COMMENTARY

Proteinaceous elicitor from a secretion of egg-laying insect herbivore induces plant emission that attracts egg parasitoids

Jarmo K. Holopainen ®

Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland

Correspondence

Jarmo K. Holopainen, Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland.
Email: jarmo.holopainen@uef.fi

The coevolution and arm's race between plants and their herbivores have been a driving force for plant chemical ecology research (Agrawal & Zhang, 2021; Jones et al., 2021). Chemical defence compounds produced by plants may directly affect the feeding or egg-laying of insects by disturbing their behaviour and food digestion or indirectly by attracting the natural enemies of the herbivorous insect (Johnson, 2011). Plants respond rapidly by activating induced chemical defence when they are injured by ovipositor or by mouthparts and due to the chemical compounds released by the herbivore. Damage can be mechanical, for example, by chewing mouthparts of insects, which result in the breakup of cell membranes and rapid release of the so-called green leaf volatiles (Ameye et al., 2018). In addition, plant responses can be caused by elicitors that constitute insect-derived compounds such as saliva, regurgitant or ovipositor secretions that activate various plant defence pathways (Jones et al., 2021).

Chemical defence of conifers is based on terpenoids, such as mono-, di- and sesquiterpenes, that form the oleoresin-mediated defence. Mono- and sesquiterpenes maintain the fluidity of oleoresin and their volatility promote the crystallisation of oleoresin diterpenes to resin plugs as it occurs after attack by bark beetles (Keeling & Bohlmann, 2006). Resin-based chemical defence is a trait that has been 'broken' by herbivorous insects that had evolved special counter-defence against such resin compounds.

In this issue, Hundacker et al. (2021) report a specific annexin-like protein called diprionin from the ovipositor secretion of the common pine sawfly *Diprion pini*. This protein was fractionated from the ovipositor gland secretion that was released by sawfly females when they drill their eggs in pine needles (Figure 1). Earlier studies have shown that the ovipositor secretion of *D. pini* induces the emission of the sesquiterpene *E*-β-farnesene (Mumm et al., 2003) and

this volatile compound acts as an attractant for a tiny parasitoid wasp *Closterocerus ruforum* (Mumm et al., 2005; Beyaert et al., 2010). The parasitoid wasp female inserts its own small eggs in the sawfly eggs. When a larva develops, it consumes the sawfly embryo and pupates inside the host egg. Instead of needle-damaging sawfly larva, a parasitoid wasp emerges from the sawfly egg. In behavioural tests, Hundacker et al. (2021) found that diprionin, when applied on pine needles, acted as an elicitor rendering pine needles attractive for *C. ruforum*. The novelty of the study is that no other insect-produced annexin protein is known so far to be involved in direct or indirect defensive response of plants to herbivory.

Hundacker et al. (2021) also analysed comprehensively at the transcriptome level the plant defence responses to natural *D. pini* egg deposition and diprionin treatment. Terpene biosynthesis and reactive oxygen species signalling genes were similarly affected by both treatments. A gene of monoterpenoid synthase pathway geranyl pyrophosphatase (GPP) was up-regulated relative to transcript abundance in wounded-plus-buffer-treated trees, but farnesyl pyrophosphatase (*FPP*) was not responding. Against the original hypothesis, the expression sequence of *PAL* (phenylalanine ammonia lyase), a key enzyme for biosynthesis phenylpropanoids, was down-regulated by *D. pini* egg deposition and diprionin application. However, concentrations of any end products of terpene or phenylpropanoid biosynthesis pathways in cellular tissues of needles were not studied in this study.

For an ecologist, the most interesting result of this study is that not the insect egg, but a specific chemical compound produced by the ovipositor secretion gland acts as an elicitor of indirect defence in the host plant. Elicitors that make herbivore-attacked plants attractive to an egg parasitoid can be extremely efficient because egg parasitoids can kill the herbivore larvae before it can cause any
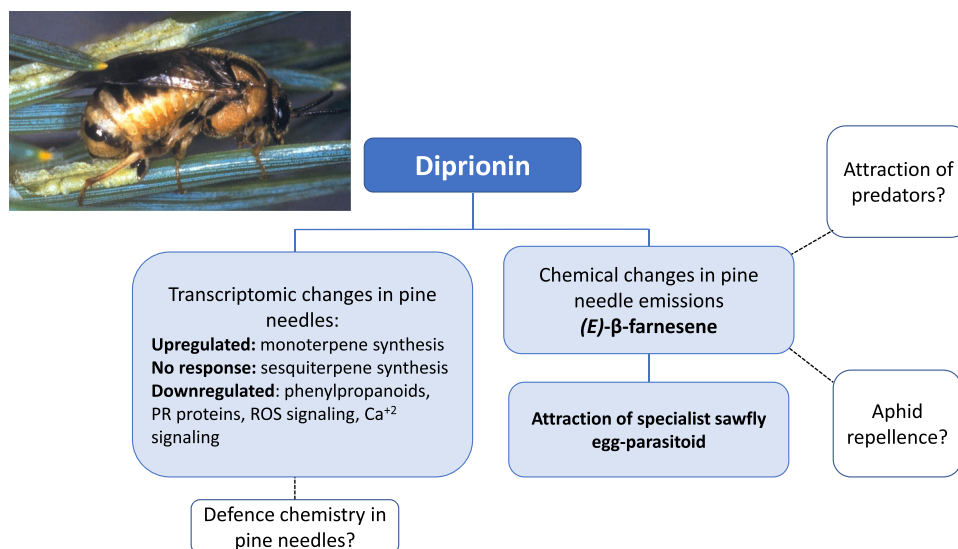
FIGURE 1 *Diprion pini* egg deposition with ovipositor secretion containing diprionin on Scots pine needles. The light blue boxes indicate the molecular, chemical and ecological responses of pine to diprionin as measured by Hundacker et al. (2021). The text boxes with question marks are suggested ecological interactions to be tested with diprionin for a better understanding of natural elicitor effects in pine forests

feeding damage to a host plant. Additionally, the herbivore controlling capacity of egg parasitoids is more efficient than that of larval parasitoids (Cusumano et al., 2012). However, in nature, the first requirement for the efficiency of indirect plant defence based on natural enemies is that these are available at the site where the herbivore is attacking. During long-lasting forest insect outbreaks, the role of egg and larval parasitoids to control the herbivore becomes more important at the end of the outbreak period when parasitoid populations have better colonised the area (Vindstad et al., 2010). In an active biocontrol, egg parasitoids can be released in forests at an early stage of an outbreak, for example, from drones to achieve more efficient herbivore control (Martel et al., 2021). In normal population densities of *D. pini* and the egg parasitoid, the capacity of diprionin to induce *E*-β-farnesene emissions and attract egg parasitoids might not be so deleterious for the herbivore offspring as during outbreaks when parasitoid population density is higher. On the other hand, during outbreaks, 'birth control' by egg parasitoids might prevent the high larval density of the herbivore population and therefore avoid food shortage. This strategy will ensure that the larger surviving sawfly larvae have enough food and they can develop to final instar and pupate. In this case, the oviposition-induced volatile *E*-β-farnesene signal to egg parasitoids may be beneficial to all participants of this tritrophic signalling chain.

The ovipositor extract to cover the eggs deposited in the slit pine needle probably has originally evolved as a trait to give protection to eggs against desiccation and mechanical protection against various predators. The capacity of ovipositor secretion with diprionin to elicit induced *E*-β-farnesene emission has probably evolved later and benefited more the host plant than *D. pini*. As diprionin production has survived as a trait in *D. pini* evolution, *E*-β-farnesene induction after oviposition should provide some competitive advantage for neonatal

larvae that emerge from eggs. One possible explanation could be the repellent effect against competing herbivores because *E*-β-farnesene emitted by pine is the same compound that is produced by aphids as an alarm pheromone (Beale et al., 2006). When *E*-β-farnesene is released by an aphid that is attacked by a parasitoid or predator, it will cause an escape reaction by other aphids. If diprionin-induced *E*-β-farnesene has a similar repellent effect on pine aphids, *D. pini* female may create a competition-free space for its offspring. For example, some pine needle aphids such as *Eulachnus agilis* can cause needle chlorosis and early dropping of pine needles (Bliss et al., 1973) and may reduce the quality of food for neonatal sawfly larvae. Induced *E*-β-farnesene emissions may also affect the predation risk for *D. pini* by general predators such as ants (Lindstedt et al., 2006) and birds (Lindstedt et al., 2011). With birds, the olfactory response has been shown, but the volatile cue compounds were not identified (Mantyla et al., 2020).

Transcriptomics analysis of plant chemical defence response to diprionin in pine needles by Hundacker et al. (2021) revealed that most of the genes activated (priming effect) by natural egg deposition were also activated by diprionin. Interestingly, *GPP3* gene of monoterpene pathway (MPE) was up-regulated by both egg deposition and diprionin, but a sesquiterpene pathway (MVA) gene *FPP* was up-regulated only by egg deposition and expression of an (*E*)-β-farnesene synthase (*TPS5*) was not activated by either of the treatments. As opposite to monoterpene synthesis genes, the transcript level of phenylalanine ammonia lyase (PAL), a key gene for phenylpropanoid production was down-regulated. In an earlier study (Bittner et al., 2019), up-regulation of PAL gene was found in Scots pine if the plant was exposed to *D. pini* sex pheromone without oviposition. The authors suggested that other members of the PAL gene family might show other responses to diprionin than the tested *PAL* sequence.

The study by Hundacker et al. (2021) confirmed earlier observation by Kopke et al. (2010) that *D. pini* egg deposition does not regulate expression of an (*E*)-β-farnesene synthase (*TPS5*), but still (*E*)-β-farnesene emission were responsive. Similarly, Kovalchuk et al. (2015) could not observe any correlation between the increased emission of (*E*)-β-farnesene or other sesquiterpenes and the expression levels of the terpene synthase-encoding genes in injured Scots pine bark after pine weevil feeding damage. More research is needed to better understand how oleoresin storage and flow in resin ducts affect localised terpenoid emissions.

If *D. pini* females could control the food quality of pine needles for their offspring by manipulating concentrations of secondary metabolites with oviposition secretion containing diprionin, the needle transcriptomics results by Hundacker et al. (2021) look reasonable. The detected up-regulation of the monoterpene pathway suggests increased production of oleoresin, which is a mixture of monoterpenes and diterpenes. Oleoresin is an important chemical defence of pine trees against generalist herbivores, but specialist insects like pine sawfly larvae can store oleoresin and use this sticky product as a defence against predating birds. Lindstedt et al. (2011) showed that pine sawfly pupae weights were even higher on the high-resin diet than on the low-resin diet. Down-regulation of *PAL* that controls phenylpropanoid production by diprionin suggests that reduction of phenylpropanoids in needles could also be beneficial for *D. pini* larvae, but needs more diprionin studies with other members of *PAL* gene family as concluded by Hundacker et al. (2021). High concentrations of phenylpropanoids such as myricetin-3-galactoside in foliage may reduce *D. pini* larval performance directly or indirectly by reducing needle nitrogen concentration (Roitto et al., 2009).

The findings by Hundacker et al. (2021) of the new natural elicitor may provide a valuable tool to unravel the function of the fine-tuned communication network in the terrestrial ecosystem. It is known that several plants also receive information on the potential risk of damage by the volatiles released by herbivore-attacked plants (Loreto & D'Auria, 2021; Zu et al., 2020). Conifer trees are dominating tree species in huge and highly vulnerable boreal and mountain forest ecosystems and act as an important sink and storage of carbon. These ecosystems are under the threat of rapid and combined effects of increasing temperatures and drought stress (Kharuk et al., 2021). Therefore, it is crucial to better understand the biochemistry of conifers under abiotic and biotic stresses as well as find novel methods to maintain their carbon fixing capacity. Natural elicitors such as diprionin and possible synthetic analogues could prove potential tools to control the invasive and outbreaking insect herbivores under a rapidly warming climate.

ORCID
Jarmo K. Holopainen [ID] http://orcid.org/0000-0001-5026-3245

REFERENCES
Agrawal, A.A. & Zhang, X.N. (2021) The evolution of coevolution in the study of species interactions. *Evolution*, 75, 1594–1606.

Ameye, M., Allmann, S., Verwaeren, J., Smagghe, G., Haesaert, G., Schuurink, R.C. et al. (2018) Green leaf volatile production by plants: a meta-analysis. *New Phytologist*, 220, 666–683.

Beale, M.H., Birkett, M.A., Bruce, T.J.A., Chamberlain, K., Field, L.M., Huttly, A.K. et al. (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 10509–10513.

Beyaert, I., Waschke, N., Scholz, A., Varama, M., Reinecke, A. & Hilker, M. (2010) Relevance of resource-indicating key volatiles and habitat odour for insect orientation. *Animal Behaviour*, 79, 1077–1086.

Bittner, N., Hundacker, J., Achotegui-Castells, A., Anderbrant, O. & Hilker, M. (2019) Defense of Scots pine against sawfly eggs (*Diprion pini*) is primed by exposure to sawfly sex pheromones. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 24668–24675.

Bliss, M., Yendol, W.G. & Kearby, W.H. (1973) Probing behavior of *Eulachnus agilis* and injury to Scotch pine. *Journal of Economic Entomology*, 66, 651–655.

Cusumano, A., Peri, E., Vinson, S.B. & Colazza, S. (2012) Interspecific extrinsic and intrinsic competitive interactions in egg parasitoids. *BioControl*, 57, 719–734.

Hundacker, J., Bittner, N., Weise, C., Bröhan, G., Varama, M. & Hilker, M. (2021) Pine defense responses to eggs of an herbivorous sawfly are elicited by an annexin-like protein. *Plant, Cell & Environment* (in press). https://doi.org/10.22541/au.162796610.03372484/v1

Johnson, M. (2011) Evolutionary ecology of plant defences against herbivores. *Functional Ecology*, 25, 305–311.

Jones, A.C., Felton, G.W. & Tumlinson, J.H. (2021) The dual function of elicitors and effectors from insects: reviewing the 'arms race' against plant defenses. *Plant Molecular Biology* (in press). Available from: https://doi.org/10.1007/s11103-021-01203-2

Keeling, C.I. & Bohlmann, J. (2006) Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytologist*, 170, 657–675.

Kharuk, V.I., Im, S.T., Petrov, I.A., Dvinskaya, M.L., Shushpanov, A.S. & Golyukov, A.S. (2021) Climate-driven conifer mortality in Siberia. *Global Ecology and Biogeography*, 30, 543–556.

Kopke, D., Beyaert, I., Gershenzon, J., Hilker, M. & Schmidt, A. (2010) Species-specific responses of pine sesquiterpene synthases to sawfly oviposition. *Phytochemistry*, 71, 909–917.

Kovalchuk, A., Raffaello, T., Jaber, E., Kerio, S., Ghimire, R., Lorenz, W.W. et al. (2015) Activation of defence pathways in Scots pine bark after feeding by pine weevil (*Hylobius abietis*). *BMC Genomics*, 16, 352.

Lindstedt, C., Huttunen, H., Kakko, M. & Mappes, J. (2011) Disengtangling the evolution of weak warning signals: high detection risk and low production costs of chemical defences in gregarious pine sawfly larvae. *Evolutionary Ecology*, 25, 1029–1046.

Lindstedt, C., Mappes, J., Paivinen, J. & Varama, M. (2006) Effects of group size and pine defence chemicals on diprionid sawfly survival against ant predation. *Oecologia*, 150, 519–526.

Loreto, F. & D'Auria, S. (2021) How do plants sense volatiles sent by other plants? *Trends in Plant Science*, 27, 29–38. Available from: https://doi.org/10.1016/j.tplants.2021.08.009

Mantyla, E., Kipper, S. & Hilker, M. (2020) Insectivorous birds can see and smell systemically herbivore-induced pines. *Ecology and Evolution*, 10, 9358–9370.

Martel, V., Johns, R.C., Jochems-Tanguay, L., Jean, F., Maltais, A., Trudeau, S. et al. (2021) The use of UAS to release the egg parasitoid *Trichogramma* spp. (hymenoptera: Trichogrammatidae) against an agricultural and a forest pest in Canada. *Journal of Economic Entomology*, 114, 1867–1881.

Mumm, R., Schrank, K., Wegener, R., Schulz, S. & Hilker, M. (2003) Chemical analysis of volatiles emitted by Pinus sylvestris after induction by insect oviposition. *Journal of Chemical Ecology*, 29, 1235–1252.

Mumm, R., Tiemann, T., Varama, M. & Hilker, M. (2005) Choosy egg parasitoids: specificity of oviposition-induced pine volatiles exploited by an egg parasitoid of pine sawflies. *Entomologia Experimentalis Et Applicata*, 115, 217–225.

Roitto, M., Rautio, P., Markkola, A., Julkunen-Tiitto, R., Varama, M., Saravesi, K. & Tuomi, J. (2009) Induced accumulation of phenolics and sawfly performance in Scots pine in response to previous defoliation. *Tree Physiology*, 29, 207–216.

Vindstad, O., Hagen, S.B., Schott, T. & Ims, R.A. (2010) Spatially patterned guild structure in larval parasitoids of cyclically outbreaking winter moth populations. *Ecological Entomology*, 35, 456–463.

Zu, P.J., Boege, K., del-Val, E., Schuman, M.C., Stevenson, P.C., Zaldivar-Riveron, A. et al. (2020) Information arms race explains plant–herbivore chemical communication in ecological communities. *Science*, 368, 1377–1381.