± 2

^a Expression values are mean reads per kilobase per million mapped reads from four replicates ± standard error. H = *H. solani* alone on potato and CH = *H. solani* together with *Clonostachys rosea*. Significant gene expression between samples and noninoculated control were analyzed by *t* tests, and upregulated genes were considered to be significant if they were equal to or greater than twofold ($P < 0.05$). Samples with nonsignificant gene expression: A = only one of four samples showed expression and B = only two of four samples showed expression.

of *H. solani* within the potato host, because expression of this gene is not detected at 7 dpi.

A subtilisin-like serine protease gene (g8679) was highly expressed in *H. solani* at all time points during infection, suggesting a role in pathogenicity. Several other proteases (g3300, g4598, g5509, g6982, g7768, and g8622) were also expressed. Proteases constitute a significant part of many fungal secretomes and play an important role in the fungal infection process of plants, nematodes, and insects (Li et al. 2010; Muszewska et al. 2011; Rovenich et al. 2014). The *StuA* gene in *H. solani* (g3617) was expressed significantly at all time points of infection. The APSES family protein *StuA* belongs to a conserved class of transcription factors that are unique to fungi, and are known to regulate key developmental processes in ascomycetes (Zhao et al. 2015). *StuA* proteins are involved in pathogenicity in several fungi (García-Pedrajas et al. 2010; Lysøe et al. 2011; Tong et al. 2007).

Several other genes encoding potential pathogenicity factors such as heat-shock proteins (g2782, g5760, and g6875), an endochitinase (g8281), a polyketide synthase (g9412), and a non-ribosomal peptide synthetase (g3526) were also expressed in *H. solani* during infection (Table 1).

Gene expression in the biocontrol agent *C. rosea* was boosted upon coinoculation with the pathogen *H. solani*.

The amount of *C. rosea* DNA was not significantly influenced at 7, 14, and 28 dpi by the presence of *H. solani* (Fig. 2B). However, the detected gene expression in *C. rosea* was clearly boosted at 7 and 14 dpi when it was inoculated together

with *H. solani* (CH), compared with when *C. rosea* was the only inoculant (C), indicating a direct effect of the biocontrol agent on the pathogen (Fig. 2A). The most upregulated *C. rosea* gene at CH7 compared with C7 encodes a putative S-glutathione synthase (BN869_T00005941_1) (Fig. 3), a gene that was expressed in only one of four biological replicates at C7 (Supplementary File S4). Glutathione is an important antioxidant in fungi and other organisms, preventing damage to cellular components caused by reactive oxygen species (ROS) (Pompella et al. 2003). Other genes from *C. rosea* potentially involved in cellular response to oxidative stress were also upregulated at CH7, such as genes encoding methionine-R-sulfoxide reductase (BN869_T00006749_1) and α -trehalose-phosphate synthase (BN869_T00002672_1). These upregulated ROS defense genes in *C. rosea* were most probably due to increased host defense response against *C. rosea* because of mycoparasitism activity toward *H. solani*. One of the *C. rosea* genes with the highest expression during mycoparasitism encodes a putative methyltransferase (BN869_T00005904_1), similar to the global epigenetic regulator *Lae1/LaeA* found in *Trichoderma*, *Fusarium*, *Aspergillus*, and numerous other species of Ascomycetes. *Lae1/LaeA* is known to regulate several activities, such as secondary metabolism (Bok and Keller 2004), asexual development and mycoparasitism (Aghchegh et al. 2013), and expression of cellulases and hemicellulases (Seiboth et al. 2012) as well as virulence (Wiemann et al. 2010). The *Lae1* ortholog of *C. rosea* had no significant expression in C7 (expressed in only 1/4 of the replicates) and low expression at C14. During the interaction with *H. solani*, it was high and stably expressed at all time points (Fig. 3), supporting a possible function in mycoparasitism.

Fig. 3. Relative gene expression (reads per kilobase per million mapped reads [RPKM]) from four biological replicates $\pm$ standard error in *Clonostachys rosea* after interaction with potato tubers for 7, 14, and 28 days. Genes encoding S-glutathione synthase (BN869_T00005941_1), methyltransferase *LAE1* (BN869_T00005904_1), Subtilin-like serine protease (BN869_T00008490_1), and regulator of G-protein signaling (BN869_T00004558_1). C = *C. rosea* alone and CH = *C. rosea* with *Helminthosporium solani*.

Several protease genes were upregulated in *C. rosea* when interacting with *H. solani* and, thus, the gene ontology (GO) category “peptidase activity” (GO:0008233) was significantly enriched ($P = 5.9\text{E-}6$) in *C. rosea* CH7 compared with C7. Previous studies demonstrate the role of extracellular proteases in a number of fungal biocontrol agents: *T. virens*, *T. harzianum*, *T. asperellum*, *T. flavus*, and *Verticillium biguttatum* that parasitize pathogenic fungi or oomycetes such as *Rhizoctonia solani*, *F. oxysporum*, *B. cinerea*, *S. sclerotiorum*, or *P. ultimum* (Daguette et al. 2014). One example in *C. rosea* is a gene encoding a subtilisin-like serine protease (BN869_T00008490_1) that was clearly upregulated after 7 days of incubation when coinoculated with *H. solani* (CH7) (Fig. 3). A subtilisin-like serine protease from the biocontrol agent *T. harzianum* T88 has previously been found to exert broad-spectrum antifungal activity against *Alternaria alternata*, *F. oxysporum*, *R. solani*, *S. sclerotiorum*, and *Cytospora chrysosperma* (Yan and Qian 2009).

A gene encoding a putative regulator of G-protein signaling in *Clonostachys rosea* (BN869_T00004558_1) was clearly upregulated at early stages (CH7 and CH14) during coinoculation with *H. solani* compared with when *C. rosea* was the only inoculant (Fig. 3), supporting a role in mycoparasitism. The mycoparasitic attack involves cross-talk between host and pathogen, with similar processes as described for other pathogenic fungi such as infection-related morphogenesis, the production of hydrolytic enzymes involved in host invasion, and mycotoxin synthesis (Omann and Zeilinger 2010). G-protein signaling plays an essential role in the different processes, and

is believed to be involved in mycoparasitism in *Trichoderma* spp. by regulating antifungal compounds and cell-wall-degrading enzymes (do Nascimento Silva et al. 2009; Reithner et al. 2005).

Transcriptomic response of potato tubers to microorganisms and *C. rosea* biocontrol-activated defense against the pathogen *H. solani*.

Hierarchical clustering of the whole potato transcriptome showed that tubers inoculated with *C. rosea* clustered with the mock-inoculated potato control at all time points (Fig. 4A). In the potato-*C. rosea* interaction, most differentially expressed (up- and downregulated) potato genes were detected relatively early (7 dpi). The potato response decreased three- to fourfold with respect to the number of genes differentially expressed at 14 and 28 dpi (Fig. 4B). Overall, this suggests that the biocontrol agent does not significantly influence potato gene expression over time.

One of the goals of this work was to investigate whether *C. rosea* could activate defense genes in potato, as reported by Dubey et al. (2013), and that seem to be the case. *C. rosea* alone (or together with *H. solani*) activated several genes in potato at 7 dpi, which were not activated by *H. solani* alone (Table 2). Most of these genes are typically connected to plant defense, such as those annotated as pathogenicity related genes, lipid transporters (Kader 1996; Salcedo et al. 2007), glucan endo-1,3- β -glucosidase (Meirinho et al. 2010), and drug resistance (Table 2). These results clearly strengthen the hypothesis that *C. rosea* could activate resistance in potato.

Fig. 4. Potato transcriptome in response to inoculation of potato tubers with *Helminthosporium solani* (H) or *Clonostachys rosea* (C) after incubation for 7, 14, and 28 days. K = mock-inoculated controls. **A**, Hierarchical clustering of the transcriptome. **B**, Venn diagrams indicating number of significant up- and downregulated genes compared with mock-inoculated controls. Significant gene expression between samples and noninoculated control were analyzed by *t* tests, and upregulated genes were considered to be significant if they were equal to or greater than twofold ($P < 0.05$).

Expression of potato genes after inoculation with the pathogen *H. solani* clustered with the mock-inoculated control at 7 dpi but clearly separated from the control at 14 and 28 dpi (Fig. 4A). The potato transcriptome shifted from a situation where most genes were downregulated at 7 dpi (H7), toward most genes upregulated at 28 dpi (H28) (Supplementary File S5). A noticeable result is that many of the GO categories enriched among the downregulated genes at H7 were also enriched among the upregulated genes at H28. Examples of such genes are those involved in potato defense response, protein phosphorylation, phosphorus metabolic process, phosphate-containing compound metabolic process response to biotic stimulus, cell recognition, response to stress, multiorganism process, response to stimulus, response to external biotic stimulus, and response to other organisms (Supplementary File S6). This major shift in gene expression is most likely due to regulation of signaling pathways. A pronounced downregulation of genes encoding mitogen-activated protein kinases (MAPK) in potato, especially MAPK kinase kinase (MAPKKK), was found at 7 dpi in the presence of C, H, and CH (Fig. 5A). At 14 dpi, downregulation of MAPK was observed only in the samples inoculated with H and CH. MAPK-mediated signaling is an essential step in establishing resistance to pathogens (Pitzschke et al. 2009). The potato defense response seemed to be suppressed by *C. rosea* and *H. solani* at 7 dpi, possibly through fungal host regulation by effector proteins (Pitzschke et al. 2009; Rovenich et al. 2014). For potato tubers inoculated with *C. rosea*, the downregulation of most genes ceased at 14 dpi, whereas genes encoding MAPK in the tuber samples coinoculated with *H. solani* continued to be downregulated. To examine this in detail, we did real-time quantitative polymerase chain reaction (qPCR) analysis of a selected *MAPKKK* gene (PGSC0003DMG402008032) that had an expression pattern typical for all MAPK, including also earlier time points after inoculation. The results clearly verified the RNA-seq results, and showed that downregulation of this *MAPKKK* occurred very early (1 dpi) after inoculation with *H. solani* (Fig. 5B). Interestingly, the gene encoding the ethylene-responsive transcription factor (PGSC0003DMG400017233) followed exactly the *MAPKKK* expression pattern, suggesting that there is a tight connection between these genes or their products (Fig. 5C), and that MAPK cascades are involved in ethylene signaling or biosynthesis, as discussed by (Hahn and Harter 2009). Ethylene regulates a large number of plant processes, including defense against pathogens, and the observed downregulation in

response to *H. solani* infection might be a result of effector-mediated manipulation of the ethylene pathway (Kazan and Lyons 2014). Our results differ from a study done on tomato by Mouekouba et al. (2014), where expression of MAPK in *Solanum lycopersicum* was upregulated in the presence of *C. rosea*. This could be explained by differences in host pathosystem, tissue used, and time frame of the experiment (Kazan and Lyons 2014).

A pronounced upregulation of genes encoding enzymes involved in the terpenoid biosynthesis via the methylerythritol 4-phosphate pathway was observed 28 dpi with *H. solani* and partly after mixed inoculation with *H. solani* and *C. rosea* (Fig. 6). Plants contain a wide range of terpenoid (isoprenoid) compounds with functions in primary and secondary metabolism, and these contribute to plant defense (Vranová et al. 2012). *H. solani* had the highest numbers of detected genes 28 dpi (Fig. 2A) and concentration of DNA (Fig. 2B), and upregulation of methylerythritol 4-phosphate pathway is probably a direct response to the pathogen.

To validate some of the RNA-seq data, we used real-time qPCR on selected genes from potato, *C. rosea*, and *H. solani* (Supplementary File S7). These results show that all selected genes are expressed and, therefore, support the findings from RNA-seq. For potato and *H. solani*, the same expression patterns were found by qPCR as by RNA-seq; however, some of the selected genes from *C. rosea* showed a slightly different expression pattern in the qPCR compared with RNA-seq results.

Conclusions.

Silver scurf of potato caused by *H. solani* is highly reduced when inoculated together with the biocontrol agent *C. rosea*. The reductions of symptoms are probably due to a combination of mechanisms such as a direct biocontrol effect through mycoparasitism and indirect with *C. rosea* stimulation of plant defense mechanisms and microbial competition for nutrients, space, and antibiosis.

MATERIALS AND METHODS

Fungal strains.

C. rosea isolate IK726 was obtained from Swedish University of Agricultural Sciences. The isolate has been evaluated extensively for its biocontrol efficiency (Jensen et al. 2007).

Table 2. The most upregulated biocontrol-activated genes putatively involved in defense in potato at 7 days postinoculation^y

Potato CDS	Putative function ^z	K7	H7	C7	CH7
PGSC0003DMC400051612	PDR protein 1	2.6 ± 1.7	0.5 ± 0.2	17.9 ± 5.0	13.1 ± 3.2
PGSC0003DMC400056120	Fatty acid	4.4 ± 3.0	3.3 ± 2.2	64.4 ± 20.2	61.1 ± 11.3
PGSC0003DMC400033771	PR protein	20.1 ± 11.3	8.4 ± 3.5	106.1 ± 37.3	86.6 ± 22.1
PGSC0003DMC400033772	PR protein	33.6 ± 16.9	12.0 ± 5.4	145.1 ± 49.8	125.5 ± 33.3
PGSC0003DMC400043059	PR protein	24.2 ± 3.8	11.2 ± 2.0	50.7 ± 20.2	62.2 ± 9.5
PGSC0003DMC400051976	Glucan endo	11.9 ± 6.1	7.6 ± 2.3	54.3 ± 13.2	54.3 ± 13.5
PGSC0003DMC400022730	NLT protein 2	6.7 ± 1.5	6.9 ± 1.7	7.8 ± 0.7	43.0 ± 11.6
PGSC0003DMC400054441	NLT protein 2	18.8 ± 22.7	22.7 ± 3.6	24.9 ± 3.7	137.6 ± 36.2
PGSC0003DMC400023388	Osmotin-like protein	0.4 ± 0.24	0.32 ± 0.24	4.2 ± 1.19	3.45 ± 1.16
PGSC0003DMC400001479	Patatin-like protein	1.43 ± 0.50	0.47 ± 0.15	5.47 ± 0.39	4.13 ± 0.74
PGSC0003DMC400005489	Trypsin inhibitor	1.77 ± 0.55	0.93 ± 0.67	8.48 ± 3.21	10.43 ± 2.77
PGSC0003DMC400024677	Cytochrome p450	5.71 ± 1.59	2.39 ± 0.45	13.44 ± 4.58	15.35 ± 1.97
PGSC0003DMC400004343	Extensin-like protein	2.8 ± 0.61	2.45 ± 0.68	13.36 ± 4.98	12.74 ± 1.95

^y K7 = mock-inoculated potato (control), H7 = potato inoculated with *Helminthosporium solani*, C7 = potato inoculated with *Clonostachys rosea*, and CH7 = potato inoculated with both *C. rosea* and *H. solani*. Expression values are mean reads per kilobase per million mapped reads from four biological replicates ± standard error. Significant gene expression between samples and noninoculated control were analyzed by *t* tests, and upregulated genes were considered to be significant if they were equal to or greater than twofold ($P < 0.05$).

^z PDR = pleiotropic drug resistance, Fatty acid = omega-6 fatty acid desaturase, PR = pathogenesis related, Glucan endo = glucan endo-1,3-β-glucosidase, and NLT = nonspecific lipid transfer.

H. solani N09-HS070 was isolated during a survey of skin blemish diseases in Norway and was obtained from our culture collection (Nærstad et al. 2012).

Inoculation of potato with *C. rosea* and *H. solani*.

For production of inoculum, *C. rosea* IK726 and *H. solani* N09-HS070 were grown on potato dextrose agar plates at room temperature (approximately 20°C) for 14 days and 8 weeks, respectively. The inocula were produced by suspending the

spores in sterile distilled water to spore concentrations of 100,000 spores/ml for *C. rosea* and 10,000 spores/ml for *H. solani*. 'Berber' potato was surface sterilized before inoculation with four different treatments: (i) *H. solani* (H), (ii) *C. rosea* (C), (iii) a 50:50 mixture of *C. rosea* and *H. solani* (CH), and (iv) sterile distilled water (mock control). The tubers were tumbled in a plastic bag with 100 ml of spore suspension for 1 min and placed on metal or plastic grids in trays lined with moist filter paper. The trays were covered with black plastic and

Fig. 5. Downregulation of potato mitogen-activated protein kinases (MAPK) and ethylene-responsive transcription factor 7 and 14 days postinoculation (dpi) with *Helminthosporium solani* and *Clonostachys rosea*. **A**, Summary of relative gene expression from RNA-seq (reads per kilobase per million mapped reads RPKM) for all potato genes encoding MAPK. Significant gene expression between samples and noninoculated control were analyzed by *t* tests, and upregulated genes were considered to be significant if they were equal to or greater than twofold ($P < 0.05$). **B** and **C**, Relative gene expression of a single MAPK kinase kinase (MAPKKK) gene (PGSC0003DMG402008032) and an ethylene-responsive transcription factor gene (PGSC0003DMG400017233) measured by real-time quantitative polymerase chain reaction (qPCR) and normalized to the potato actin gene (PGSC0003DMG400003985). For the qPCR results, we also included other time points; however, experiments with samples K3 and CH3 were not performed and, hence, could not be included in the figure. K = mock control, C = *C. rosea*, and CH = *C. rosea* and *H. solani*. Numbers indicate days postinoculation and error bars = standard error.

incubated at 16°C in the dark up to 50 days. Tubers were investigated for presence of conidiophores from *H. solani* at each harvest point (see below) and after 50 days of incubation.

RNA and DNA sample preparation and sequence analysis.

The genome sequences of *S. tuberosum*, *C. rosea*, and *H. solani* are available. For RNA-seq, four biological samples (potato peel) were taken from each treatments 7, 14, and 28 dpi. For isolation of DNA and RNA for qPCR, other time points were also included. The tubers were peeled immediately and the peels were snap frozen in liquid nitrogen and stored at -80°C. RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis) and DNA was extracted using the Qiagen DNeasy plant mini kit (Qiagen, Valencia, CA, U.S.A.). RNA and DNA qualities were determined using the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, U.S.A.), and RNA qualities were determined by the Agilent 2100 Bioanalyzer (Santa Clara, CA, U.S.A.). Sequencing was performed at the Norwegian Sequencing Center (Oslo, Norway) on the Illumina HiSeq platform using the 100-bp paired-end protocol. The sequences are available in The European Nucleotide Archive under the project identification number PRJEB8925.

Reads were trimmed and RNA-seq analysis for all 48 samples was performed with CLC Genomics Workbench v8.0 (CLC Bio, Aarhus, Denmark) using genes from the three organisms as templates. For the transcriptome of *H. solani*, gene prediction of the assembly AWWW01000000 (Mattupalli et al. 2014) was carried out using Augustus v.3.03 (Stanke et al. 2004), with *Fusarium graminearum* as a training set. For the transcriptome of *S. tuberosum* and *C. rosea*, we used PGSC_DM_v3.4_cds.fasta (<http://solanaceae.plantbiology.msu.edu>) and CrosV1.transcripts.fasta (Karlsson et al. 2015), respectively, as templates. RNA-seq analysis for potato was done using default settings. For the two microorganisms, we adjusted similarity fraction to 0.9 (default = 0.8) in CLC Genomics Workbench to get more stringent conditions. Gene expression was calculated using the reads per kilobase per million mapped reads method. Differential expression between samples was analyzed by *t* tests, and up- or downregulated genes were considered to be significant if they were equal to or greater than twofold ($P < 0.05$). The RNA-seq gene expression results for the microorganisms were examined manually and had 99 to

100% match to the nucleotide gene sequences from the respective fungus. The software Venny (Oliveros 2015) was used to make Venn diagrams of the differentially expressed transcripts. Functional annotation and GO enrichment analysis of all three transcriptomes were performed using Blast2GO (Conesa et al. 2005).

To validate the results from the RNA-seq, real-time qPCR was performed on selected genes from *C. rosea*, *H. solani*, and *S. tuberosum*. CLC Main Workbench v7 (CLC Bio, Aarhus, Denmark) was used to design primers for selected targets and housekeeping genes. The PCR (20 µl) contained 500 nM each primer, 2 µl of cDNA, and 10 µl of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, U.S.A.). qPCR analysis was performed in two technical replicates for each of the three biological replicates using the real-time instrument CFX96 (Bio-Rad). Genomic DNA and water were included as positive and negative controls, respectively, for each primer pair. Melting curve analysis was carried out to evaluate undesired amplifications and signals from primer dimers. To normalize target gene expression, we used actin housekeeping gene for all three organisms: *H. solani* (g1275), *C. rosea* (BN869_T00009809_1), and *S. tuberosum* (PGSC0003DMC400007099). Normalized and relative gene expression were analyzed using Bio-Rad CFX manager 3.1.

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LITERATURE CITED

- Aghchegh, R. K., Druzhinina, I. S., and Kubicek, C. P. 2013. The putative protein methyltransferase LAE1 of *Trichoderma atroviride* is a key regulator of asexual development and mycoparasitism. *PLoS One* 8: e67144.
- Avis, T. J., Martinez, C., and Tweddell, R. J. 2010. Integrated management of potato silver scurf (*Helminthosporium solani*). *Can. J. Plant Pathol.* 32:287-297.
- Bok, J. W., and Keller, N. P. 2004. LaeA, a regulator of secondary metabolism in *Aspergillus* spp. *Eukaryot. Cell* 3:527-535.
- Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., and Robles, M. 2005. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21: 3674-3676.
- Daguerrre, Y., Siegel, K., Edel-Hermann, V., and Steinberg, C. 2014. Fungal proteins and genes associated with biocontrol mechanisms of soil-borne pathogens: A review. *Fungal Biol. Rev.* 28:97-125.
- do Nascimento Silva, R., Steindorff, A. S., Ulhoa, C. J., and Félix, C. R. 2009. Involvement of G-alpha protein GNA3 in production of cell wall-degrading enzymes by *Trichoderma reesei* (*Hypocrea jecorina*) during mycoparasitism against *Pythium ultimum*. *Biotechnol. Lett.* 31: 531-536.
- Droce, A., Sørensen, J. L., Sondergaard, T. E., Rasmussen, J. J., Lysøe, E., and Giese, H. 2017. PTR2 peptide transporters in *Fusarium graminearum* influence secondary metabolite production and sexual development. *Fungal Biol.* 121:515-527.
- Dubey, M. K., Broberg, A., Jensen, D. F., and Karlsson, M. 2013. Role of the methylcitrate cycle in growth, antagonism and induction of systemic defence responses in the fungal biocontrol agent *Trichoderma atroviride*. *Microbiology* 159:2492-2500.
- Errampalli, D., Saunders, J. M., and Holley, J. D. 2001. Emergence of silver scurf (*Helminthosporium solani*) as an economically important disease of potato. *Plant Pathol.* 50:141-153.
- Firman, D. M., and Allen, E. J. 1995. Transmission of *Helminthosporium solani* from potato seed tubers and effects of soil conditions, seed inoculum and seed physiology on silver scurf disease. *J. Agric. Sci.* 124: 219-234.
- García-Pedrajas, M. D., Baeza-Montañez, L., and Gold, S. E. 2010. Regulation of *Ustilago maydis* dimorphism, sporulation, and pathogenic

Fig. 6. Potato terpenoid biosynthesis is upregulated when *Helminthosporium solani* is present at 28 days postinoculation. Summary of relative gene expression (reads per kilobase per million mapped reads [RPKM]) from four biological replicates $\pm$ standard error for all methylerythritol 4-phosphate genes in potato. Significant gene expression between samples and non-inoculated control were analyzed by *t* tests, and upregulated genes were considered to be significant if they were equal to or greater than twofold ($P < 0.05$). K = mock control, C = *Clonostachys rosea*, and CH = *C. rosea* and *H. solani*.

- development by a transcription factor with a highly conserved APSES domain. *Mol. Plant-Microbe Interact.* 23:211-222.
- Gijzen, M., and Nürnberger, T. 2006. Nep1-like proteins from plant pathogens: Recruitment and diversification of the NPP1 domain across taxa. *Phytochemistry* 67:1800-1807.
- Hahn, A., and Harter, K. 2009. Mitogen-activated protein kinase cascades and ethylene: Signaling, biosynthesis, or both? *Plant Physiol.* 149: 1207-1210.
- Hauser, M., Narita, V., Donhardt, A. M., Naider, F., and Becker, J. M. 2001. Multiplicity and regulation of genes encoding peptide transporters in *Saccharomyces cerevisiae*. *Mol. Membr. Biol.* 18:105-112.
- Jellis, G. J., and Taylor, G. S. 1977. The development of silver scurf (*Helminthosporium solani*) disease of potato. *Ann. Appl. Biol.* 86: 19-28.
- Jensen, B., Knudsen, I. M. B., and Jensen, D. F. 2000. Biological seed treatment of cereals with fresh and long-term stored formulations of *Clonostachys rosea*: Biocontrol efficacy against *Fusarium culmorum*. *Eur. J. Plant Pathol.* 106:233-242.
- Jensen, D. F., Knudsen, I. M. B., Lübeck, M., Mamarabadi, M., Hockenhull, J., and Jensen, B. 2007. Development of a biocontrol agent for plant disease control with special emphasis on the near commercial fungal antagonist *Clonostachys rosea* strain 'IK726'. *Australas. Plant Pathol.* 36:95-101.
- Jung, B., Kim, S., and Lee, J. 2014. Microcycle conidiation in filamentous fungi. *Mycobiology* 42:1-5.
- Kader, J. C. 1996. Lipid-transfer proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:627-654.
- Karlsson, M., Durling, M. B., Choi, J., Kosawang, C., Lackner, G., Tzelepis, G. D., Nygren, K., Dubey, M. K., Kamou, N., Levasseur, A., Zapparata, A., Wang, J., Amby, D. B., Jensen, B., Sarrocco, S., Panteris, E., Lagopodi, A. L., Pöggeler, S., Vannacci, G., Collinge, D. B., Hoffmeister, D., Henrissat, B., Lee, Y. H., and Jensen, D. F. 2015. Insights on the evolution of mycoparasitism from the genome of *Clonostachys rosea*. *Genome Biol. Evol.* 7:465-480.
- Kazan, K., and Lyons, R. 2014. Intervention of phytohormone pathways by pathogen effectors. *Plant Cell* 26:2285-2309.
- Knudsen, I. M. B., Hockenhull, J., and Jensen, D. F. 1995. Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: Effects of selected fungal antagonists on growth and yield components. *Plant Pathol.* 44:467-477.
- Koch, E., Schmitt, A., Stephan, D., Kromphardt, C., Jahn, M., Krauthausen, H. J., Forsberg, G., Werner, S., Amein, T., Wright, S. A. I., Tinivella, F., Gullino, M. L., Roberts, S. J., van der Wolf, J., and Groot, S. P. C. 2010. Evaluation of non-chemical seed treatment methods for the control of *Alternaria dauci* and *A. radicina* on carrot seeds. *Eur. J. Plant Pathol.* 127:99-112.
- Li, J., Yu, L., Yang, J., Dong, L., Tian, B., Yu, Z., Liang, L., Zhang, Y., Wang, X., and Zhang, K. 2010. New insights into the evolution of subtilisin-like serine protease genes in *Peizomycotina*. *BMC Evol. Biol.* 10:68.
- Liu, J., Cao, Y., and Xia, Y. 2010. *Mmc*, a gene involved in microcycle conidiation of the entomopathogenic fungus *Metarhizium anisopliae*. *J. Invertebr. Pathol.* 105:132-138.
- Luongo, L., Galli, M., Corazza, L., Meekes, E., De Haas, L., Van der Plas, C. L., and Kohl, J. 2005. Potential of fungal antagonists for biocontrol of *Fusarium* spp. in wheat and maize through competition in crop debris. *Biocontrol Sci. Technol.* 15:229-242.
- Lysøe, E., Pasquali, M., Breakspear, A., and Kistler, H. C. 2011. The transcription factor FgStuAp influences spore development, pathogenicity, and secondary metabolism in *Fusarium graminearum*. *Mol. Plant-Microbe Interact.* 24:54-67.
- Mamarabadi, M., Jensen, B., Jensen, D. F., and Lübeck, M. 2008. Real-time RT-PCR expression analysis of chitinase and endoglucanase genes in the three-way interaction between the biocontrol strain *Clonostachys rosea* IK726, *Botrytis cinerea* and strawberry. *FEMS Microbiol. Lett.* 285: 101-110.
- Mattupalli, C., Glasner, J. D., and Charkowski, A. O. 2014. A draft genome sequence reveals the *Helminthosporium solani* arsenal for cell wall degradation. *Am. J. Potato Res.* 91:517-524.
- Meirinho, S., Carvalho, M., Dominguez, A., and Choupina, A. 2010. Isolation and characterization by asymmetric PCR of the ENDO1 gene for glucan endo-1,3- β -D-glucosidase in *Phytophthora cinnamomi* associated with the ink disease of *Castanea sativa* Mill. *Braz. Arch. Biol. Technol.* 53:513-518.
- Mérida, C. L., and Loria, R. 1994. Survival of *Helminthosporium solani* in soil and *in vitro* colonization of senescent plant tissue. *Am. Potato J.* 71: 591-598.
- Møller, K., Jensen, B., Andersen, H. P., Stryhnz, H., and Hockenhull, J. 2003. Biocontrol of *Pythium tracheiphilum* in Chinese cabbage by *Clonostachys rosea* under field conditions. *Biocontrol Sci. Technol.* 13: 171-182.
- Mouekoub, L. D. O., Zhang, L., Guan, X., Chen, X., Chen, H., Zhang, J., Zhang, J., Li, J., Yang, Y., and Wang, A. 2014. Analysis of *Clonostachys rosea*-induced resistance to tomato gray mold disease in tomato leaves. *PLoS One* 9:e102690.
- Muszewska, A., Taylor, J. W., Szczesny, P., and Grynberg, M. 2011. Independent subtilase expansions in fungi associated with animals. *Mol. Biol. Evol.* 28:3395-3404.
- Nærstad, R., Dees, M. W., Le, V. H., Holgado, R., and Hermansen, A. 2012. Occurrence of skin blemish diseases (scab and scurf) in Norwegian potato production. *Potato Res.* 55:225-239.
- Oliveros, J. C. 2015. VENN: An interactive tool for comparing lists with Venn Diagrams. Online publication. <http://bioinfo.cnb.csic.es/tools/venny/index.html>
- Omman, M., and Zeilinger, S. 2010. How a mycoparasite employs g-protein signaling: Using the example of *trichoderma*. *J. Signal Transduct.* 2010: Article 123126. doi:10.1155/2010/123126
- Oome, S., Raaymakers, T. M., Cabral, A., Samwel, S., Böhm, H., Albert, I., Nürnberger, T., and Van den Ackerveken, G. 2014. Nep1-like proteins from three kingdoms of life act as a microbe-associated molecular pattern in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 111: 16955-16960.
- Pitzschke, A., Schikora, A., and Hirt, H. 2009. MAPK cascade signalling networks in plant defence. *Curr. Opin. Plant Biol.* 12:421-426.
- Pompella, A., Visvikis, A., Paolicchi, A., De Tata, V., and Casini, A. F. 2003. The changing faces of glutathione, a cellular protagonist. *Biochem. Pharmacol.* 66:1499-1503.
- Reithner, B., Brunner, K., Schuhmacher, R., Peissl, I., Seidl, V., Krška, R., and Zeilinger, S. 2005. The G protein alpha subunit Tga1 of *Trichoderma atroviride* is involved in chitinase formation and differential production of antifungal metabolites. *Fungal Genet. Biol.* 42:749-760.
- Roberti, R., Veronesi, A., Cesari, A., Cascone, A., Di Berardino, I., Bertini, L., and Caruso, C. 2008. Induction of PR proteins and resistance by the biocontrol agent *Clonostachys rosea* in wheat plants infected with *Fusarium culmorum*. *Plant Sci.* 175:339-347.
- Rodríguez, D. A., Secor, G. A., Gudmestad, N. C., and Grafton, K. 1995. Screening tuber-bearing *Solanum* species for resistance to *Helminthosporium solani*. *Am. Potato J.* 72:669-679.
- Rodríguez, M. A., Cabrera, G., Gozto, F. C., Eberlin, M. N., and Godeas, A. 2011. *Clonostachys rosea* BAFC3874 as a *Sclerotinia sclerotiorum* antagonist: Mechanisms involved and potential as a biocontrol agent. *J. Appl. Microbiol.* 110:1177-1186.
- Rovenich, H., Boshoven, J. C., and Thomma, B. P. H. J. 2014. Filamentous pathogen effector functions: Of pathogens, hosts and microbiomes. *Curr. Opin. Plant Biol.* 20:96-103.
- Salcedo, G., Sánchez-Monge, R., Barber, D., and Díaz-Perales, A. 2007. Plant non-specific lipid transfer proteins: An interface between plant defence and human allergy. *Biochim. Biophys. Acta* 1771:781-791.
- Saxena, R. K., Khurana, N., Kuhad, R. C., and Gupta, R. 1992. D-Glucose soluble starch, a novel medium for inducing microcycle conidiation in the genus *Aspergillus*. *Mycol. Res.* 96:490-494.
- Schisler, D. A., Slininger, P. J., Kleinkopf, G., Bothast, R. J., and Ostrowski, R. C. 2000. Biological control of fusarium dry rot of potato tubers under commercial storage conditions. *Am. J. Potato Res.* 77:29-40.
- Seiboth, B., Karimi, R. A., Phatale, P. A., Linke, R., Hartl, L., Sauer, D. G., Smith, K. M., Baker, S. E., Freitag, M., and Kubicek, C. P. 2012. The putative protein methyltransferase LAE1 controls cellulase gene expression in *Trichoderma reesei*. *Mol. Microbiol.* 84:1150-1164.
- Slininger, P. J., Schisler, D. A., Eirjesson, L. D., Brandt, T. L., Frazier, M. J., Woodell, L. K., Olsen, N. L., and Kleinkopf, G. E. 2007. Biological control of post-harvest late blight of potatoes. *Biocontrol Sci. Technol.* 17:647-663.
- Slininger, P. J., Schisler, D. A., Shea-Anders, M. A., Sloan, J. M., Woodell, L. K., Frazier, M. J., and Olsen, N. L. 2010. Multi-strain co-cultures surpass blends for broad spectrum biological control of maladies of potatoes in storage. *Biocontrol Sci. Technol.* 20:763-786.
- Sophianopoulou, V., and Dhalluin, G. 1995. Amino acid transporters of lower eukaryotes: Regulation, structure and topogenesis. *FEMS Microbiol. Rev.* 16:53-75.
- Stanke, M., Steinkamp, R., Waack, S., and Morgenstern, B. 2004. AUGUSTUS: A web server for gene finding in eukaryotes. *Nucleic Acids Res.* 32:W309-W312.
- Sutton, J. C., Li, D. W., Peng, G., Yu, H., Zhang, P., and Valdebenito-Sanhueza, R. M. 1997. *Gliocladium roseum* a versatile adversary of *Botrytis cinerea* in crops. *Plant Dis.* 81:316-328.
- Tong, X., Zhang, X., Plummer, K. M., Stowell, K. M., Sullivan, P. A., and Farley, P. C. 2007. GcSTUA, an APSES transcription factor, is

- required for generation of appressorial turgor pressure and full pathogenicity of *Glomerella cingulata*. *Mol. Plant-Microbe Interact.* 20:1102-1111.
- Vranová, E., Coman, D., and Gruissem, W. 2012. Structure and dynamics of the isoprenoid pathway network. *Mol. Plant* 5:318-333.
- Wiemann, P., Brown, D. W., Kleigrewe, K., Bok, J. W., Keller, N. P., Humpf, H. U., and Tudzynski, B. 2010. FfVell and FfLae1, components of a velvet-like complex in *Fusarium fujikuroi*, affect differentiation, secondary metabolism and virulence. *Mol. Microbiol.* 77: 972-994.
- Yan, L., and Qian, Y. 2009. Cloning and heterologous expression of SS10, a subtilisin-like protease displaying antifungal activity from *Trichoderma harzianum*. *FEMS Microbiol. Lett.* 290:54-61.
- Zhang, L., Yang, J., Niu, Q., Zhao, X., Ye, F., Liang, L., and Zhang, K. Q. 2008. Investigation on the infection mechanism of the fungus *Clonostachys rosea* against nematodes using the green fluorescent protein. *Appl. Microbiol. Biotechnol.* 78:983-990.
- Zhao, Y., Su, H., Zhou, J., Feng, H., Zhang, K. Q., and Yang, J. 2015. The APSES family proteins in fungi: Characterizations, evolution and functions. *Fungal Genet. Biol.* 81:271-280.