COMMUNICATION
Guillermo H. Jimenez-Aleman, Wilhelm Boland et al.
JA-Ile-macrolactones uncouple growth and defense in wild tobacco
Small molecules capable of uncoupling growth-defense in plants are currently not known. In this study, for the first time, semi-synthetic analogues of the phytohormone JA-Ile are employed to uncouple growth and defense responses in wild tobacco. The JA-Ile analogues are easily synthesized from inexpensive substrates via olefin metathesis.

Plant growth is often compromised when the plant immune system is activated in response to environmental challenges such as pathogens and herbivores. These negative associations between growth and defense are, to an important extent, controlled by phytohormones such as jasmonates (Scheme 1).\(^1\)–\(^3\) \(N\)-Jasmonoyl-l-isoleucine (JA-Ile, 4) is the most active endogenous jasmonate. This molecule mediates several processes such as development of reproductive organs\(^4\) and activation of plant defenses against necrotrophic pathogens and herbivores.\(^5\)–\(^6\) These properties of JA-Ile (4) could be exploited in agricultural systems, but the activation of the jasmonate signaling (JA-signaling) often results in the reduction of plant growth and fitness, drawbacks which restrain the use of bioactive jasmonates for crop protection.\(^7\)–\(^8\) In a recent study, genetic manipulation of the JA-signaling was used to uncouple growth and defense in Arabidopsis thaliana.\(^3\) The loss-of-function mutants did not have five of the thirteen JASMONATE-ZIM-DOMAIN (JAZ) proteins – which act as co-receptors of JA-Ile (4) in the JA-signaling pathway. Although genetic manipulation is a promising approach in this context, it may be limited by both the lack of molecular tools in many plant species of economic interest and the still strong popular opposition to genetically-modified crops. An alternative to overcome these limitations could be the use of small molecules (e.g. analogs of phytohormones) in order to uncouple growth and defense in wild type crop plants.

Several analogs of JA-Ile (4) have been employed to manipulate the JA-signaling pathway.\(^9\)–\(^12\) Based on this, and on preliminary observations in a previous work with \(\omega\)-modified jasmonates (macrolactones 9 and 9a, Scheme 2),\(^1\)\(^3\) the possibility of uncoupling growth from defense responses by using JA-Ile (4) analogs was explored. Diastereomeric JA-Ile-lactones 9 and 9a are a new class of JA-Ile derivatives capable of inducing nicotine accumulation in Nicotiana attenuata leaves to a similar extent as endogenous bioactive jasmonates do.\(^1\)\(^3\) In this study, N. attenuata plants were treated with macrolactones 9 and 9a and the performance of Manduca sexta caterpillars on treated plants, plant growth rates and reproductive output were evaluated. In addition, a new synthetic route (Scheme 2) to JA-Ile-lactones 9 and 9a was developed.

The \(Z\)-selective cross-metathesis of (±)-2 and but-3-en-1-yl acetate (both compounds commercially available and inexpensive) afforded compound (±)-10 in excellent yield (>80%) and \(Z\)-selectivity (>90%). Saponification of (±)-10 (>85% yield), conjugation of (±)-3 to \(Z\)-Ile, and macrolactonization of (±)-8 as previously described\(^1\)\(^3\) afforded enantiomerically pure macro lactones 9 and 9a in only three linear steps (Scheme 2).

In agreement with our previous study,\(^1\)\(^3\) macro lactones 9 and 9a induced the accumulation of nicotine (Fig. 1) – MeJA (2) is converted into bioactive JA-Ile (4) \textit{in vivo} and was therefore employed as the positive control.\(^1\)\(^4\) Moreover, nicotine was not induced in plant genotypes impaired in JA-Ile (4) perception (irCOI1 plants), indicating that JA-Ile-lactones 9 and 9a are perceived by \(N.\) \textit{attenuata} plants in a similar manner as the endogenous bioactive jasmonate JA-Ile (4) (Fig. 1). Interestingly, caffeoylputrescine was induced by MeJA (2) but not by the lactones 9 or 9a (Fig. 1). These outcomes suggest that (i) the lactones can selectively induce nicotine and, perhaps, other plant defenses, (ii) additional elements induced by endogenous jasmonates (4) but not by the lactones 9 or 9a are required for a full induction of jasmonate-dependent defensive secondary metabolites.\(^1\)\(^5\)

Interestingly, nicotine induced by the lactones was accompanied by a decrease in both \(M.\) \textit{sexta} caterpillar mass

\(^{†}\) Present address: Institute of Plant Sciences, Biotic Interactions. University of Bern. Altenbergrain 21, 3013 Bern, Switzerland.
gain and survivorship (Fig. 2). *Manduca sexta* is a specialist insect very tolerant to nicotine, which supports our hypothesis that the lactones activate additional plant defenses apart from nicotine or that nicotine is detrimental for *M. sexta* at the observed concentrations. Strikingly, contrary to MeJA (2), macrolactones 9 and 9a stimulated plant growth at the early developmental stage and did not affect seed capsule formation (Fig. 3). At the maturing stage, the height of plants treated with
the lactones was not different from control plants, but MeJA (2) treated plants were considerably smaller (Fig. 4). These results show that the induction of defenses by the macrolactones 9 or 9a is not accompanied by a decrease in plant growth or fitness as it is generally observed after treatment with endogenous or synthetic jasmonates known to date.1

Our study suggests that N. attenuata plants can simultaneously defend themselves against the attack of the caterpillar and maintain the capability to grow. In N. attenuata, overproduction of jasmonates results in stem stunting due to cross-talk of bioactive jasmonates with other phytohormones.16,17 On the other hand, exogenously applied jasmonates are metabolized in vivo into bioactive jasmonates, resulting in elevated levels of these compounds within plant tissues.18,19 Therefore, it was hypothesized that, contrary to MeJA (2), macrolactones 9 and 9a activate jasmonate-related defenses without increasing the levels of endogenous jasmonates, which results in defended plants with normal growth rates (no interference of endogenous jasmonates with other phytohormones). In agreement with this hypothesis, it was observed that endogenous JA (1) and JA-Ile (4) levels were similar in control and lactone-treated plants, while a significant increase of these compounds was observed in MeJA (2) treated plants (Fig. S1†).

Together these results indicate that JA-Ile-lactones may promote the interaction between the jasmonate receptor COI1 and singular JAZ proteins (repressors of JA-signaling and co-receptors of JA-Ile),20 which results in the observed phenotypes. The recent report on the Arabidopsis thaliana mutant (lacking five of the thirteen JAZs), where growth and defense are uncoupled, supports this hypothesis.3 However, the possibility that JA-Ile-lactones (9) and (9a) exert their action via a non-canonical pathway cannot be ruled out at this point. Likewise, a direct toxicity of lactones 9 and 9a for insects cannot be fully discarded, although their concentration in the leaf tissue is very low (pmoles per gram of fresh weight).

In conclusion, we have developed brief synthetic route based on Z-selective olefin cross-metathesis which allows the preparation of macrolactones 9 and 9a in only three linear steps. The pioneer studies with these compounds open the possibility of uncoupling defense and growth in plants by using small molecules. This idea, a ligand based strategy to activate particular plant responses, might be also applicable to other phytohormones such as gibberellins and brassinosteroids, both occurring in high diversity within plant tissues.
Additionally, the presented ligand-based manipulation of the JA-signaling pathway constitutes a promising approach for (i) plant protection that could reduce the use of both pesticides and genetically modified crops, (ii) the use of rationally designed jasmonates as molecular tools to better understand the mechanisms governing growth-defense tradeoffs in plants. The molecular basis underpinning the findings presented here constitute exciting avenues for future research.

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