



Bioactivity of Acyl Solamines from Leaves of *Solanum bulbocastanum* against *Alternaria solani* and *Botrytis cinerea*

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Abstract

Wild potatoes contain valuable genetic diversity that can help to develop resistance to pathogens in cultivated *Solanum tuberosum*, a globally important crop. This study investigates the bioactivity of acyl solamines from *Solanum bulbocastanum* leaves against *Alternaria solani*, the causal agent of early blight on potato and tomato, and the generalist fungal phytopathogen *Botrytis cinerea*. An enrichment and purification procedure for acyl solamines was developed. The resulting acyl solamine fraction contained more than 90% fatty acyl solamines with a chain length of 12–18 carbon atoms. The C16:3-solamine was the dominant compound. In agar dilution assays, the isolated acyl solamine fraction showed significant mycelial growth inhibition for both pathogens already at concentrations lower than those found in *S. bulbocastanum* leaves. Half-maximal inhibitory concentrations were 150 mg/L for *A. solani* and 94 mg/L for *B. cinerea*. Possible modes of action and the potential use of long-chain fatty acyl solamines in potato production and integrated pest management are discussed.

Keywords *Alternaria solani* · Antifungal natural products · *Botrytis cinerea* · Long-chain fatty acyl solamines · Wild potato

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Introduction

The cultivated potato, *Solanum tuberosum*, is one of the world's most important crops. More than 374 million tons were produced on over 16 million hectares in (FAO 2022). Only maize, wheat, and rice contribute more to the global food supply (FAO, 2022). But the process of crop domestication and the associated selection for quality and yield has, in many cases, led to a decrease in the genetic diversity of plants and their resistance to pathogens (Smýkal et al. 2018). This has resulted in potatoes being a highly pathogen-infested crop. Up to 50 different pests and diseases attack *S. tuberosum*. Besides bacterial and viral diseases, nematodes and arthropods, fungal diseases play an important role in potato production (Fiers et al. 2011; Kapsa 2008; Salazar 2006; Savary et al. 2019).

Alternaria solani is the causal agent of early blight, which infects solanaceous crops worldwide. It is a necrotrophic member of the *Fungi imperfecti* and follows a typical life cycle involving extensive spore production, dispersal, germination, and infection (Chaerani & Voorrips 2006; Schmey et al. 2024). *A. solani* is a polycyclic pathogen because it can infect the same field several times within a growing season, making it difficult to control. It infects the foliage, stems, and tubers, causing dark, concentric lesions on leaves that can coalesce, leading to significant defoliation and yield loss. Infected tubers show brownish sunken circular lesions with the underlying tissue becoming corky. Despite its name, the disease usually starts later in the growing season as the fungus prefers to infect mature leaves around the time of tuber initiation (Horsfield et al. 2010). The economic impact of early blight is profound with yield losses of up to 50% in potatoes (Leiminger & Hausladen 2012).

Early blight is mainly controlled by cultural practices and plant protection products (PPPs), as no fully resistant potato varieties are available (Wolters et al. 2021). The use of PPPs against *A. solani* depends on the active ingredient, with 3–4 applications of systemic PPPs or spraying of protectant type PPPs in 1- or 2-week cycles. Decision support systems aim to reduce the application of PPPs to a minimum dose but maximum effect (Abuley et al. 2023). It should be noted that almost as many PPP formulations have been registered for use against early blight as for all other potato pathogens combined (Tsedaley 2014). The extensive use of PPPs has in many cases led to an increase in PPP resistance in *A. solani* (Deising et al. 2008; Schmey et al. 2024). PPP resistance in *A. solani* has been reported against various compounds and is constantly evolving (Gudmestad et al. 2013; Nottensteiner et al. 2019; Nuwamanya et al. 2022).

B. cinerea is a ubiquitous necrotrophic ascomycete that causes grey mould. With significant pre- and post-harvest losses, infestations result in substantial economic losses (Dean et al. 2012). In potato production, *B. cinerea* is of less economic importance, but potato-growing areas with humid and cold climates and with long growing seasons are affected. *B. cinerea* can infect all parts of the potato plant (Humphreys-Jones 1982; INRAe 2019). The symptoms of infection on leaves are very similar to those of *Phytophthora infestans* (Humphreys-Jones 1982). No major genes for complete resistance to *B. cinerea* are known (Sun et al.

2017). As the pathogen uses universal mechanisms to induce necrosis, conferring plant resistance to *B. cinerea* by single genes is considered impossible (Cheung et al. 2020).

The management of grey mould in agriculture relies on PPPs, too (Rosslenbroich & Stuebler 2000). However, *B. cinerea* rapidly develops resistance against fungicides (Fernández-Ortuño et al. 2015; Hahn 2014). For example, after the introduction of benzimidazole PPPs, resistant strains of *B. cinerea* were found in the field 2 years later; dicarboximide resistant strains evolved within 5 years (Deising et al. 2008). Thus, active PPPs are available against *B. cinerea*, but strict resistance management is required to save their activity (Hahn 2014).

The increase in PPP resistance in pests and pathogens and more severe pest outbreaks due to climate change require new measures to ensure food and environmental safety. Integrated Pest Management (IPM) is the most promising approach, and the EU has emphasized IPM as an integral part of its Sustainable Use Directive. IPM aims to prevent severe disease and pest outbreaks using strategies such as crop rotation, balanced fertilization, push–pull strategies, and biological components to reduce chemical pesticides to a minimum (Deguine et al. 2023, 2021; Richard et al. 2022; Tripathi et al. 2022). The use of resistant or tolerant crops is a fundamental approach in IPM. However, many crop resistances have been lost during domestication. A survey of more than 900 potato lines did not reveal any line with complete resistance to *Alternaria solani* (Boiteux et al. 1995), and no single resistance factor (one gene conferring resistance) has yet been described in the literature (Schmey et al. 2024).

Wild crop relatives or ancestors are a major resource for resistance breeding, as they provide a vast pool of genetic variability to improve modern crops (Ali et al. 2022; Blossei et al. 2022; Crossley et al. 2018; Li et al. 2022). Resistance to *A. solani* has been observed in the wild species *Solanum berthaultii*, *Solanum commersonii*, and *Solanum chacoense* (Alam 1985; Wolters et al. 2021) and in some *Solanum hougasii*–*Solanum tuberosum* hybrid clones (Haynes & Qu 2016). *Solanum bulbocastanum* has a broad range of resistance to pests and pathogens (Brown et al. 2009; Chen et al. 2003; Jansky et al. 2009; Lokossou et al. 2010a, b; Song et al. 2003; van der Vossen et al. 2003). Some factors such as resistance genes (*R* genes) against *Phytophthora infestans* have been identified in *S. bulbocastanum* and shown to confer enhanced resistance in potato plants (Haesaert et al. 2015; Lokossou et al. 2010a, b; Oosumi et al. 2009; Park et al. 2005; Song et al. 2003; van der Vossen et al. 2003; van der Vossen et al. 2005).

Bioactive metabolites such as hydroxycinnamic acid amides or steroidal glycoalkaloids can also contribute to the resistance of wild and cultivated potato against pests and pathogens (Dahlin et al. 2017; Dobritsch et al. 2016; Wolters et al. 2023; Yogendra et al. 2014). However, literature on chemical resistance mechanisms of *S. bulbocastanum* is scarce (Aversano et al. 2017; Caruso et al. 2013; Paraschiv et al. 2022). The lysophosphatidylcholine LPC 17:1 from the leaf surface of *S. bulbocastanum* was identified and shown to inhibit *P. infestans* spore germination as well as mycelial growth (Gorzolka et al. 2021).

Recently, numerous acyl solamines have been discovered in leaves of some *S. bulbocastanum* accessions with total concentrations of 1–2% of leaf dry matter

(Gorzolka et al. 2025). The quantitatively dominating long-chain fatty acyl solamine (C16:3(7Z,10Z,13Z)-solamine) accounting for approximately 90% of the total acyl solamine content was isolated from *S. bulbocastanum* WKS31741 and structurally characterized. Application of C16:3(7Z,10Z,13Z)-solamine on *S. tuberosum* leaf disks significantly reduced the feeding of Colorado potato beetles (*Leptinotarsa decemlineata*) and potato flea beetles (*Epidrix papa*). C16:3(7Z,10Z,13Z)-solamine reduced *P. infestans* sporangia germination in vitro and plant leaf infection (Gorzolka et al. 2025). The isolation of the C16:3(7Z,10Z,13Z)-solamine from leaf material for bioassays is cost-intensive and laborious. In contrast, the enrichment of acyl solamines using a solid-phase extraction procedure is a simple and fast alternative. With this approach, the bioactivity of the complete spectrum of leaf acyl solamines can be tested for bioactivity against pathogens and pests.

The objective of this study was to assess the bioactivity of an acyl solamine fraction derived from *Solanum bulbocastanum* leaves against *Alternaria solani*, a serious potato phytopathogen, and *Botrytis cinerea*, a common broad-spectrum pathogen. An enrichment procedure for acyl solamines from leaf extracts was established. The resulting fraction was characterized for its composition and tested in agar dilution assays for its mycelial growth inhibitory effect on both pathogens. The mode of action of long-chain fatty acyl solamines and their relevance for potato resistance breeding and crop protection are discussed.

Materials and Methods

Plant Material

Seeds of the Mexican wild potato *Solanum bulbocastanum* accession WKS31741 were received from the Federal ex situ Gene Bank of the Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany). A clonal line (*blb2G*) was maintained as in vitro culture at the Julius Kühn Institute (Sanitz, Germany). Plants were regenerated as described (Thieme et al. 1997). After 3–4 weeks, plants were transferred to potting soil (“Fruhstorfer Topferde Typ T”; HAWITA Gruppe GmbH) and grown in a climate chamber (16 h light/20 °C; 8 h dark/18 °C; relative humidity ~40%) for 10 weeks. Plants were watered daily as required and fertilized once per week (0.2% WUXAL Super, Hauert MANNA Duengerwerke GmbH). *Solanum tuberosum* ‘Maxi’ and ‘Gala’ plants were grown from seed tubers under the same conditions. Fresh leaves were harvested, deep-frozen at –80 °C, freeze-dried, vacuum-packed in plastic bags, and stored at room temperature until further processing.

Extraction and Enrichment of Acyl Solamines

Ten grams of freeze-dried *S. bulbocastanum* leaves were homogenized using a tube mill (IKA Tube-Mill 100). The plant material was mixed with 100 mL 80% aqueous methanol (MeOH), sonicated for 10 min (bath temperature < 25 °C), and stirred for

2 h at room temperature. The mixture was vacuum-filtered, and the remaining plant material was re-extracted using the same procedure.

The pooled crude extract was acidified to pH 2 using 2 M HCl and successively subjected to strong cation exchange solid-phase extraction (SPE) using a 60-mL cartridge packed with 8 g Strata-X-C polymeric sorbent (particle size 33 μm , Phenomenex). The sorbent was conditioned and equilibrated using 100 mL MeOH and 80% aqueous MeOH, respectively. Afterwards, the acidified extract was passed under the application of a slight vacuum through the sorbent, which was then washed twice using 100 mL 80% aqueous MeOH containing 0.1 M HCl and 100 mL MeOH. Finally, metabolites were eluted using 100 mL MeOH containing 5% of an aqueous ammonia solution (25% (w/w)). The obtained eluate was vacuum-concentrated at 30 °C until dryness and stored under an atmosphere of nitrogen at –20 °C until further use.

Reversed-phase solid phase extraction was performed using two 60-mL cartridges each packed with 10 g Strata C18-E silica-based sorbent (particle size 55 μm , Phenomenex). The sorbent was conditioned with 100 mL acetonitrile (ACN) and equilibrated with 5% aqueous ACN. The dried fraction obtained from strong cation exchange SPE was dissolved in 100 mL 5% aqueous ACN. Using a slight vacuum, 50 mL each of this solution was passed through the cartridge. The sorbent was washed with 100 mL 5% aqueous ACN acidified with 0.5% formic acid (FA). Acyl solamines were eluted with 100 mL 70% aqueous ACN containing 0.5% FA. The eluates were combined and vacuum-concentrated at 30 °C to give 0.12 g (1.2% leaf dry matter) of a green-brownish oily residue, which was stored under an atmosphere of nitrogen at –20 °C until further use.

LC–MS Analysis of Acyl Solamines

Liquid chromatography-mass spectrometry analyses were performed on an Infinity 1290 series UHPLC system equipped with an Acquity UPLC HSS T3 column (2.1 mm $\times$ 100 mm, particle size 1.8 μm , pore size 100 Å, Waters) and hyphenated to a 6550 iFunnel Q-TOF mass spectrometer (Agilent Technologies) via a dual jet stream electrospray ion source with instrument settings as described (Gorzolka et al. 2025). In brief, metabolites were separated using a binary eluent system consisting of 0.1% FA in water (eluent A) and 0.1% FA in ACN (eluent B). The following linear gradient at a flow rate of 500 $\mu\text{L}/\text{min}$ was applied: 0–1 min, isocratic 5% B; 1–15 min, linear to 60% B; 15–18 min, linear to 95% B; 18–19 min, isocratic 95% B; 19–21 min, isocratic 5% B. The column temperature was maintained at 40 °C. Mass spectra were acquired in positive or negative ion mode from m/z 50–1700 with an acquisition rate of 3 Hz. Collision-induced dissociation tandem mass spectra were acquired applying collision energies of 15, 25, and 35 V in auto-MS² mode and targeted-MS² mode as described (Gorzolka et al. 2025).

Crude methanolic leaf extracts (50 g leaf dry mass/L) and purified acyl solamine fractions (500 mg/L in 80% aqueous MeOH) from three independent preparations were analysed. All samples were serially diluted (1/10, 1/100, 1/1000) with

80% aqueous MeOH and subjected together with the undiluted sample to LC–MS analysis.

Annotation and Relative Quantification of Acyl Solamines

Raw data inspection, compound spectrum extraction, compound annotation, compound database search, and generation of molecular formulas were done in MassHunter Qualitative Analysis software (version B.07.00, Agilent Technologies). Quantification of acyl solamines was done in MassHunter Quantitative Analysis (version B.07.00, Agilent Technologies). A self-built combinatorial compound database of 148 acyl solamines comprising fatty acyl solamines (chain length: 6, 8, 10, ..., 20, double bond equivalents: 0–4, oxygenations: 0–3) and hydroxycinnamoyl solamines was generated in MS Excel (Microsoft Corporation) and imported in MassHunter Personal Compound Database Library Manager (version B.07.00, Agilent Technologies) (Gorzolka et al. 2025).

For annotation of acyl solamines, compound spectra with a peak volume of > 2000 counts were deconvoluted within a retention time range of 0.4–18 min using the function “find compounds by molecular feature.” Determined molecular masses were matched (mass tolerance ± 5 ppm) against the constructed acyl solamine compound database. In order to validate database hits, the extracted compound spectra were evaluated for the presence of $[M+H]^+$ and $[M+2H]^{++}$ ions, which both form upon positive ion electrospray ionization of acyl solamines. In addition, collision-induced dissociation tandem mass spectra obtained from putative protonated acyl solamines were examined for the presence of characteristic ions at m/z 216.243 ($C_{12}H_{30}N_3^+$, [solamine+H] $^+$), m/z 171.186 ($C_{10}H_{23}N_2^+$, [solamine- C_2H_7N +H] $^+$), m/z 126.128 ($C_8H_{16}N^+$, [solamine-2 C_2H_7N +H] $^+$), and m/z 100.112 ($C_6H_{14}N^+$).

To estimate the proportion of acyl solamines in the total signal intensity of an LC–MS chromatogram, the fraction of the total peak volume of all annotated acyl solamines to the total peak volume of all deconvoluted compounds was calculated. For relative quantification of annotated acyl solamines, extracted ion chromatograms of $[M+H]^+$ ions were integrated. Peak integrals of chromatographically separated isomers were summed up. In the case of detector saturation, abundant acyl solamines were relatively quantified in correspondingly diluted samples.

Pathogen Strains and Cultivation Conditions

Alternaria solani (isolate 774_1, isolated in Hamerstorf in Lower Saxony in 2015) was kindly provided by Dr. Hans Hausladen (Technical University Munich). Pre-cultures of *A. solani* were grown on synthetic nutrient-poor agar (SNA) (Nirenberg 1981) for 2 weeks at 22.5 °C in the dark. *Botrytis cinerea* (isolate JKI-Nr. 68731) was obtained from the mycological culture collection of the Julius Kühn Institute (Berlin). Precultures were grown on SNA for 10 days at 22.5 °C in the dark.

Detached Leaf Assays

A. solani (isolate 774_1) was grown on SNA as described above. Agar from 4-week-old plates was cut into small pieces, which were transferred to new SNA plates (cutting promoted sporulation). After two further weeks of incubation, agar pieces with conidia were mixed with sterile tap water in a centrifuge tube and filtered through double-layered gauze (250- μ m mesh width). Microscopy of the filtrate showed conidia with hardly any mycelium. The conidia density was adjusted to 4000 conidia/mL.

Healthy, fully expanded leaves of 8-week-old plants of *S. tuberosum* ‘Gala,’ ‘Maxi,’ and *S. bulbocastanum* WKS31741 *blb2G* were harvested and placed abaxial side up in a sterilized plastic box. Petioles were embedded in wet tissue. A 20- μ L droplet of the conidia suspension was placed on the abaxial side of each leaf, approximately at the midpoint of the leaf right of the midrib. The plastic boxes were closed and incubated in a phytochamber using settings as described above for plant growth. The embedding tissues for the petioles were moistened on a daily basis as required. Lesions were monitored for 3 weeks, and photos of the leaves and leaf surfaces (microscopy) were taken.

Bioactivity of Acyl Solamines against *Alternaria solani* and *Botrytis cinerea*

The agar-dilution assays were performed on potato dextrose agar (PDA) which was prepared from 39 g/L potato extract glucose agar (Carl Roth GmbH + Co. KG) and 5 g/L Oxoid technical agar (agar no. 3, Fisher Scientific). Five millilitres of PDA was aliquoted into 20-mL test tubes and autoclaved. The isolated acyl solamine fraction was dissolved in ethanol, and stock solutions with concentrations of 35, 25, 15, 10, 5, 2.5, and 0.5 g/L were prepared. Test tubes with sterilized PDA were incubated at 70 °C in a water bath. After melting of the medium, 50 μ L of an ethanolic acyl solamine solution (treatment, T) or 50 μ L ethanol (control, C) were added (final acyl solamine concentrations in treated PDA: 350, 250, 150, 100, 50, 25, 5 mg/L). The spiked medium was briefly vortexed, poured into a plastic Petri dish (diameter 5 cm), and left to solidify. As positive control, PDA containing 5 mg/L tebuconazole was used. Mycelium containing agar plugs from *A. solani* or *B. cinerea* precultures were punched out from the inner region of the preculture using a cork borer and placed in the centre of the PDA test plates. Test plates were incubated at 22.5 °C in the dark for about 7–10 days in *A. solani* tests and for 2–3 days in *B. cinerea* tests. The test was terminated before the mycelia on any test plate reached the edge of the plate. This final time point was evaluated to determine growth inhibition. For *A. solani*, four independent experiments were performed. In each experiment, five plates were evaluated per treatment ($N_{\text{total}} = 20$). For *B. cinerea*, three independent experiments from three separate *B. cinerea* precultures were performed. In each experiment, five plates were evaluated per treatment ($N_{\text{total}} = 15$) except for $T = 150$ mg/L ($N_{\text{total}} = 14$).

Test plates were scanned (resolution 600 dpi) with a reference scale, and the resulting pictures were analysed using ImageJ. The final circumference of the

mycelium and the circumference of the inoculum were marked using the polygon tool, and the resulting areas were determined. Both areas were converted into radii ($r = (\text{area}/\pi)^{1/2}$) and subtracted to give the final radial growth of the mycelium. Growth inhibitory activities were determined separately for each independent experiment using the formula $[1 - r_T/r_{C,\text{mean}}] \times 100\%$, where r_T is the radial growth of the mycelium of the treatment and $r_{C,\text{mean}}$ the average radial growth of the mycelium of the controls. Growth inhibitory activities were statistically evaluated by one-way ANOVA and Tukey's honestly significant difference (HSD) test using functions from the R package 'agricolae' (de Mendiburu & Yaseen 2020). Dose–response relationships were fitted by a weighted 5-parameter logistic regression model using the function `nplr` from the R package 'nplr' (Commo & Bot 2016).

Results

Enrichment of Acyl Solamines from Leaves of *Solanum bulbocastanum*

Freeze-dried and homogenized leaf material from *S. bulbocastanum* WKS31741 *blb2G* was extracted with aqueous methanol. UHPLC/ESI-QTOFMS-based metabolite analysis of the extract showed very abundant chromatographic peaks belonging to protonated fatty acyl solamines, which are (as alkaloids bearing two dimethyl-amino moieties) exclusively detectable in the positive ion mode (Fig. 1A–C, Online Resource Fig. 1).

To enrich the dominating fatty acyl solamines, methanolic leaf extracts were subjected to strong cation exchange (SCX) solid-phase extraction (SPE). After elution of neutral and acidic components, the bound basic components were eluted with a solution of ammonia in methanol. UHPLC/ESI-QTOFMS-based analysis of this eluate showed the presence of acyl solamines and other metabolites such as amino acids and biogenic amines. To remove these more polar components, a second fractionation was performed using an endcapped silica-based C18 reversed-phase (RP) sorbent. Unwanted polar components were eluted with 5% aqueous acetonitrile, while fatty acyl solamines were obtained after elution with 70% aqueous acetonitrile. It should be noted that eluents have to be acidified with at least 0.5% formic acid to suppress the interaction between residual silanols and amines and to facilitate effective elution. Figure 1 presents exemplary total ion chromatograms of the crude leaf extract and the enriched acyl solamine fraction after SCX and RP SPE. Determination of the peak volume of all annotated acyl solamines in relation to the peak volume of all deconvoluted components suggests an enrichment of acyl solamines from 64% in the crude extract to 96% in the enriched fraction (Fig. 1E). Repeated execution of the developed enrichment procedure indicated a good repeatability with respect to yield and composition (Fig. 1E). The average yield of the enriched acyl solamine fraction was $1.2 \pm 0.1\%$ of leaf dry matter ($n = 3$).

Using UHPLC/ESI-QTOFMS, a total of 33 acyl solamines were annotated and relatively quantified within the enriched fraction. Acyl solamines with even-numbered long-chain fatty acyl moieties (LCFA-solamines, C14–C18) with up to four double bond equivalents and even-numbered medium-chain fatty acyl moieties (MCFA-solamines,

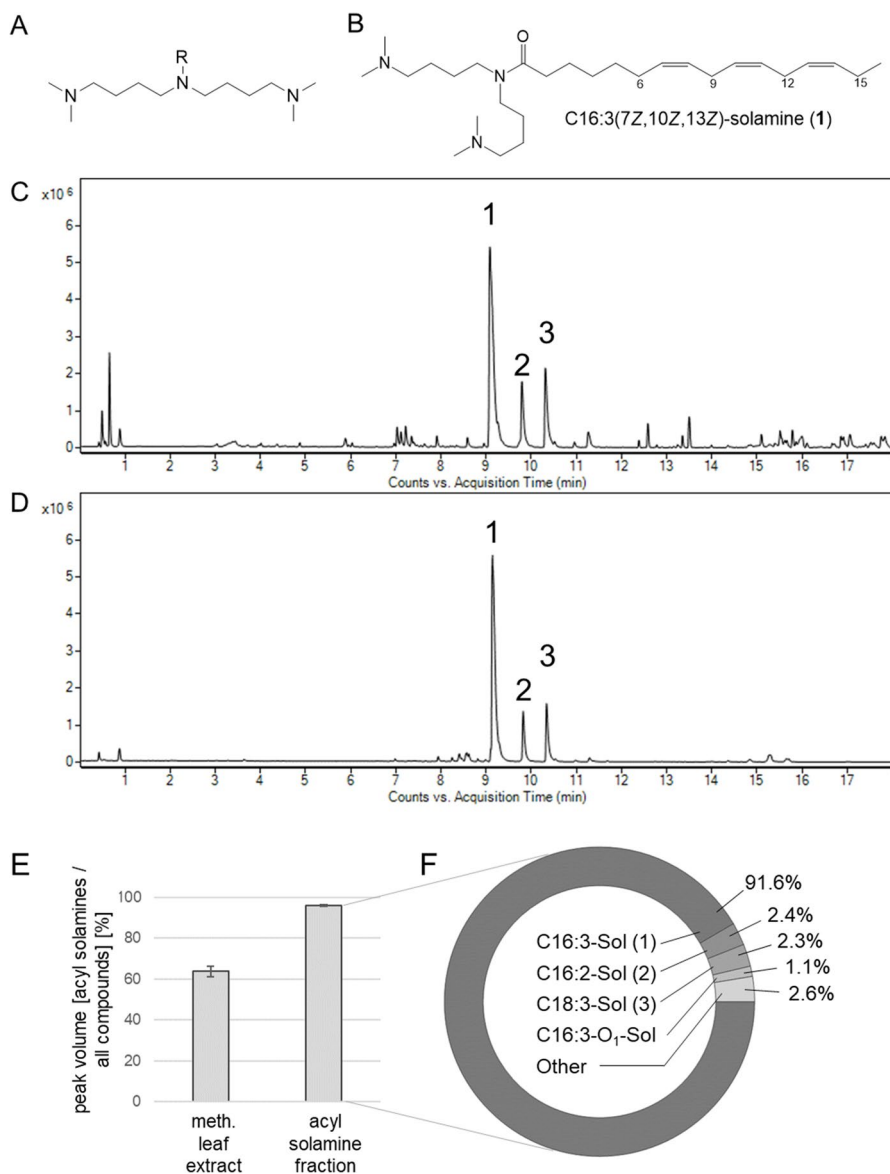


Fig. 1 Enrichment and analysis of acyl solamines from methanolic leaf extracts of *S. bulbocastanum* WKS31741 blb2G. **A** Generalized structure of acyl solamines. R: fatty acyl C(6–18):(0–4)-O_{0–3} or (hydroxy)cinnamoyl. **B** Structure of C16:3-solamine (1) as revealed by NMR (Gorzolka et al. 2025). **C** Total ion chromatogram obtained from a methanolic *S. bulbocastanum* leaf extract (5 g/L). **D** Total ion chromatogram obtained from the enriched acyl solamine fraction (50 mg/L). Chromatograms were obtained by UHPLC/ESI-QTOFMS operated in positive ion mode. The three most abundant fatty acyl solamines are labelled. **E** Proportions of the peak volume of the annotated acyl solamines in the peak volume of all deconvoluted components. Means and standard deviations of three preparations are shown. **F** Relative quantification of annotated acyl solamines in the enriched fraction. Means of three preparations are shown. Detailed data can be found in Online Resource Table 1

C6-C10) with up to two double bond equivalents were annotated. In addition, acyl solamines with oxygenated, unsaturated medium- and long-chain fatty acyl moieties as well as cinnamoyl- and feruloyl-solamine were detected (Fig. 1D, Online Resource Table 1). With more than 90% of the total peak area, C16:3-solamine was the quantitatively dominating acyl solamine, followed by C16:2-solamine (2.4%), C18:3-solamine (2.3%), and C16:3-O₁-solamine (1.1%). The relative intensity of each of the remaining 29 acyl solamines was below 0.5% (Online Resource Table 1). In total, these minor components accounted for only 2.6% of the total acyl solamine peak area.

Bioactivity of the Enriched Acyl Solamine Fraction Isolated from *S. bulbocastanum* Leaves against *Alternaria solani*

A detached leaf infection assay using a conidia suspension of *A. solani* demonstrated a much higher resistance of *S. bulbocastanum* WKS31741 *blb2G* in comparison to *S. tuberosum* ‘Maxi’ and ‘Gala’ (Fig. 2). *Solanum tuberosum* leaves showed first symptoms of infection 4–5 days post inoculation and clear symptoms after 8–10 days post inoculation. At this time point, brown necrotic lesions of 0.5–1 cm diameter were observed at the position of the infection droplet with yellowish surrounding leaf tissue. In contrast, no or very slight symptoms (small brown spots of < 2 mm size, no or few yellowish surrounding leaf tissue) were observed on *S. bulbocastanum* leaves. Microscopic analyses of the leaf surfaces revealed that conidia germination and mycelial development of *A. solani* occurred on leaves of both species (Online Resource Fig. 2).

A



B

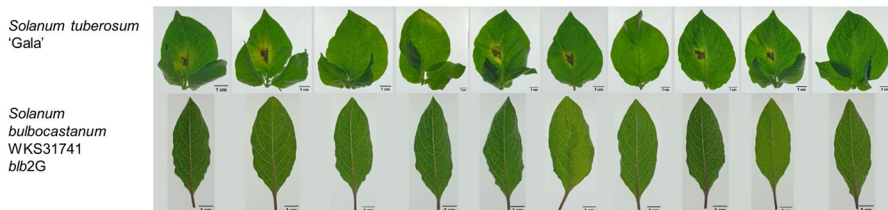


Fig. 2 Detached leaf infection assays of **A** *Solanum tuberosum* ‘Maxi’ and *Solanum bulbocastanum* WKS31741 *blb2G* and **B** *Solanum tuberosum* ‘Gala’ and *Solanum bulbocastanum* WKS31741 *blb2G* with 20 μ L conidia suspension of *Alternaria solani* isolate 774_1 (4000 conidia/mL) placed as a droplet right next to the mid rib. Photos were taken 9 days post-inoculation in (A) and 21 days post inoculation in **B**

To investigate a potential role of acyl solamines accumulating in leaves of *S. bulbocastanum* blb2G as a resistance factor, the effect of the enriched acyl solamine fraction on the mycelial growth of *A. solani* was investigated using an agar dilution assay. The highest concentration of the acyl solamine fraction applied in the assay (350 mg/L) was three to four times lower than the native total level of acyl solamines in *S. bulbocastanum* leaves (~1200 mg/kg fresh weight). Figure 3 presents the dose-growth inhibition relationship obtained from four independent experiments.

Mycelial growth was significantly inhibited at 100 mg/L (Fig. 3A) with a statistically significant concentration-dependent growth inhibition in the concentration range of 100–350 mg/L (Fig. 3, Tukey's HSD test, $p \leq 0.05$). At a

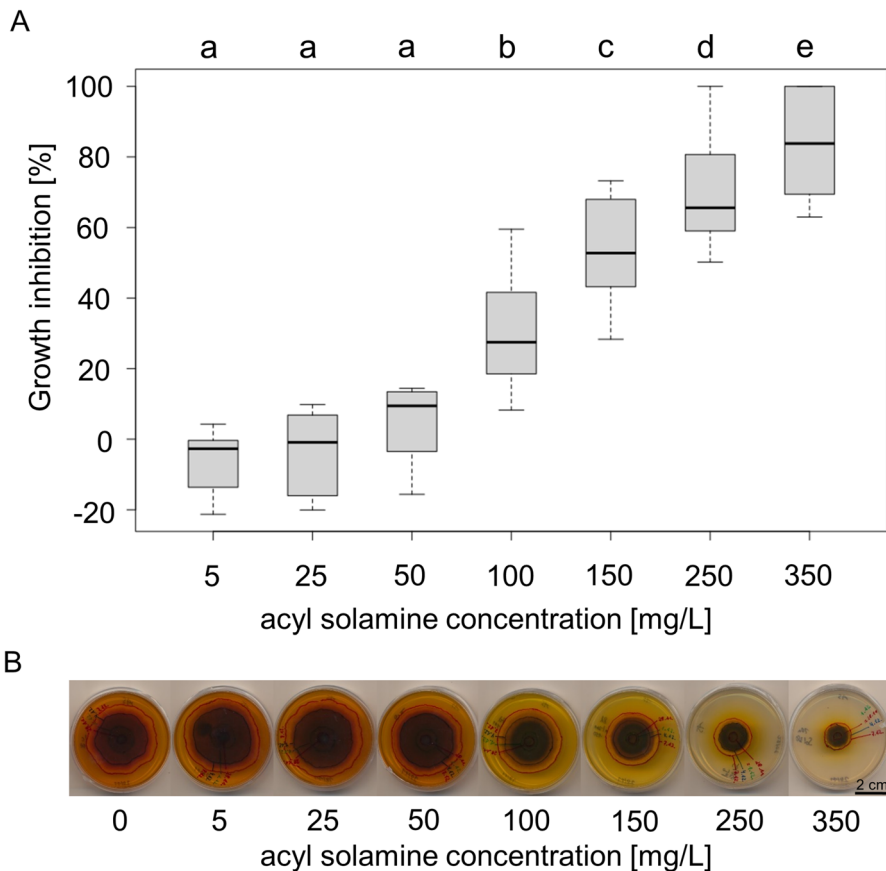


Fig. 3 Effect of the enriched acyl solamine fraction isolated from *S. bulbocastanum* leaves on the mycelial growth of *A. solani*. **A** Inhibition of mycelial growth depending on the concentration of the enriched acyl solamine fraction in the culture medium. Four independent experiments with each five technical replicates per treatment were carried out ($N_{\text{total}}=20$). The horizontal solid lines of the boxes indicate the median and the interquartile range (IQR). The whiskers represent the last data point within $1.5 \times \text{IQR}$. Treatments not sharing any letter are significantly different (Tukey's HSD test, $p \leq 0.05$). **B** Representative agar plates of *A. solani* growing for 9 days on PDA spiked with different amounts of the enriched acyl solamine fraction

concentration of 350 mg/L, 45% (9 of 20) of the replicates showed complete inhibition of *A. solani* mycelial growth (data not shown). Using a logistic regression model (Online Resource Fig. 3), the half-maximal inhibitory concentration (IC₅₀) was determined to be 150 mg/L (95% confidence interval 92–250 mg/L).

Bioactivity of the Enriched Acyl Solamine Fraction Isolated from *S. bulbocastanum* Leaves against *Botrytis cinerea*

To test whether the acyl solamine fraction also had growth inhibitory activity against a generalist phytopathogenic fungus, analogous agar dilution assays were performed with *B. cinerea* (Fig. 4). In comparison to *A. solani*, *B. cinerea* grew much faster on

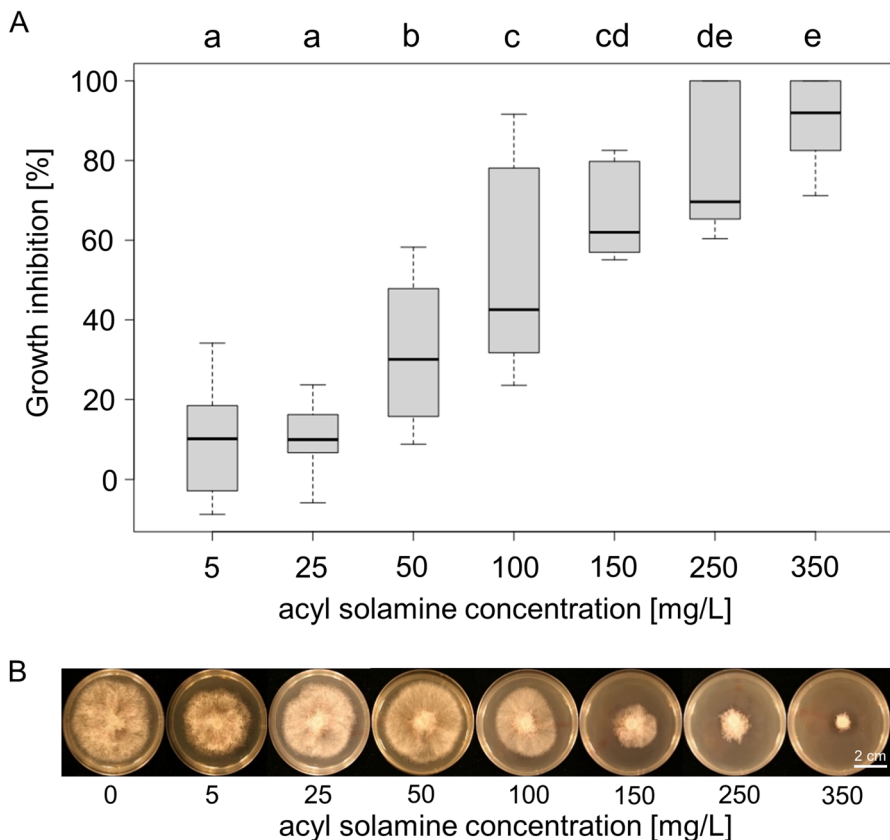


Fig. 4 Effect of the enriched acyl solamine fraction isolated from *S. bulbocastanum* leaves on the mycelial growth of *B. cinerea*. **A** Inhibition of mycelial growth depending on the concentration of the enriched acyl solamine fraction in the culture medium. Three independent experiments were performed with five technical replicates per treatment ($N_{\text{total}}=15$, except 150 mg/L: $N=14$). The horizontal solid lines of the boxes indicate the median and the interquartile range (IQR). The whiskers represent the last data point within $1.5 \times \text{IQR}$. Treatments not sharing any letter are significantly different (Tukey's HSD test, $p \leq 0.05$). **B** Representative agar plates of *B. cinerea* growing for 3 days on PDA spiked with different amounts of the enriched acyl solamine fraction

PDA control plates. Statistically significant growth inhibitory effects were registered at a concentration of 50 mg/L (Fig. 4) which increased further with higher acyl solamine concentrations. At a concentration of 350 mg/L, 40% of the replicates showed no mycelial growth even after prolonged incubation for 10 days beyond the duration of the test (data not shown). The IC₅₀ was determined to be 94 mg/L (95% confidence interval 43–220 mg/L) (Online Resource Fig. 4).

Discussion

Enrichment of Acyl Solamines from *Solanum bulbocastanum* Leaves

Acyl solamines were previously isolated from *Solanum tripartitum* (Kupchan et al. 1967, 1969), *Solanum betaceum* (Evans et al. 1972), *Solanum carolinense* (Evans & Somanabandhu 1977), and *S. bulbocastanum* (Gorzolka et al. 2025) and detected in several further *Solanum* species (Evans & Somanabandhu 1980; Gorzolka et al. 2025). While most of the analyses revealed the accumulation of acyl solamines in root tissue, their presence in leaves or aerial parts was only documented for *S. tripartitum*, *S. radicans* (Evans & Somanabandhu 1980), and *S. bulbocastanum* (Gorzolka et al. 2025). Leaf material of three *S. bulbocastanum* accessions was reported to contain approximately 1.5–2.2% of leaf dry matter in mainly long-chain fatty acyl solamines, whereas leaves of nine other investigated *Solanum* species of the section Petota (tuber-bearing *Solanum* species) did not accumulate detectable amounts of any acyl solamines (Gorzolka et al. 2025). Since *S. bulbocastanum* is known for its multiple pest and pathogen resistances, it was hypothesized that this highly abundant alkaloid class might serve as a resistance determinant *in planta*. In order to investigate the bioactivity of acyl solamines *in vitro*, a simple and reliable extraction and enrichment protocol starting from dry *S. bulbocastanum* leaf material was established in this study. The obtained enriched acyl solamine fraction was then characterized by UHPLC/ESI-QTOFMS and tested for its potential to inhibit the mycelial growth of *Alternaria solani* and *Botrytis cinerea*.

Our approach for enrichment of acyl solamines is based on a two-step solid-phase extraction procedure. The first step employs a SCX sorbent and targets the basic solamine head group bearing two dimethylamino moieties. To separate co-purified polar and highly nonpolar basic metabolites from acyl solamines, a second enrichment step using an RP C18 sorbent was applied. UHPLC/ESI-QTOFMS-based analyses performed in both ion modes suggest that the obtained fraction was nearly exclusively composed of acyl solamines. However, given the inherent limitations of the detection capabilities of LC/ESI-MS, the presence of other components cannot be fully excluded. The obtained average yield (1.2% of leaf dry matter) of acyl solamines from leaves of *S. bulbocastanum* WKS 31741 blb2G was in good agreement to the previously reported total content of acyl solamines in leaves of this accession (1.7% leaf dry matter) (Gorzolka et al. 2025), which indicates a sufficient process efficiency of the established extraction and enrichment protocol. A comparison of LC-MS data obtained from the crude leaf extract and the enriched fraction suggests that the relative proportions of acyl solamines were not significantly altered

by the enrichment procedure. In the crude leaf extract and in the enriched fraction, long-chain fatty acyl solamines accounted for > 99% of the total content of acyl solamines. It should be noted that, in contrast to many other *Solanum* species, leaves of *S. bulbocastanum* WKS 31741 *blb2G* do not accumulate substantial amounts of steroidal glycoalkaloids. Thus, if the developed protocol is applied to plant material containing both steroidal glycoalkaloids and fatty acyl solamines, co-enrichment of both alkaloid classes has to be expected. To separate both alkaloid classes, the second RP SPE has to be optimized or substituted by preparative RP HPLC.

Bioactivity of Long-Chain Fatty Acyl Solamines

Biotests with the acyl solamine fraction demonstrated a clear inhibition of *Alternaria solani* and *Botrytis cinerea* mycelial growth with similar IC₅₀ values (100–150 mg/L). The cationic, amphiphilic structure of long-chain fatty acyl solamines suggests membrane disintegration as the primary mode of action. Previous studies investigated the bioactivities of the long-chain fatty acyl solamine solapalmitine (C16:0-solamine) from leaves of *S. tripartitum*. Solapalmitine was shown to alter the membrane permeability and impeded the cell proliferation of *Escherichia coli* (Silver & Kralovic 1969). Human nasopharyngeal carcinoma cells (KB) released magnesium ions upon solapalmitine treatment, detached from the surface and were susceptible to trypan blue staining, an indicator for dead cells (Silver et al. 1970). Solapalmitine and solapalmitenine (C16:1-solamine) exhibited cytotoxicity against KB cells and inhibited in vivo growth of a carcinosarcoma in rats (Kupchan et al. 1972, 1969). The relationship between cytotoxicity and structural features of the acyl and the polyamine moiety was investigated for solapalmitine-related fatty acyl triamines (Kupchan et al. 1972). It was shown that the fatty acyl moiety requires a length of at least 12 C atoms for maximal cytotoxicity against KB cells, whereas changes in the polyamine moiety had no significant impact. Based on these observations, it was concluded that the cytotoxicity of solapalmitine and related acyl triamines is due to their ability to alter cell permeability properties (Silver & Kralovic 1969; Silver et al. 1970).

Fengycins are a class of antifungal lipopeptides from *Bacillus subtilis* that affect many filamentous fungi, e.g. they inhibit *A. solani* mycelial growth and conidia germination (Zhang et al. 2022). Fengycin A is composed of a long-chain β -hydroxy fatty acid which is conjugated to a decapeptide moiety (Sur et al. 2018), leading to an amphiphilic molecule like the acyl solamines. According to membrane intercalation simulations, lipopeptides like fengycins can integrate in membranes within milliseconds and provoke disorder on phosphatidylethanolamine, phosphatidylglycerole, and phosphatidylcholine membranes (fungal and microbial membranes) (Horn et al. 2013; Sur et al. 2018). Other short cationic lipopeptides (tetrapeptides with two lysines containing an *N*-terminal bound saturated fatty acyl moiety (C12–C16)) inhibited in vitro the growth of *Alternaria alternata*, *B. cinerea*, *Cochliobolus heterostrophus*, *Pseudomonas syringae*, and *Agrobacterium tumefaciens*. The authors observed severe effects of these

synthetic lipopeptides on the membranes of the pathogens including shrinkage, cell lysis, and spore lysis. These effects indicate membrane disruption as the main mode of action. The observed MICs were often between 3 and 50 μM in the liquid culture assays (Makovitzki et al. 2007). Due to the general similarity of the molecular structure and known bioactivities, the effect of long-chain fatty acyl solamines on membranes might be similar to that observed for cationic lipopeptides. The positively charged solamine head group could interact with negatively charged components of the cell membrane (e.g. phosphatidylglycerol) and insertion of the hydrophobic residue leads to disruption of the membrane integrity, collapse of the transmembrane electrochemical gradients, and finally cell death.

With such an unspecific mode of action, long-chain fatty acyl solamines are toxic for many types of cells and microorganisms including human cell lines (Silver et al. 1970), bacteria (Silver & Kralovic 1969), oomycetes (Gorzolka et al. 2025), and filamentous fungi (this study). It would be interesting to investigate how far long-chain fatty acyl solamines affect further pathogenic, but also beneficial microorganisms in their interaction with *S. bulbocastanum*. Differences in the sensitivity towards long-chain fatty acyl solamines are likely due to distinct general membrane compositions, the presence of repair mechanisms, or acyl solamine metabolizing enzymes.

The bioactivity of acyl solamines may also depend on the polyamine moiety. In fungal pathogens, polyamines regulate pathogenicity by mediating sporulation, appressoria formation, and effector production (Augustyniak et al. 2024; Jiménez-Bremont et al. 2014; Yi et al. 2024). Spermidine and spermine, two of the most prevalent polyamines in plants, both inhibited the spore germination and mycelial growth of *Fusarium oxysporum*, *Septoria linicola*, and *Alternaria* spp. in microdilution assays. Spermidine had the highest inhibitory effect with an IC_{50} value of 20 mg/L in vitro (Alves da Costa Ribeiro Quintans et al., 2022). Two spermidine analogues were shown to reduce the infection of host plants by *Erysiphe graminis* f.sp. *hordei*, *Uromyces viciae-fabae*, and *Podosphaera leucotricha* (Mackintosh et al. 1997). Coumaroyl and caffeoyl spermidine conjugates reduced mycelial growth of *Pyrenophora avenae* and powdery mildew in planta (Walters et al. 2001). Although the antifungal activity of spermidine or its analogues was clearly demonstrated, the mode of action of these polyamines has yet to be determined. The polyamine residue of acyl solamines might exhibit similar bioactivity. Biotests with unconjugated solamine (4,4'-bis(dimethylamino)dibutylamine) would provide more information and exclude membrane disruption as a mode of action. To our knowledge, no studies have been performed yet regarding this question.

Contribution of Fatty Acyl Solamines to Resistance of *S. bulbocastanum* against *A. solani*

Our work suggests that fatty acyl solamines contribute at least partially to the resistance of *S. bulbocastanum* WKS31741 *blb2G* against *A. solani*. While additional immune mechanisms may confer resistance in detached leaf assays, the performed in vitro assay clearly revealed the antifungal properties of long-chain fatty acyl solamines against *A. solani* and *B. cinerea*. The acyl solamines were extracted from healthy leaves obtained from plants grown in a climate chamber.

This indicates that high levels of acyl solamines were constitutively produced in leaf tissue of *S. bulbocastanum*. Therefore, in an attempt to infect *S. bulbocastanum* leaves, especially necrotrophic fungi such as *Alternaria* or *Botrytis* sp. could come in contact with a high concentration of fatty acyl solamines (1200 mg/kg) immediately after induced host cell lysis. This concentration is -according to our study- sufficient to inhibit fungal growth and proliferation, since already 350 mg/L was found to suppress growth in vitro. The investment of metabolic resources in the massive production of long-chain fatty acyl solamines in the leaf tissue might have increased *S. bulbocastanum* WKS31741 survival against pathogen attacks and led to adaptation due to natural selection. This hypothesis is supported by the absence of any steroidal glycoalkaloids in the leaves of this *S. bulbocastanum* accession. In *Solanaceae*, steroidal glycoalkaloids were often regarded as important resistance factors against pests and pathogens (Calf et al. 2018; Fabian et al. 2022; Milner et al. 2011; Wolters et al. 2023). The absence of these alkaloids in the leaves of *S. bulbocastanum* WKS31741 *blb2G* in favour of long-chain fatty acyl solamines may suggest a functional compensation and a prominent role in plant defence.

Potential Application of Fatty Acyl Solamines in Integrated Plant Protection

There is an urgent need for the reduction of fungicides and pesticides in potato crop protection while maintaining potato yields and quality. Currently, conventional potato production relies on the frequent use of PPPs (Adolf et al. 2020). This leads to the development of resistance in potato pests, limiting the number of effective active ingredients. Long-chain fatty acyl solamines could be part of integrated pest management strategies. They could be used as botanical fungicides or be co-formulated with common fungicides to act as emulsifiers and surfactants while providing additional antifungal activity. Such formulations could be useful for resistance management, as synergistic effects of fungicides and solamines could allow both concentrations to be reduced to a minimum. The biosynthetic pathway of long-chain fatty acyl solamines could be implemented in cultivated potatoes to produce plants with enhanced resistance against fungi, microbial pathogens, and insect pests. Unfortunately, *S. bulbocastanum* and *S. tuberosum* are incompatible and techniques such as somatic hybridization (Rakosy-Tican et al. 2015) or bridge crossing (Behling et al. 2024) will be required to overcome crossing barriers. Other wild potato species need to be screened for their ability to constitutively accumulate high levels of long-chain fatty acyl solamines in their leaves in order to identify compatible donors for breeding programmes. This would be a strategy not only for potato, but for *Solanaceae* crops in general. Breeders could use acyl solamines as metabolic compound resistance markers in breeding programmes, or the as yet unknown structural genes of acyl solamine biosynthesis could serve as genetic resistance markers. Further research on acyl solamines is needed to evaluate and implement such approaches in agriculture.

Conclusion

This study extends the knowledge on long-chain fatty acyl solamines from *S. bulbocastanum* leaves by demonstrating their antifungal activity against the necrotrophic phytopathogenic fungi *A. solani* and *B. cinerea*. The results suggest that long-chain fatty acyl solamines partially contribute to the resistance of *S. bulbocastanum* leaves to *A. solani* and *B. cinerea*, which has significant potential for application in potato breeding and sustainable agriculture.

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Data Availability All the data relevant to the experiments are included in the manuscript or as Online Resource.

Declarations

Conflict of interest The authors declare no competing interests.

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