Research Article

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Revisiting steroidal glycoalkaloids as hatching stimulants for *Globodera rostochiensis* and *Globodera pallida*

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Abstract

BACKGROUND: Potato production faces significant challenges from pests, particularly potato cyst nematodes (PCNs) such as *Globodera rostochiensis* and *Globodera pallida*. These nematodes are classified as regulated quarantine pests due to their detrimental effect on potato yields, yet populations continue to persist in the soil despite stringent control measures. PCNs can survive for long periods in the soil and hatch in response to root exudates containing hatching factors. The differences in hatching behavior and susceptibility between *G. rostochiensis* and *G. pallida* complicate management strategies, especially as the effectiveness of nematicides and resistant cultivars decline.

RESULTS: Steroidal glycoalkaloids (SGAs) can exhibit considerable hatching activity, and the hatching stimulatory effects of SGAs was shown to clearly differ between these two nematode species, including differences at gene expression levels.

CONCLUSION: Assessment of changes in *G. rostochiensis* and *G. pallida* relative hatching-related gene expression in response to SGAs provides further insight into their different responses to hatching stimuli.

Supporting information may be found in the online version of this article.

Keywords: plant parasitic nematodes; hatching; Solanaceae; steroidal glycoalkaloids; gene expression

1 INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's most important tuber crop and the third most important food crop, playing an important role in meeting food needs for human consumption.[1] The Food and Agriculture Organization of the United Nations (FAO) estimates that more than 375 million tons of potatoes were produced worldwide in 2022,[2] with China being the largest producer. Although Switzerland accounts for only a small share of global potato production (382 029 metric tons),[3] potatoes are the third most important arable crop in Switzerland, with a self-sufficiency of 90%, higher than any other crop.[4]

The main constraints to potato production are insects, nematodes, fungi and other pathogens. Nematodes alone are responsible for yield losses of up to 23% and are the most important potato pests. In particular, *Globodera rostochiensis* (Wollenweber) Behrens, and *Globodera pallida* (Stone) Behrens (potato cyst nematodes – PCNs) are responsible for large losses in the production of industrial, seed and staple potatoes, especially when there is a lack of management to control their spread.[5–7] Due to their devastating impact on potato production, PCNs are regulated quarantine pests in Switzerland and are under strict quarantine measures in most potato producing countries in the world.[8,9] Despite these regulatory measures to control PCNs, *G. rostochiensis* and *G. pallida* populations are still found in Swiss soils.[10]

PCNs can persist in the soil for many years, even in the absence of a suitable host, due to a combination of different survival systems like dormancy, diapause and quiescence.[11] Furthermore, as the name implies, a cyst protects the eggs, and hatching of second stage juveniles (J2s) from encysted eggs occurs mainly after exposure to host root leachate, which typically contains not only multiple hatching factors (HFs), but also hatching stimulants and hatching inhibitors.[12,13] Therefore, one approach to control PCNs is to stimulate early hatching of the encysted eggs[14] in the absence of their host plants. However, *G. rostochiensis* and *G. pallida* show different levels of spontaneous hatching, selectivity in response to HFs, optimal hatching temperature, and susceptibility to resistant potato varieties.[15] These differences between the two PCN species have selected for the presence of *G. pallida* in mixed populations,[10] and now growers face a serious problem as management of *G. pallida* by nematicides, resistant cultivars, or crop rotation is less effective than that of *G. rostochiensis*.[15] This, together

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with the phasing out of chemical nematicides, has led to an increased search for alternatives to control PCNs.

Artificial HFs such as sodium metavanadate and picrolonic acid, have been studied and stimulate hatching by different mechanisms.[16] While the former induces egg mortality, the latter does not. Sodium metavanadate is a HF for both species, whereas picrolonic acid is only active in *G. rostochiensis*.[17,18] Due to binding to soil particles, the use of picrolonic acid has been ruled out for use in the field, despite its ability to reduce PCN populations by up to 33%.[19]

Natural HFs from root leachates have been studied for many years, and the molecular weights and empirical formulas of several compounds have been determined,[20–22] including solanoeclepin A[5] and, more recently, solanoeclepin B.[23,24] In addition to some hormones and amino acids, steroidal glycoalkaloids (SGAs) such as α-solanine and α-chaconine show weak but still significant hatching stimulating activity.[13,25]

SGAs are secondary metabolites mainly produced by plants of the Solanaceae family, consisting of a nitrogen-containing aglycone (steroidal alkaloid) moiety derived from cholesterol (27-carbon backbone cholestane), classified as the solanidane (potato; Fig. 1(A)) or spirolosane (tomato and eggplant; Fig 1(B)–(D)) type, and a glycosidic moiety (carbohydrate side chain attached at the C3-OH position), which can consist of one to five saccharides (Fig. 1(E),(F)).[26–29] The variety of possible combinations of these groups creates structures with different reactivities, of which hundred have been described so far.[30–33]

Recently, the contribution of SGAs to the hatching stimulatory activity of *G. rostochiensis* was further investigated, and structure–activity relationship analysis revealed that the hatching stimulatory activity of SGAs depends on their glycone and aglycone moieties.[34] The aglycone moieties alone showed little hatching stimulatory activity, suggesting that the glycone moieties of the SGAs are necessary for the hatching stimulatory process. Shimizu *et al.*,[34] also proposed that chacotriose is more active than solatriose between the glycone moieties attached at the C3-OH position of the aglycones, while the stereochemistry in the rings E-F of the aglycone moiety also affects the hatching stimulating activity. Solasonine [(22*R*,25*R*)-spirolosane type (solasodine) + solatriose] stimulates higher hatching than α-solamarine [(22*S*,25*S*)-spirolosane type (dehydrotomatidine) + solatriose] when hatching of *G. rostochiensis* was evaluated. However, although the aim was not to evaluate the structure–activity, a recent publication investigating the effect of SGAs on hatching and reproduction of *G. pallida* shows the opposite, α-solamarine induced a higher hatching than solasonine, followed by α-solamargine [(22*R*,25*R*)-spirolosane type (solasodine) + chacotriose].[35]

In this sense, we aimed to further investigate the hatching stimulatory potential of SGAs on both *G. rostochiensis* and *G. pallida* and to evaluate the changes in the early onset of gene expression of selected genes involved in hatching in response to SGAs, in an

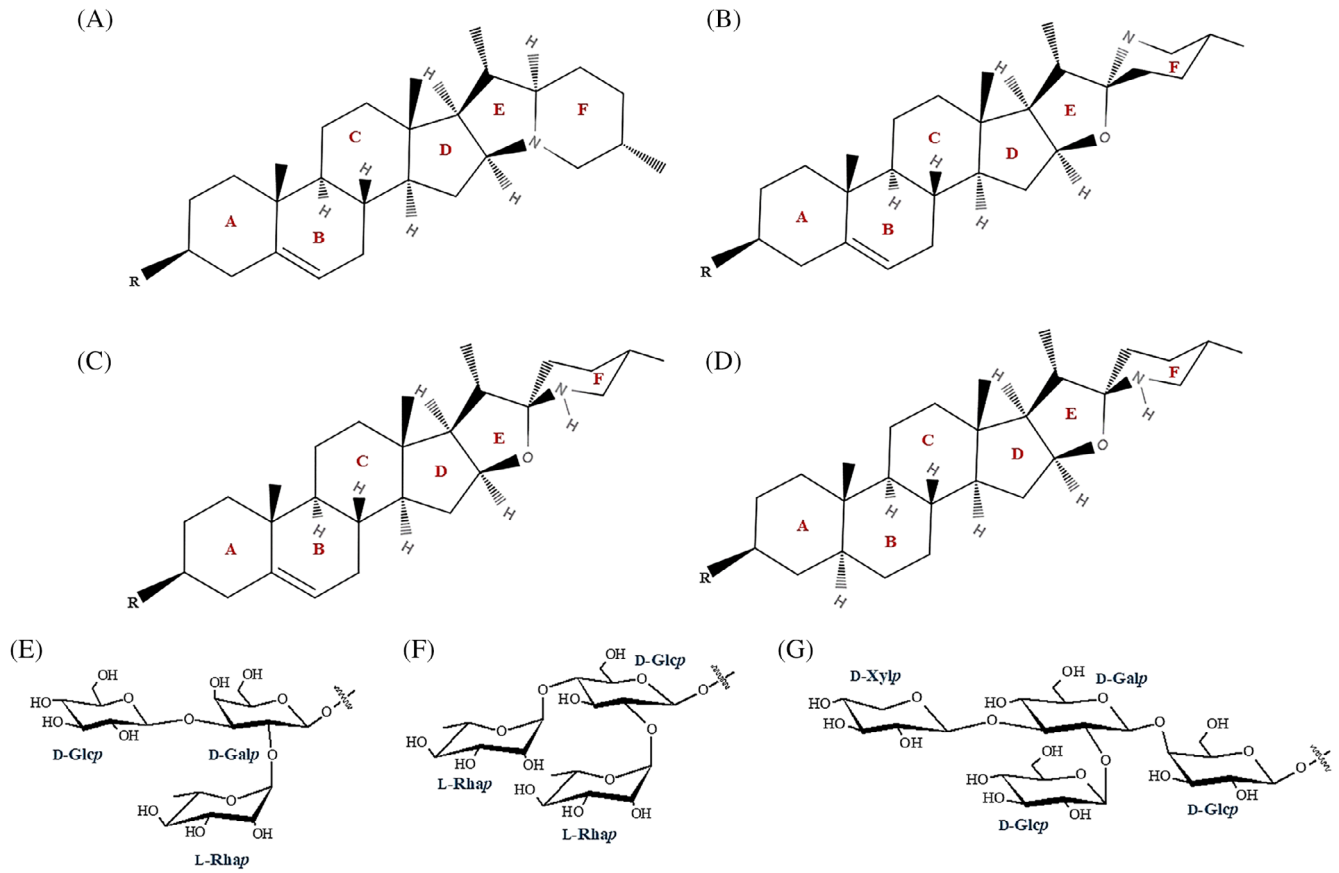

Figure 1. Structural diversity of the main steroidal glycoalkaloids found in potato, tomato and eggplant. Aglycone moieties: (A) solanidane type – solanidine, (B) (22*R*,25*R*)-spirolosane type – solasodine, (22*S*,25*S*)-spirolosane type – (C) dehydrotomatidine and (D) tomatidine. Glycosidic moieties: (E) {*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-D-galactose} – solatriose, (F) {*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-D-glucose} – chacotriose and (G) {*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-D-galactose} – lycotetraose. R indicates the C3-OH position where the glycosidic side chain is attached. Letters in blue (A–D) are used to denominate the rings in the steroidal alkaloid (aglycone) moiety.

attempt to understand how *G. rostochiensis* and *G. pallida* respond to different hatching stimuli.

2 MATERIALS AND METHODS

2.1 Nematodes

Populations of *G. rostochiensis* (pathotype Ro1) and *G. pallida* (pathotype Pa2/3) originating in Germany (VP ZK/S2016; Germany) were reared on potato cv. Désirée under glasshouse conditions set at 22 °C (±2 °C), 60% humidity and a day/night cycle of 16 h:8 h. Cysts from different generations (technical replicates; $n = 3$) of the same population (biological replicates; $n = 5$), not older than 1 year and with sizes between 300 µm and 500 µm were used for hatching assays.[36] Prior to use, cysts were pre-soaked on filter paper discs saturated with distilled water in Petri dishes at room temperature (~22 °C) for 7 days.[25]

2.2 Hatching factors (HFs)

Sodium metavanadate (Sigma-Aldrich, St Louis, MO, USA) and the SGAs: α-chaconine, α-solanine, α-solamargine, solasodine, tomatine, tomatidine (Extrasynthese, Lyon, France), and (25*R*)-3β{*O*-β-D-glucopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 4)-[*O*-α-L-rhamnopyranosyl-(1 → 2)]-α-L-rhamnopyranosyl}-22αN-spirosol-5-ene isolated from leaves of *Blumea lacera* (SGA-*Bl*)[37] (Fig. 2 (A)–(G), respectively), were used as HFs in the hatching stimulation activity assay.

2.3 Potato root exudate – production and extraction

For the production of potato root exudate, used as a positive control for hatching assays, ten tubers of the potato variety Désirée were individually planted 5 cm below the surface in 13 cm diameter pots filled with prewashed gravel. Five pots per tray (30 cm × 36 cm) were kept in a growth chamber at 23 °C ± 2 °C, 16 h photoperiod and 60% relative humidity. Sprouted, surface-sterilized tubers of similar physiological age and size were used. Four weeks after planting and the emergence of the first leaves, each pot was watered with 200 mL of 1% nutrient solution (Wuxal Super liquid NPK fertilizer, 8% N, 8% P_2O_5, 6% K_2O; Aglukon, Germany) every 2 days for 4 weeks. The exudates were collected at each nutrient application. The exudates from each of the ten pots were polled, filtered through paper filters and through 0.22 µm polyethersulfone (PES) filters (Sartolab® BT Vacuum Filtration), followed by rotary evaporation (30 °C) to reduce the initial volume (8000 mL) to 50 mL and stored at 4 °C for a maximum of 2 days.

Root exudate was then extracted by adding 2.5 mL (*v/v*) of charged Amberlite XAD-4 resin (Sigma Aldrich).[36] After 24 h incubation at 20 °C with regular gentle agitation, the resin was recovered by filtration, washed with distilled water to remove unbound material, and eluted with 30 mL of methanol. The eluent was rotary evaporated to dryness at 30 °C; the residue was suspended in 10 mL of distilled water, frozen, lyophilized,[38] and five concentration dilutions were prepared from a 1000-ppm stock solution (0.01, 0.1, 1.0, 10, and 100 ppm).

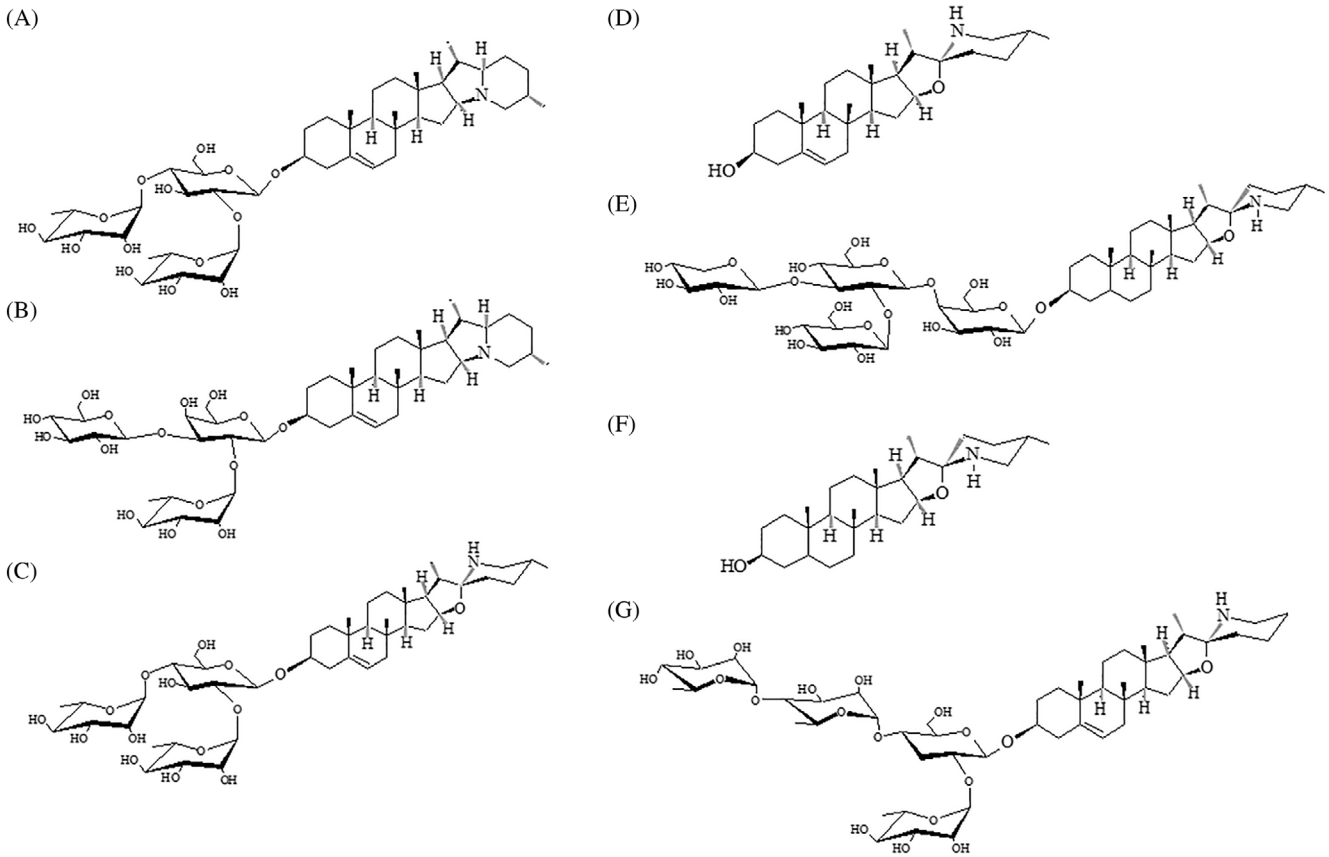

Figure 2. Chemical structure of the steroidal glycoalkaloids and steroidal alkaloids tested as hatching stimulants of *Globodera rostochiensis* and *Globodera pallida*. (A) α-Chaconine, (B) α-solanine, (C) α-solamargine, (D) solasodine, (E) tomatine, (F) tomatidine and (G) SGA-*Bl*.

2.4 Hatching-stimulation activity assay

Cysts of *G. rostochiensis* and *G. pallida* (five biological replicates and three technical replicates) were pre-soaked in water for 7 days (hydration) in the dark at 20 °C, followed by treatments with water, sodium metavanadate, potato root exudate or the earlier-mentioned SGAs, for 24 and 48 h. Treatments were performed in 48-well plates using 300 µL aqueous solutions of each of the HFs in five concentration dilution series prepared from a 10 000-ppm stock solution (0.1, 1.0, 10, 100, and 1000 ppm). Tap water was used as a negative control. Hatched J2s were counted under a light microscope.

2.5 Viability assay

At the end of the hatching assay, the cysts were crushed to release the unhatched eggs/J2 and 30 µL of a Nile blue A staining solution (10% aqueous dimethyl sulfoxide)[39] was added to the wells. For control, unstained eggs were mixed with a drop of distilled water. The plates were kept in the dark overnight. Viable (unstained) and non-viable (stained) eggs and J2s were counted under a light microscope. The hatching rate was calculated as a percentage [number of hatched J2/total number of eggs or J2 (hatched and unhatched) × 100] and the results are presented as the mean ± standard deviation, $n = 3$.

2.6 Principal component analysis (PCA)

Principal component analysis (PCA) was performed using the Principal Component Analysis Calculator.[40] The mean hatch rates obtained for the treatments with the different concentrations of the selected steroidal alkaloids and SGAs ($n = 3$) were used to compare the responses of *G. rostochiensis* and *G. pallida*.

2.7 Temporal gene expression analysis

For temporal gene expression analysis, ten cysts each of *G. rostochiensis* and *G. pallida*, were used per treatment: dry, soaked in water for 7 days (hydration), incubated in water or in 1 mg mL^{-1} solution of α-chaconine, α-solanine, α-solamargine, solasodine, sodium metavanadate or Désirée root exudate for 24 and 48 h. After each treatment, the cysts were snap frozen in liquid nitrogen and stored at −20 °C. Cysts were crushed in 1.5 mL tubes using a pestle (EK-10539; Thomas Scientific) and processed using the RNeasy Plus Micro Kit (Qiagen, Hilden, Germany) for RNA extraction. Extracted RNA was quantified using NanoDrop One One/OneC Microvolume Ultraviolet (UV)-Visible Spectrophotometer measurements (Thermo Fisher Scientific, Reinach, Switzerland) and quality assessed by agarose gel electrophoresis. Complementary DNA (cDNA) synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA).

Quantitative polymerase chain reaction (qPCR) Primers (Sigma-Aldrich, Switzerland) (Supporting Information Table S1) were designed from selected nucleotide sequences using Primer3web (version 4.1.0; Whitehead Institute for Biomedical Research). The primer software was set to achieve amplification sizes of 100–120 bp, a GC content of 45–60%, a primer sequence length of 20 nt, and a melting temperature of 63 °C. Housekeeping genes were selected from previously published work.[41] The specificity of the selected primer sequences for *G. rostochiensis* and *G. pallida* (Table S1) was confirmed *in silico* using the National Center for Biotechnology Information (NCBI) BLAST function and the WormBase database (version: WBPS15). Primer specificity was further tested by conventional PCR and fragments were visualized by agarose gel electrophoresis.

The qPCR analyses were performed according to the iTaq™ Universal SYBR® Green Supermix protocol (Bio-Rad) and optimized for a primer melting temperature of 63 °C on the Roche LightCycler 480. The Roche LightCycler 480 program was used to analyze and verify the melting peak and temperature for each qPCR run. Each experiment was normalized to the reference gene expression of *AMA-1* (AMAnitin resistance family member), *GR* (glutathione reductase) and *PMP-3* (putative ABC transporter). Relative changes in expression levels were analyzed in Excel using $2^{-\Delta\Delta Ct}$.[42]

2.8 Heat map

The Heatmapper online tool[43] was used to generate heat maps related to the relative gene expression analysis of *G. rostochiensis* and *G. pallida*. Hierarchical average linkage was used as the clustering method, and distance measurement was performed using the Euclidian algorithm. The relative gene expression levels of the selected genes were compared with those obtained for the dry cysts. From there, genes were presented as suppressed (pink to light pink) or overexpressed (light green to green).

2.9 Statistical analysis

Hatching rate and gene expression level results were analyzed using univariate one-way analysis of variance (ANOVA). Significant differences between treatments and controls were determined using Tukey's honestly significant difference (HSD) *post hoc* multiple range test at a 5% significance level and 95% confidence interval.

3 RESULTS AND DISCUSSION

3.1 Hatching and viability assay

Cysts used for hatching assays contained an average of 479 ± 8.08 and 384 ± 16.17 eggs/J2 per cyst, with viability of 97.8% ± 0.91% and 96.4% ± 2.64% for *G. rostochiensis* and *G. pallida*, respectively, consistent with reports of 300–700 eggs per cyst.[44–48] Egg viability ranged from 95.4% ± 3.37% and 97.5% ± 2.51% for *G. rostochiensis* and *G. pallida*, respectively.[49]

In general, potato and other Solanaceae plants are known to produce SGAs, their aglycones, and other classes of compounds,[50–52] including solanoeclepin A[5] and the recently reported solanoeclepin(s) B.[23,24]

Solanoeclepin A has been reported to be the most potent HF for PCNs. However, Vlaar *et al.*[23] showed that a root exudate fraction containing a compound with *m/z* 526.18 (solanoeclepin B), predicted to have a high structural similarity to solanoeclepin A, stimulated a higher hatching rate than the fraction containing only solanoeclepin A. Besides solanoeclepin(s), the SGAs α-solanine and α-chaconine are among the PCN HFs identified from potato root exudates.[13] Notably, hatching in response to these glycoalkaloids appears to be faster in *G. rostochiensis*,[25] potentially reflecting its preference for potato species with high glycoalkaloids content, as found in the Andean region.[53,54]

PCA of the hatching stimulation rates promoted by the tested steroidal alkaloids and SGAs showed that the differences were mainly explained by the first principal component (PC_1, 84.19%) (Fig. 3(A)). However, there was a strong difference in hatching stimulation between sodium metavanadate (blue cluster) and the SGAs with a solanidane type aglycone moiety (red cluster) or (22*S*,25*S*)-spirolosane type aglycone moiety (red cluster), compared to Désirée root exudate (orange cluster) and (22*R*,25*R*)-spirolosane type aglycone moiety (green cluster). No differences were observed with respect to PCN species, except for

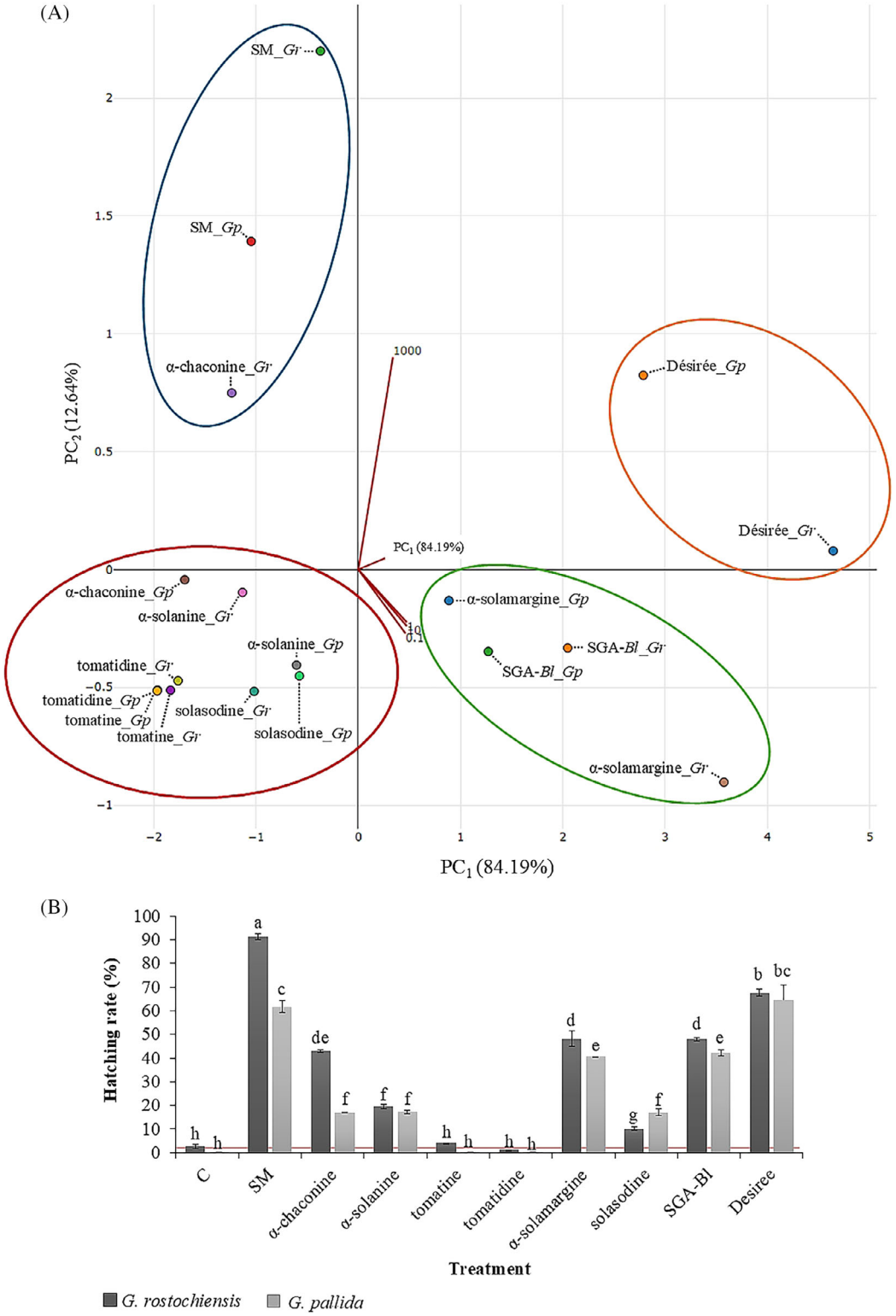

Figure 3. Hatching stimulatory activity of steroidal glycoalkaloids (SGAs) on *Globodera rostochiensis* and *Globodera pallida*. (A) Principal component analysis (PCA) plot showing general hatching differences (blue, red, green and orange oval shapes) between *G. rostochiensis* and *G. pallida* induced by treatments with different concentrations of sodium metavanadate (SM), α-chaconine, α-solanine, α-solamargine, solasodine, tomatine, tomatidine or SGA-*Bl* (0.1, 1.0, 10, 100 and 1000 ppm), and Désirée root exudate (0.01, 0.1, 10, and 100 ppm). (B) Percentage hatching rate of *G. rostochiensis* and *G. pallida*, in response to treatments with SM, α-chaconine, α-solanine, α-solamargine, solasodine, tomatine, tomatidine or SGA-*Bl* at a concentration of 1000 ppm, and Désirée root exudate at a concentration of 100 ppm. The dashed red line indicates the level of spontaneous hatching in tap water. Error bars represent standard deviations of replicates ($n = 3$). Statistical significance was calculated by one-way ANOVA with *post hoc* Tukey–Kramer HSD test, $P < 0.05$. Means followed by the same letter are not significantly different.

G. rostochiensis, which, when treated with α-chaconine clustered with both *G. rostochiensis* and *G. pallida* treated with sodium metavanadate (blue cluster) (Fig. 3(A)).

Overall, hatching rates followed expected trends.[34,35,52] *Globodera rostochiensis* exhibited higher spontaneous hatching (2.5% ± 0.97%) than *G. pallida* (0.1% ± 0.01%) in tap water control (Fig. 3(B)), although the difference was not statistically significant.

Désirée root exudate, a positive control, showed the highest hatching stimulation (68.3% ± 0.01% for *G. rostochiensis*; 67.6% ± 1.50% for *G. pallida*), with no statistically significant differences between both species. Désirée is the main variety used in different publications to collect root exudates for PCN hatching stimulation and is reported to contain medium-high levels of solanoeclepin A in its root exudate (~70 pmol g^{-1} of fresh weight).[55] This could partially explain the observed higher hatching rate, as solanoeclepin A has shown a remarkable hatching stimulating activity, especially to *G. rostochiensis*.[14,56] However, *G. pallida* has shown different hatching responses to solanoeclepin A. Sakata *et al.*[56] showed a hatching rate of approximately 20% when an egg solution was treated with solanoeclepin A at 10 ppb, which was much lower than the hatching rate achieved by treatment with tomato root diffusate. Guerrieri *et al.*[57] reported a hatching rate of approximately 80% for *G. pallida* eggs treated with solanoeclepin A at a concentration of 5000 pM (~2.5 ppb). This discrepancy may be attributed to differences in the *G. pallida* populations used in these studies. Guerrieri *et al.*[57] used an avirulent population of *G. pallida* (D383) from the Netherlands, whereas Sakata *et al.*[56] did not specify the pathotype or population of *G. pallida* used in their study.

While the levels of steroidal alkaloids or SGAs in potato root exudate are not specified, it is known that these compounds are present in root exudates of Solanaceae plants. Although SGAs exhibit a lower hatching stimulating ability compared to solanoeclepins, they remain important HFs for PCNs. The significant impact of SGAs structural complexity on PCN hatching underscores the need for further investigation, even in light of recent findings.[34]

At 1000 ppm, the hatch rate of most treatments, except α-solanine, tomatine, and tomatidine, was significantly different when *G. rostochiensis* was compared to *G. pallida* (Table S2). Sodium metavanadate induced 91.4% ± 1.22% and 61.8% ± 2.69% hatching in *G. rostochiensis* and *G. pallida*, respectively (Fig. 3(B)). Sodium metavanadate softens the PCN eggshell, making it more flexible prior to hatching. Unlike potato/tomato root leachates, which alter the permeability of the egg's three-layer membrane and release trehalose by activating osmotic changes through the diffusion of calcium ions into the soil.[58,59] This leads to water absorption through the egg membrane, initiating the hydration process,[59,60] which ends cyst diapause and leads to the hatching of J2s.[16,48,61]

Among the steroidal alkaloids tested, solasodine [(22*R*,25*R*)-spirolosane type; (10.1% ± 0.82% for *G. rostochiensis* and 17.1% ± 1.32% for *G. pallida*)] promoted a higher hatching rate than tomatidine [(22*S*,25*S*)-spirolosane type; (1.0% ± 0.03% for *G. rostochiensis* and 0.1% ± 0.04% for *G. pallida*)] (Fig. 3(B)). This demonstrates that, as proposed by Shimizu *et al.*[34] for *G. rostochiensis*, the stereochemistry of the rings E, F (Fig. 1) in the aglycone moiety alone, without interference from the glycosidic moiety, affects the hatching stimulatory activity of both *Globodera* species.

The SGAs, α-chaconine (42.8% ± 0.56% for *G. rostochiensis* and 17.0% ± 0.23% for *G. pallida*) and α-solanine (19.4% ± 1.00% for *G. rostochiensis* and 17.2% ± 0.81% for *G. pallida*) induced significantly higher hatching rates than the water control (2.5% ± 0.97% for *G. rostochiensis* and 0.1% ± 0.01% for *G. pallida*) (Fig. 3(B)). Contrasting with the findings of Byrne *et al.*,[25] where only *G. rostochiensis* hatching was significantly different from the water treatment. As α-chaconine and α-solanine share the same aglycone moiety (solanidane-type) but have different glycosidic moieties attached to the C3-hydroxyl group of the aglycone, chacotriose and solatriose, respectively, their difference in hatching stimulation can be attributed to the influence of the glycosidic moieties. Chacotriose is responsible for a higher hatching stimulation than solatriose,[34] especially for *G. rostochiensis*, while no significant difference is observed for *G. pallida* (Table S1). α-Chaconine can bind to the hatching receptors at lower concentrations, suggesting it has a more suitable configuration than α-solanine, which must be saturated to bind to the hatching receptors.[52] Since, both share the same aglycone moiety, the type and configuration of the glycosidic moieties (e.g., chacotriose – {*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-D-glucose} for α-chaconine, and solatriose – {*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-D-galactose} for α-solanine) may determine the ability of SGA to bind to receptors and stimulate hatching. The rhamnose moiety of SGA has been shown to be important for other biological activities, such as anticancer activity. By comparing SGA with and without rhamnose moieties, it has been shown that rhamnose affects the dihedral angle of the glycosidic linkage, which influences binding to the receptor sites of cell membranes and thus enhances biological activity.[62–65] The influence of rhamnose may also influence the hatching stimulating activity of PCNs, where, α-chaconine (with two rhamnose residues) has shown higher potency than α-solanine (with one rhamnose residue).

Among the SGAs with a spirolosane-type aglycone moiety, α-solamargine and the SGA-*BI* induced higher hatching rates in both, *G. rostochiensis* (48.2% ± 3.42% and 47.9% ± 0.83%, respectively) and *G. pallida* (40.6% ± 0.10% and 42.1% ± 1.27%, respectively), even at the lower concentrations tested (Supporting Information Fig. S1 and Table S1), with no significant differences between the two treatments, but significantly different between the two *Globodera* species (Fig. 3(B) and Table S1). Since both SGAs consist of a (22*R*,25*R*)-spirolosane type aglycone moiety and differ only slightly in the glycosidic moiety, it can be assumed that in this particular case the difference in the structure of the glycosidic moiety (addition of an α-L-rhamnopyranosyl unit) did not play a role in influencing the hatching rate. For *G. pallida*, α-solamargine hatching rates were consistent with those reported by Sivasankara Pillai and Dandurand[35] at the same concentration (100 ppm; Fig. S1) and were not significantly different from hatching rates induced by solasonine [(22*R*,25*R*)-spirolosane type (solasodine) + solatriose]. This suggests that in the case of SGAs with a (22*R*,25*R*)-spirolosane type aglycone, the influence of the glycosidic moiety does not appear to affect hatching stimulation as observed for SGAs with a solanidane-type aglycone moiety. However, solasonine has been reported to stimulate the highest hatching rate in *G. rostochiensis* (~80% at a concentration of 5 μM).[34] However, unlike most other studies, Shimizu *et al.*[34] evaluated the hatching stimulatory effect on an egg suspension, making comparison to 'natural' cysts difficult.

Although the hatching stimulation rates promoted by SGAs are considered to be 'low', a better understanding of how the structural differences of the hatching inducing or inhibiting compounds affect hatching in *G. rostochiensis* and *G. pallida* is needed. Torto *et al.*[48] suggest that SGAs, such as α-chaconine

and α-solanine, may induce PCN hatching in a manner similar to solanoeclepin A and glycinoeclepins A, B, and C due to their highly oxygenated structures. This property would allow water to be bound, and consequently small amounts of these compounds would be absorbed through the egg membrane and induce hatching. However, several other variables may influence the ability of these compounds to induce PCN hatching. For example, solanoeclepin A stimulated approximately 80% hatching of *G. rostochiensis* eggs at a 10 ppb, but only approximately 20% hatching stimulation was achieved for *G. pallida* at the same concentration.[56] However, when the avirulent *G. pallida* D383 population was tested, up to approximately 80% hatching stimulation was achieved at 5000 pM (~2.5 ppb),[57] suggesting that hatching stimulation may depend on the pathotype and/or virulence status of the *Globodera* species. In contrast, no such trend has been observed for SGAs, although plant developmental stages appear to influence metabolite concentrations in the rhizosphere, for example, α-solanine is present in higher concentrations in plants at early developmental stages.

It can be said that recent publications[34,35,48,52] have shed some light on the structure–activity relationship of glycoalkaloids and aglycones on hatching stimulation/inhibition of *G. rostochiensis* and *G. pallida*. However, *in vitro* hatching assays still lack the full complexity that exists in the field[66]: soil type, temperature, rhizosphere microbiome, host plant cultivar, plant developmental stage, humidity, and the natural differences between *G. rostochiensis* and *G. pallida* that affect population dynamics, pathotype/virulence, among others.

3.2 Gene expression analysis

PCN hatching involves a complex series of physiological and behavioral changes, including increased eggshell permeability, activation of the larval state, and eclosion,[67,68] likely reflected in early gene expression alterations. Differences in *G. rostochiensis* and *G. pallida* sensitivity to specific SGAs or variations in their gene regulatory networks, may lead to species-specific gene expression profiles. Understanding these gene expression changes, and the differential responses to SGAs or other HFs, could aid in the development of targeted management strategies. However, few studies have been dedicated to understanding the gene expression changes associated with the hatching process,[68–71] mainly because there are still many gaps in knowledge regarding the genes involved in the onset of the hatching process. To address this, 21 genes (Table S1) involved in different aspects of the hatching process were analyzed in *G. rostochiensis* and *G. pallida*.

The $2^{-\Delta\Delta Ct}$ values obtained for dry cysts of *G. rostochiensis* and *G. pallida* were used as a base line for the different treatments. Changes in the expression of the selected genes, as shown in the heat map (Fig. 4), are expressed relative to the gene expression of dry cysts. Overall, most genes showed low (pink to light pink) to moderate (light green) expression, with only a few showing increased expression (green, for both *G. rostochiensis* and *G. pallida*, *exp-B3* showed the strongest expression among all treatments Fig. 4(A),(B), respectively).

In *G. rostochiensis*, *hcdh*, and *adh* were strongly expressed after solasodine and α-solanine treatments, while *prdx-2* and *NEP-1* were generally suppressed. Hydration alone suppressed most genes but triggered marked increases in relative gene expression, such as for *exp-B3* (Fig. 4(A)), along with *hcdh*, *eng* and *bgal*-1 at 48 h. α-Solanine, solasodine and α-solamargine significantly upregulated oxidative stress-related and cell expansion genes at both time points. Whereas α-chaconine and hydration caused initial suppression, followed by a gradual recovery, especially for genes like *sod*. Sodium metavanadate affected *adh* similarly to α-solanine and hydration. Désirée root exudate, used as a control, mildly suppressed key genes involved in antioxidant defense (*sod*, *prdx-2*) and cell wall degradation (*cht-2*, *eng*), with minimal effect on lipid metabolism (*elo-3*). It also activated the expression of *btb/*

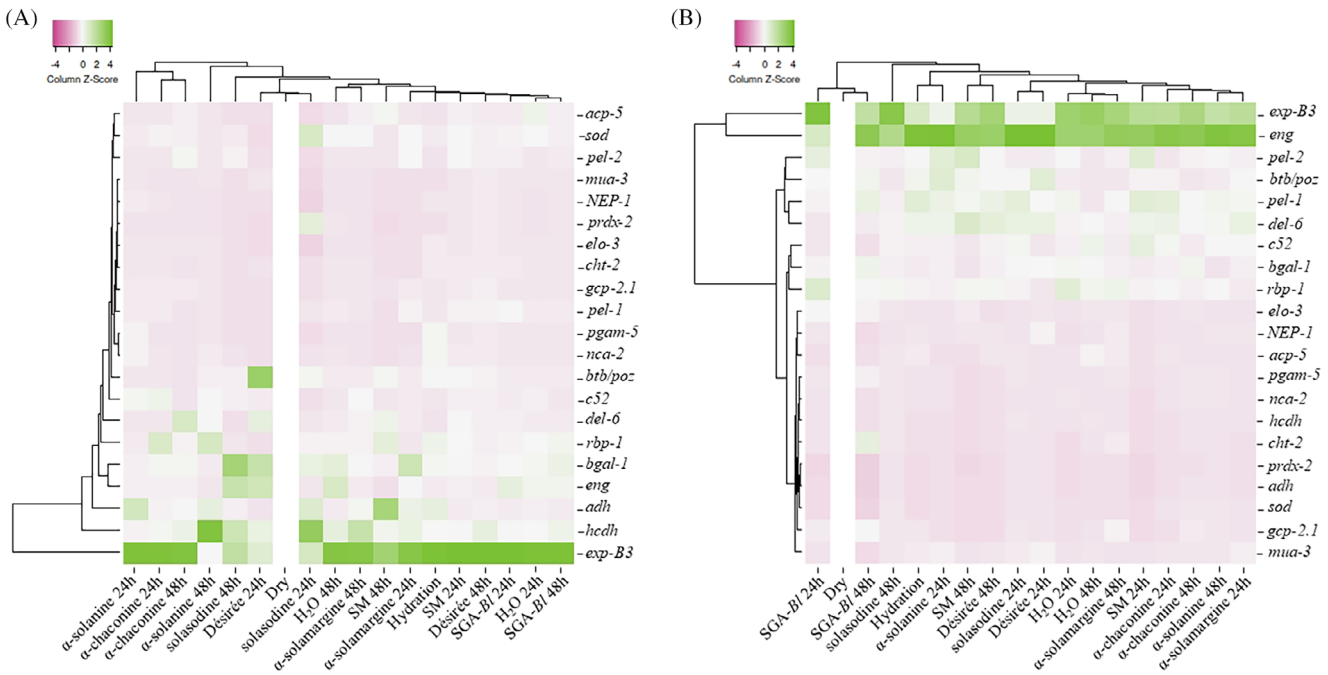

Figure 4. Heat map showing expression data for selected genes (rows) and treatments (columns), for *Globodera rostochiensis* (A) and *Globodera pallida* (B). Expression values, for 24 h or 48 h, are expressed relative to the reference genes (listed in Supporting Information Table S2) and sample (ΔΔCt).

btz, a gene involved in several developmental processes, which was repressed after 48 h.

In *G. pallida*, *eng*, *pel-2*, and *btb/btz* showed strong expression in many of the treatments, especially in dry cysts and in response to SGA-*Bl*. In contrast, *pgam-5, acp-5, elo-3*, and *NEP-1* were more suppressed, especially after treatments with α-chaconine at 24 h and α-solanine at both 24 and 48 h (Fig. 4(B)). As in *G. rostochiensis*, hydration initially suppressed many genes, but after 48 h, some genes, like *cht-2* and *eng* showed increased expression. Responses to α-solanine, α-chaconine and α-solamargine varied, but *exp-B3* and *eng* showed significant up-regulation (Fig. 4(B)). Unlike *G. rostochiensis*, *G. pallida* showed more stable or modest changes in gene expression after Désirée root exudate treatment.

3.3 Comparative analysis of relative gene expression and biological context

PCNs have coevolved with their hosts, allowing them to develop remarkable strategies to ensure their reproductive success and survivability. By synchronizing their hatching with the presence of suitable hosts, *G. rostochiensis* and *G. pallida* can survive in a desiccated state in the soil for decades.[72,73] During cyst dormancy (desiccated state), reactive oxygen species (ROS) detoxification mechanisms are impaired, leading to up-regulated enzymatic antioxidant pathways.[68] The antioxidant system in nematodes is synergistically operated by superoxide dismutase (SOD), catalase, glutathione peroxidase and peroxiredoxin. While SOD catalyzes the dismutation of superoxide anion (O_2^-) in oxygen (O_2) or hydrogen peroxide (H_2O_2), the other radical scavenging enzymes are responsible for the detoxification of $H_2O_2^-$.[74] To compensate for the oxidative stress caused by desiccation, Duceppe *et al.*[68] found that several antioxidant enzymatic pathways, including *sod* and *adh* (alcohol dehydrogenase), were up-regulated in dry cysts compared to hydrated cysts. Similarly, *sod* and *prdx-2* were up-regulated in *G. rostochiensis* and *G. pallida* dry cysts. However, after treatment with α-chaconine and α-solamargine for 48 h, *sod* expression was significantly up-regulated in *G. rostochiensis*, but down-regulated in most treatments, especially in *G. pallida* (Fig. 5(A)). While *prdx-2* expression appeared to be suppressed in all different treatments in both species (Fig. 5(B)).

The gene expression of alcohol dehydrogenase class 3 (*adh*) was also significantly up-regulated in *G. rostochiensis* after hydration, as well as after treatment with α-solanine for 24 h and with sodium metavanadate, α-chaconine and α-solanine for 48 h (Table S3). Alcohol dehydrogenases are involved in alcohol metabolism, catalyzing the conversion of alcohols to aldehydes or ketones. While the function of alcohol dehydrogenase in plant-parasitic nematodes, including *Globodera* spp., is poorly understood, in *Caenorhabditis elegans*, alcohol dehydrogenase is involved in detoxification, metabolism, and response to oxidative stress.[75]

The differences in *sod* and *adh* expression between *G. rostochiensis* and *G. pallida* may reflect species-specific oxidative stress management mechanisms. Notably, the *G. pallida* genome contains an expanded family of ten SOD genes,[76] suggesting a potentially more complex oxidative stress response system. The up-regulation of *sod* and *adh* in *G. rostochiensis* may indicate that *G. rostochiensis* experiences a more immediate or intense oxidative stress response upon exposure to HFs, than *G. pallida*. *Globodera pallida* by instance may either not experience the same level of oxidative stress or may rely on different pathways or timing to manage ROS impairment. Peroxiredoxin is involved in the reduction of H_2O_2 to water, a step that typically follows the action of SOD. Therefore, the suppression of *prdx-2* expression in both species could indicate that H_2O_2 detoxification is not required within 24–48 h of exposure, or it may be managed by other enzymes, such as the activity of catalase or glutathione peroxidase.

In *G. rostochiensis* and *G. pallida*, hydration during the transition from dormant cyst to active hatching triggers significant gene expression changes, activating metabolic pathways that prepare the nematode larvae for hatching and parasitism. Oxidative stress-related genes are expected to be up-regulated to mitigate rehydration-induced oxidative damage. However, after the hydration period (7 days) used in the experimental setup, only *adh* was up-regulated in *G. rostochiensis* (Table S3), suggesting that oxidative stress-related gene expression may peak earlier. Whereas in *G. pallida* there is evidence of delayed oxidative stress gene expression,[76] which may contribute to its slower hatching response compared to *G. rostochiensis*.

Genes involved in calcium metabolism, essential for PCN hatching, have been reported to be up-regulated in response to hydration.[68] Some of these genes were found to be significantly up-regulated only in *G. rostochiensis* (*nca-2*), while others were found to be up-regulated only in *G. pallida* (*mua-3*) (Fig. 6).

Up-regulation of both *nca-2* and *del-6* may play a role by restoring cell membrane permeability to calcium.[68] In *G. rostochiensis*, *nca-2* was up-regulated after hydration and α-solanine treatment (24 h), indicating its involvement in early stages of hatching and stress responses (Fig. 6(A)). Meanwhile, *del-6* was up-regulated after α-chaconine treatment (48 h) in *G. rostochiensis* and after treatment with sodium metavanadate, α-chaconine, α-solanine, solasodine, α-solamargine and Désirée root exudate for 24 h and with sodium metavanadate, α-solanine, solasodine and Désirée root exudate for 48 h in *G. pallida*, suggesting a role in calcium flux regulation under cellular stress (Fig. 6(B)). Given SGAs known ability to affect membrane integrity,[35,62–64,77] their influence on *del*-6 gene expression supports its potential function in maintaining cellular balance. Additionally, *mua-3*, which has predicted calcium ion binding activity,[72] was up-regulated in *G. pallida* following hydration and treatments with α-solanine, α-solamargine, and Désirée root exudate (24 h) and with sodium metavanadate (48 h) (Fig. 6(C)). This suggests its role in calcium homeostasis and possibly developmental like cytoskeletal organization or muscle function, as reported in *C. elegans*.[78] The differential expression of calcium metabolism-related genes between *G. rostochiensis* and *G. pallida* highlights species-specific mechanisms in calcium regulation mechanisms, which could be explored for targeted pest control strategies.

One gene that appeared to be up-regulated in all different treatments was *exp-B3*, with the highest fold change in *G. rostochiensis* after α-solanine treatment (24 h) and in *G. pallida* after solasodine treatment (48 h) (Fig. 7(A)). Expansin B3, encoded by the gene *exp-B3*, is best known for its role in facilitating plant cell wall modification during nematode invasion.[79] While there is no direct evidence linking *exp-B3* expression to hatching of *Globodera* spp., its critical role comes into play after the hatching, where expansin helps newly hatched juveniles penetrate host plant roots.[80] Therefore, it is likely that the gene is overexpressed in synchrony with hatching to prepare the nematode for effective parasitism. However, *eng*, encoding a beta-endoglucanase, a key effector for host root infection,[81] appeared to be differentially expressed in *G. rostochiensis* and *G. pallida*. In *G. pallida*, *eng* was up-regulated in all treatments, except SGA-*Bl*, while in *G. rostochiensis*, it was only up-regulated after water and solasodine treatments (48 h) (Fig. 7(B)). Beta-endoglucanases, the first effectors characterized

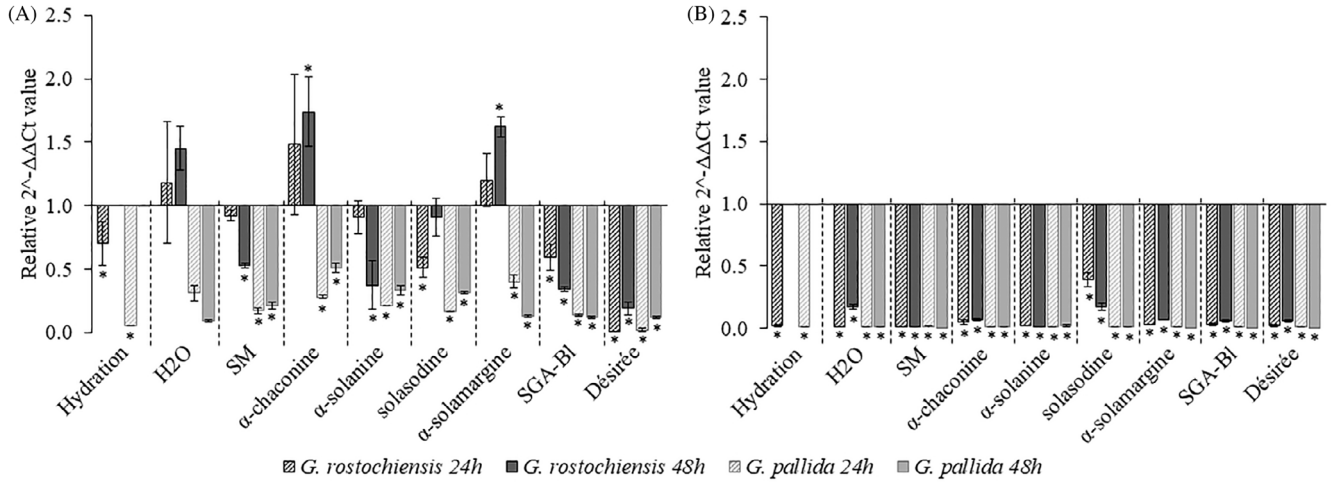

Figure 5. *Globodera rostochiensis* and *Globodera pallida sod* (A) and *prdx-2* (B) relative gene expression ($2^{-\Delta\Delta Ct}$ value) in response to different treatments: hydration (water 7 days), water, sodium metavanadate (SM), α-chaconine, α-solanine, solasodine, α-solamargine, SGA-*Bl* at a concentration of 1000 ppm, and Désirée root exudate at a concentration of 100 ppm, for 24 h or 48 h. The $2^{-\Delta\Delta Ct}$ values were normalized with respect to the relative gene expression in dry cysts (shown as line on *x*-axis). Up-regulated genes appear as bars above the *x*-axis, while down-regulated genes appear as inverted bars below the *x*-axis. Error bars represent the standard deviation of replicates ($n = 3$). Statistical significance (*) was calculated by one-way ANOVA with *post hoc* Tukey–Kramer HSD test, $P < 0.05$ (Supporting Information Table S3).

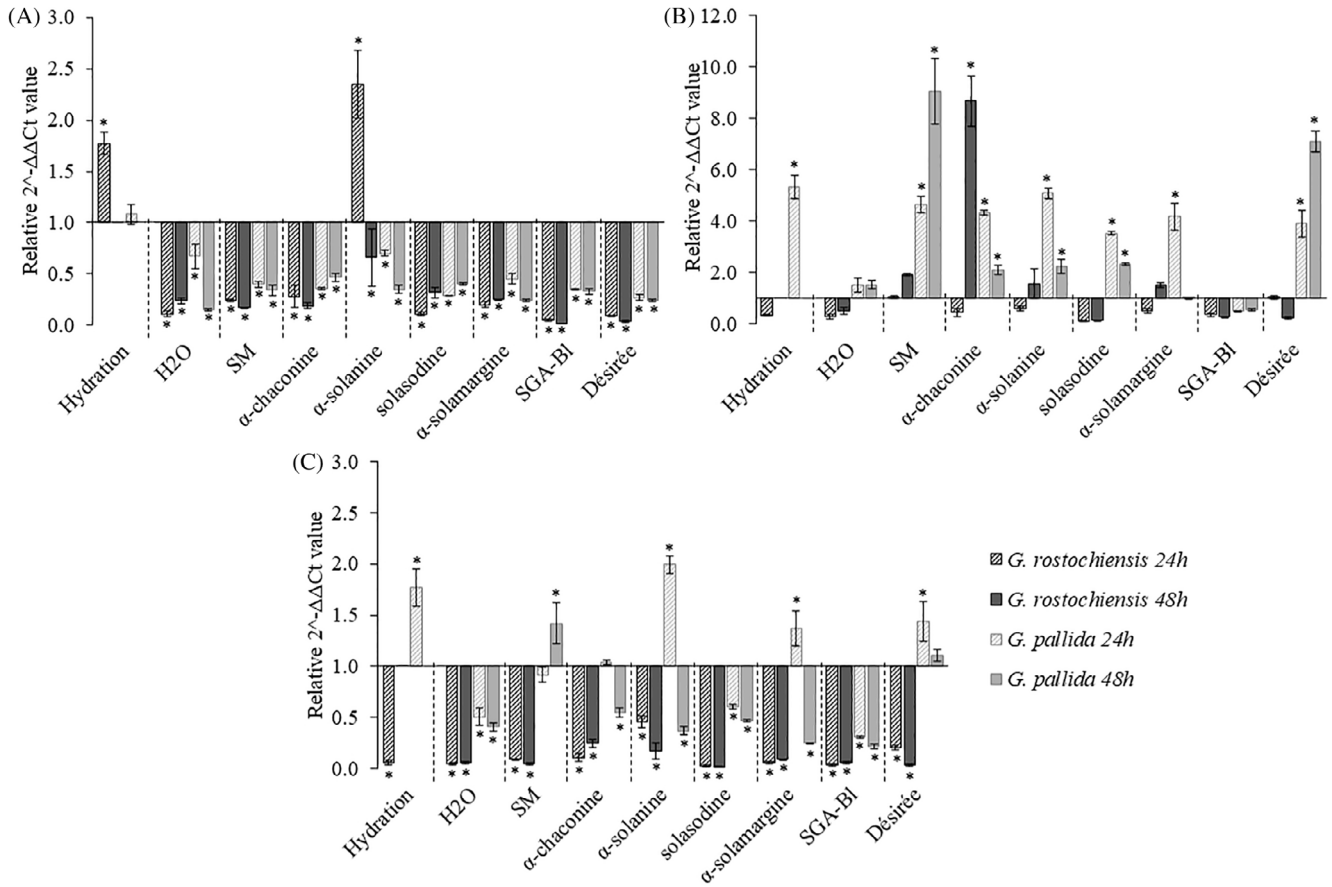

Figure 6. *Globodera rostochiensis* and *Globodera pallida nca-2* (A), *del-6* (B), and *mua-3* (C) relative gene expression ($2^{-\Delta\Delta Ct}$ value) in response to different treatments: hydration (water 7 days), water, sodium metavanadate (SM), α-chaconine, α-solanine, solasodine, α-solamargine, SGA-*Bl* at a concentration of 1000 ppm, and Désirée root exudate at a concentration of 100 ppm, for 24 h or 48 h. The $2^{-\Delta\Delta Ct}$ values were normalized with respect to the relative gene expression in dry cysts (shown as line on *x*-axis). Up-regulated genes appear as bars above the *x*-axis, while down-regulated genes appear as inverted bars below the *x*-axis. Error bars represent the standard deviation of replicates ($n = 3$). Statistical significance (*) was calculated by one-way ANOVA with *post hoc* Tukey–Kramer HSD test, $P < 0.05$ (Supporting Information Table S4).

from plant parasitic nematodes,[82] are part of a broader family of cell wall degrading enzymes, typically secreted by the nematode esophageal glands, that aid in the degradation of plant cell wall components during infection and facilitate nematode invasion.[81] Although primarily associated with host invasion, such enzymes may also play a role in hatching, as their up-regulation has been observed in *G. rostochiensis*[83] and *G. tabacum*[84] eggs before hatching.

In addition to beta-endoglucanase, Duceppe *et al.*[68] reported arabinogalactan endo-beta-galactosidase and beta-levanase invertase as additional polysaccharide-degrading enzymes up-regulated in *G. rostochiensis* after exposure to potato root diffusate for 24 h and/or 48 h. Arabinogalactan endo-beta-galactosidases hydrolyze arabinogalactans found in the cell wall of dicotyledonous plants and have so far been found to be specific for cyst nematodes (*Globodera* and *Heterodera* spp.), but not in root-knot nematodes (*Meloidogyne* spp.).[76,85] Relative gene expression analysis showed that *bgal-1*, encoding an arabinogalactan endo-beta-galactosidase, was up-regulated in *G. rostochiensis* after treatment with α-chaconine and α-solamargine for 24 h and with water, α-chaconine and solasodine for 48 h. In *G. pallida*, *bgal-1* was up-regulated following treatment with Désirée root exudate for both 24 h and 48 h, as well as after α-chaconine treatment for 48 h (Fig. 7(C)).

The gene *cht-2*, encoding a chitinase, was up-regulated only in *G. pallida* after treatment with SGA-*Bl* for 48 h, whereas its relative expression was suppressed under all other treatments (Fig. 7(D)). This contrasts with findings by Duceppe *et al.*,[68] where *cht-2* was up-regulated at both 24 h and 48 h after *G. rostochiensis* cysts were exposed to potato root diffusate. Chitinases are responsible for degrading chitin in the eggshells of plant-parasitic nematodes, a process that could be critical for hatching by allowing juvenile larvae to emerge from the cysts and initiate the infection process in the host plants. However, studies have shown that PCN eggshells remain rigid even after hatching.[86] In addition, commercial chitinase and chitinase-producing bacteria have been reported to reduce hatching of *G. rostochiensis*.[87] These findings suggest that chitinase may not play a direct role in the PCN hatching process.[54]

Pectate lyases are another class of well-characterized cell wall degrading enzymes produced by plant parasitic nematodes. They are responsible for degrading pectin, a key component of the plant cell wall, thereby facilitating the invasion of the plant roots by J2s.[88,89] Pectate lyase genes have been reported to be highly expressed in both *G. rostochiensis*[68] and *G. pallida*[71] after treatment with potato or tomato root diffusate, respectively. The relative expressions of *pel-1* and *pel-2* varied between *Globodera* species and treatments (Fig. 7(E),(F), respectively). In *G. rostochiensis, pel-1* was slightly suppressed especially after 48 h of treatment with the different selected hatching compounds (Fig. 7(E)), while *pel-2* was up-regulated only after treatment with α-chaconine for 48 h (Fig. 7(F)). In contrast, *pel-1* was up-regulated in *G. pallida*, after most treatments (Fig. 7(E)), whereas *pel-2* was up-regulated after hydration, treatment with sodium metavanadate for 24 h and 48 h, α-solanine and SGA-*Bl* for 24 h, and Désirée root exudate for 48 h (Fig. 7(F)). Previous studies have localized *pel*-1 expression to the sub-ventral esophageal gland of juvenile nematodes after hydration,[90] while *pel-2* has been associated to exposure to potato root exudates.[89]

Another effector gene, *rbp*-1 was overexpressed in *G. rostochiensis* after treatment with α-chaconine for 24 h and α-solanine for 48 h. In *G. pallida*, *rbp-1* was up-regulated after exposure to water for 24 h and after treatment with sodium metavanadate, solasodine and Désirée root exudate for 48 h (Fig. 7(G)). RBP-1, a member of the *Globodera* effector protein family, is secreted by nematodes during infection and plays a role in suppressing the host immune response.[91] RBP-1 has been shown to suppress defense-related responses by modulating plant signaling pathways, and to interact with key plant defense proteins, such as Gpa2 in potato, a known resistance gene against *Globodera*.[92] This allows the nematode to establish a feeding site (syncytium) in the vascular cylinder of the potato roots, without triggering strong plant defense mechanisms. Duceppe *et al.*,[68] reported that *rbp-1* is up-regulated after 48 h exposure to potato root diffusate, and recorded 66 different transcripts with RBP-1 BLAST results in *G. rostochiensis*, suggesting a high genetic diversity for the *rbp-1* gene.[93]

Other genes differentially expressed between *G. rostochiensis* and *G. pallida* were *hcdh* and *btb/poz*. While *hcdh* was overexpressed in *G. rostochiensis* after cysts were treated with water, α-chaconine, α-solanine, solasodine, and α-solamargine for 48 h, *btb/poz* was mainly overexpressed in *G. pallida* after hydration or treatment with α-chaconine, α-solanine, and α-solamargine for 24 h. Additionally, *btb/poz* was up-regulated in *G. pallida* following 24 h and 48 h treatment with sodium metavanadate and Désirée root exudate. Désirée treatment for 24 h, induced also an up-regulation in *G. rostochiensis* (Table S6). The gene *hcdh*, encodes an enzyme involved in fatty acid metabolism and mitochondrial energy production,[94] potentially increasing metabolic activity to meet the energy demands required for larval development, thereby facilitating hatching. In contrast, *btb/poz* encodes BTB/POZ domain-containing proteins, which mediate protein–protein interactions and play roles in various biological processes, including transcriptional regulation, cytoskeletal dynamics and ubiquitin-mediated proteolysis.[95] In *C. elegans*, BTB/POZ proteins regulate developmental pathways, such as molting.[96] This suggests that this class of proteins may be involved in regulating developmental transitions such as the first molt from first stage juvenile (J1) to the infective J2, which occurs inside the egg, or the subsequent release of larvae from the cyst. In this way, the overexpression of such genes may affect transcriptional networks that control larval development and readiness to hatch.

The other genes studied, *elo-3*, *pgam-5*, *acp-5*, and *gcp-2.1* were mainly suppressed by the different treatments. However, *c52* was notably up-regulated, especially in *G. pallida* under the different conditions and in *G. rostochiensis* after 24 h treatment with α-chaconine and α-solanine (Table S6). The functions *elo-3*, *pgam-5*, *acp-5*, *c52*, and *gcp-2.1* in *Globodera* species remain unclear. In *C. elegans*, ELO-3 regulates lifespan via behenic acid synthesis,[97] making its direct role in hatching unlikely.

Several important cellular processes, including mitophagy, apoptosis, and oxidative stress response, are regulated by a mitochondrial serine/threonine protein phosphatase encoded by *pgam-5* in *C. elegans*.[98,99] The overexpression of *pgam-5* in *G. rostochiensis* after hydration and α-solanine treatments suggests a role in mitigating oxidative stress and preparing the nematode for increased mitochondrial activity required for hatching. However, the suppression of gene expression observed for the other treatments in *G. rostochiensis* and *G. pallida* may reflect a reduced reliance on *pgam-5* function in mitigating oxidative stress.

Acid phosphatases are lysosomal enzymes involved in phosphate metabolism and cellular degradation in *C. elegans*. In *Globodera*, *acp-5* overexpression may support nutrient for hatching, whereas its suppression across multiple treatments suggests alternative nutrient mobilization mechanisms.

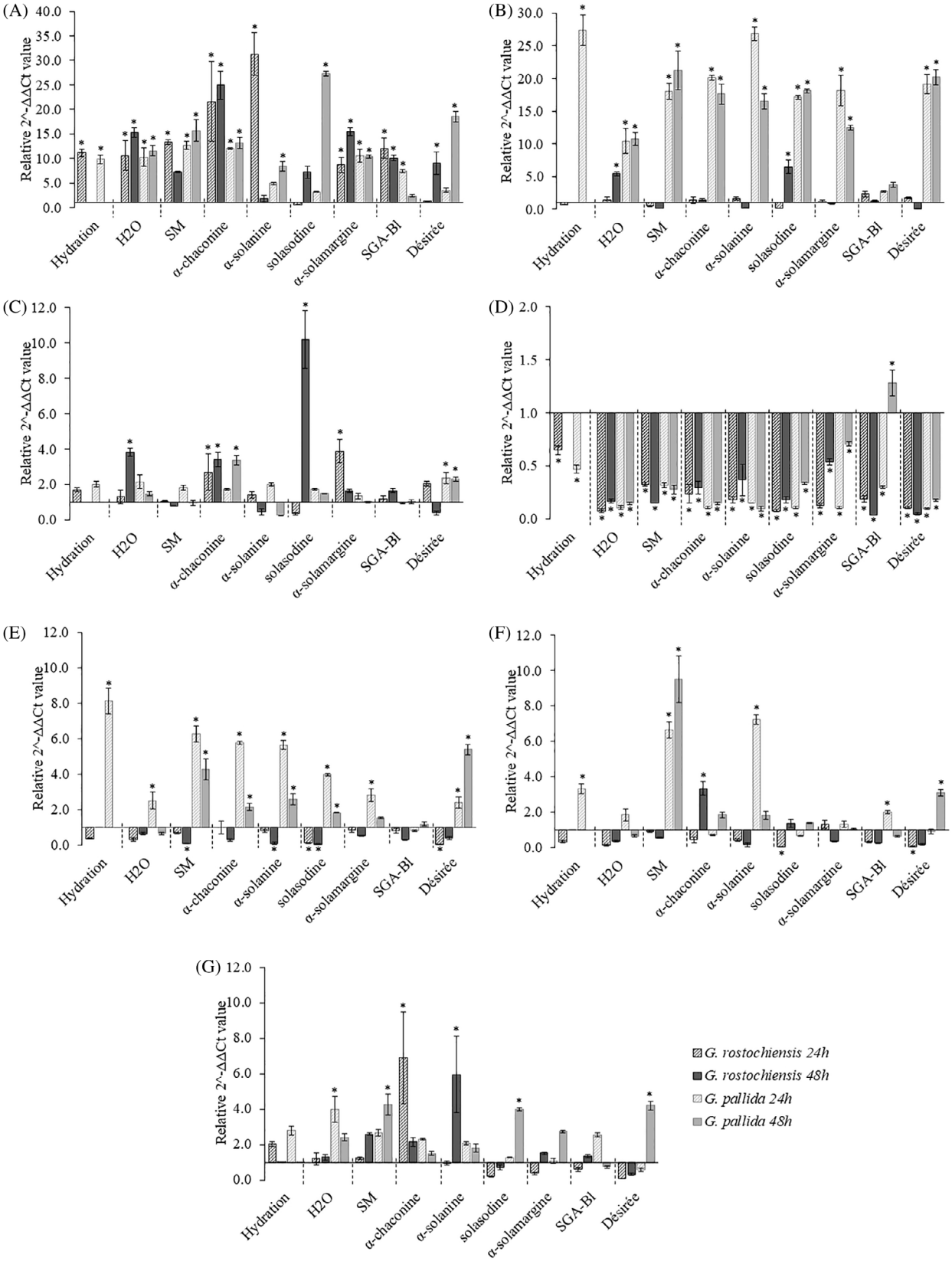

Figure 7. *Globodera rostochiensis* and *Globodera pallida exp-B3* (A), *eng* (B), *bgal-1* (C), *cht-2* (D), *pel-1* (E), *pel-2* (F), and *rbp-1* (G) relative gene expression ($2^{-\Delta\Delta Ct}$ value) in response to different treatments: hydration (water 7 days), water, sodium metavanadate (SM), α-chaconine, α-solanine, solasodine, α-solamargine, SGA-*Bl* at a concentration of 1000 ppm, and Désirée root exudate at a concentration of 100 ppm, for 24 h or 48 h. The $2^{-\Delta\Delta Ct}$ values were normalized with respect to the relative gene expression in dry cysts (shown as line on *x*-axis). Up-regulated genes appear as bars above the *x*-axis, while down-regulated genes appear as inverted bars below the *x*-axis. Error bars represent the standard deviation of replicates ($n = 3$). Statistical significance (*) was calculated by one-way ANOVA with *post hoc* Tukey–Kramer HSD test, $P < 0.05$ (Supporting Information Table S5).

Glutamate carboxypeptidase, encoded by *gcp-2.1*, is an enzyme that cleaves the neurotransmitter peptide *N*-acetyl-l-aspartyl-l-glutamate[100] and has been shown to play a role at multiple levels of *C. elegans* reproductive and post-embryonic development by mediating folate metabolism.[101] Knockout of *gcp-2.1* in *C. elegans* potentially affected the gut, muscle, and nervous systems, reducing the overall fitness of the nematodes, suggesting that it plays a critical role in maintaining physiological balance across multiple systems, and its loss amplifies vulnerability to external stress factors.[102] The suppression of *gcp-2.1* expression in *G. rostochiensis* and *G. pallida* in response to SGAs may reflect an adaptive shift prioritizing infection-related processes over metabolic or stress-regulatory functions. As feeding occurs on host plants post-establishment, gut activity may be less crucial during hatching.

Finally, very little is known about the function of *c52* in nematodes, including *C. elegans* and *Globodera* species, but it is interesting to note that its gene expression was much more altered in *G. pallida* than in *G. rostochiensis* (Table S6), emphasizing the differences between the two *Globodera* species and the large knowledge gap that still exists.

4 CONCLUSIONS

The results presented illustrate the complexity of the structure–activity relationship and highlight the role of glycosidic moieties and stereochemistry in determining the efficacy of SGAs in managing PCN infestations as targeted hatching stimulants. The structural diversity of SGAs, solanoeclepins, and related compounds suggests potential for the development of targeted nematode control strategies. Identifying the most effective structural components of these molecules could lead to new developments, particularly through the use of artificial intelligence approaches that could help determine the best synthetic analogues to enhance hatching in controlled environments, thereby reducing nematode populations before they cause crop damage.

Despite progress in understanding the structure–activity relationship of SGAs and solanoeclepins, there is still a lack of comprehensive knowledge of how these compounds interact with environmental factors, soil conditions and the broader rhizosphere ecosystem. Therefore, further research should focus on understanding how different PCN populations respond to these compounds, especially considering virulence factors, pathotypes and environmental influences.

In *G. rostochiensis* and *G. pallida*, it is very difficult to directly correlate the differential gene expression during hatching because the exact mechanisms, such as gene expression, expression localization and transcription are still understudied and poorly understood to create a gene regulatory network for better understanding the induction of PCN hatching. However, the observed differences in gene expression during hatching between *G. rostochiensis* and *G. pallida* highlight their different strategies to adapt to environmental cues and underscore the need for further investigation into the molecular mechanisms involved.

This study on PCN hatching reveals a complex interplay of physiological and genetic factors influencing the hatching process in *G. rostochiensis* and *G. pallida*. The stronger and faster increase in relative gene expression of detoxification, stress response, and host interaction genes in *G. rostochiensis* reinforces the idea that this species is able to respond more rapidly to environmental cues, implying a faster hatching rate for *G. rostochiensis* compared to *G. pallida*. This ability to handle oxidative stress, detoxify compounds, and degrade plant tissues faster than *G. pallida*, gives *G. rostochiensis* a competitive advantage in emerging from dormancy and hatching earlier when exposed to the same environmental triggers. This differential gene expression suggests that these PCN species may developed different strategies to adapt to their environment, which has important implications for their susceptibility to management strategies involving the use of HFs as shown here with SGAs. This likely reflects the different adaptability and sensitivity of these nematode species to host-derived signals and chemical treatments. *Globodera rostochiensis* appears to have a broader hatching response to a wide range of stimuli, as reflected in the gene expression profile, while *G. pallida* is more specialized, showing a stronger genetic response to host-specific signals such as those from Désirée root exudate.

Overall, Désirée root exudate appeared to provide a more optimal environment for embryonic development and hatching by maintaining low oxidative stress, controlled metabolic activity, and gradual cell wall loosening, reflected by milder changes in the relative gene expression analysis in *G. rostochiensis* and *G. pallida*. This balanced approach seems to be most conducive to high hatching activity. However, α-solamargine, SGA-*Bl*, and α-chaconine also promote significant hatching, but under more stressful conditions with increased demands for detoxification, metabolic energy, and cellular restructuring. These compounds likely accelerate the hatching process by inducing stronger stress responses, but not as efficiently or as smoothly as Désirée root exudate.

In conclusion, while considerable progress has been made in identifying HFs for *Globodera*, the genetic and molecular basis of hatching remains underexplored. Future research should prioritize the identification of key genes, explore their regulatory networks, and validate their roles in hatching, with a focus on differences between *G. rostochiensis* and *G. pallida*. This knowledge could ultimately lead to more targeted, effective and sustainable pest management strategies in agricultural practice, particularly in the management of these important agricultural pests.

AUTHOR CONTRIBUTIONS

ACR (conceptualization, data curation, formal analysis; methodology, validation, writing – original draft, writing – review and editing); ET (writing – review and editing); PD (conceptualization; methodology, validation, writing – review and editing).

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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the results of this study are available in the article and in the Supporting Information.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the author(s) used DeepL Write in order to improve the readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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