Interaction Between NaCl and Insecticide

* Continuous dependent variable of total growth (AUC).
* Categorical independent variable (treatment). Treatments are levels in a factor.
* Two insecticides, two concentrations for each (5µM and 5mM), with and without salt. Also, no salt (control) and salt.
* Salt is 0.5M NaCl.
* Ten levels in factor
  1. salt
  2. no salt
  3. thiacloprid 5µM (low)
  4. thiacloprid 5mM (high)
  5. acetamiprid 5µM (low)
  6. acetamiprid 5mM (high)
  7. thiacloprid 5µM + salt (low)
  8. thiacloprid 5mM + salt (high)
  9. acetamiprid 5µM + salt (low)
  10. acetamiprid 5mM + salt (high)
* With 9 replicates each the whole expt can fit all on one plate.
* Do three blanks with media and three blanks with NaCl media.

Are the effects of the two stressors additive or synergistic? If the effects are synergistic this improves the viability of the metabolism screen. If synergistic, by combining salt with insecticide the effect of insecticide alone has been amplified. This amplification provides a larger effect to be reversed if insecticide metabolism by a recombinant pollinator enzyme occurs.

Biological Interpretation

The main question from my results is why don’t most of the insecticides have an effect? Is it due to a lack of intracellular targets, or because their effective intracellular concentration is low, resulting from the action of efflux pumps in the plasma membrane? Can the proposed experiment lend support to either scenario? Can the effects of insecticide alone be explained by studying their effect in combination with NaCl?

Outcomes

Salt alone + insecticide alone = salt + insecticide together (additive)

Salt alone + insecticide alone < salt + insecticide together (synergistic)

Yannick’s argument – For insecticides that had no effect in isolation (e.g. acetamiprid)

*If additive result, then this shows that the insecticide (alone) enters the cell but has no effect.*

*If synergistic result, then small, previously undetected insecticide (alone) effect has been revealed through its interaction with another stressor. Shows that the small effect of the insecticide in isolation is due to efflux not a lack of intracellular targets.*

*Therefore, this experiment is an indirect way of looking at intracellular effect of the insecticide in isolation.*

This wasn’t his argument. I misunderstood. It was simply that if there is a synergistic effect between NaCl and insecticide then insecticide is having some form of effect (albeit in tandem with another stressor).

My argument –

If additive result, this only shows that there is no interaction between NaCl and insecticide stress. Nothing regarding the insecticide only intracellular effect can be concluded from this result.

If synergistic result, this only shows there is an interaction between the two stressors. Again, nothing can be inferred about the intracellular status of yeast for the insecticide only treatment.

My reasoning – There is an alternative explanation to yannick’s. For the synergistic result, this wouldn’t necessarily be uncovering a previously undetected, but present/potential, insecticide only effect. The insecticide in isolation may be present at high intracellular concentrations and be benign, and only have an effect when combined with NaCl. There are multiple examples of where two substances in isolation are harmless, but when combined are toxic, in the literature1. It’s possible that treatment with the insecticide in isolation leads to a low intracellular concentration (due to poor influx or rapid efflux) and a small effect, and combination with NaCl either facilitates insecticide entry or heightens the existing effects driven by the low intracellular insecticide concentration. But this isn’t the only explanation. Furthermore, the additive result would also be inconclusive: maybe, on treatment with only the insecticide, intracellular insecticide concentration isn’t reflective of the media concentration due to poor influx, and NaCl treatment doesn’t remedy this. In this case, the insecticide in combination with NaCl would still have no effect but may if it was present intracellularly. An alternative explanation is the insecticide in isolation treatment results in a low intracellular concentration (due to poor influx or rapid efflux) and a small, undetectable effect, but there is no interaction between this and the effect of NaCl.

An alternative/additional experiment would be to try and measure intracellular insecticide concentration using quantitative mass spec. This would at least clarify if the lack of effect for most insecticides is due to a low intracellular concentration. Of course, if their intracellular concentration is low this wouldn’t answer if they would have an effect if it were high. Moreover, my results would more relevant if there was only a low intracellular concentration. For example, with Thiacloprid I could then argue that the concentrations tested were relevant as the intracellular concentration was actually similar to those encountered in agricultural settings (for soil microbes at least). Of course, a counter argument exists where the concentrations encountered in field realistic settings are actually present at much lower levels intracellularly too.

Response to gene expression study

Yannick mentioned performing it anyway with yeast+NaCl as a control and yeast+NaCl+insecticide as the treatment. This wouldn’t necessarily inform on insecticide only effects. The observed effects may only be present in the combination of the two stressors. The interaction between insecticide and salt is only interesting for marine fungi. This isn’t what I’m interested in.

Other Options for Investigating Off Target Effects

Choose an insect cell line (potentially Sf9 (Sf21 substrain) derived from *Spodoptera frugiperda*, fall army worm moth*)* or High Five (or another cell line derived from *Trichoplusia ni*, cabbage looper) that doesn’t express nAchR (could also use these for the metabolism screen. Advantage of post-translational modifications2). Closer taxonomically to species of interest and (probably) won’t possess detoxification battery of plasma membrane transporters. Yeast are single cell organisms and require efficient detoxication mechanisms. Insect cell lines, on the other hand, unless derived from tissues associated with detoxification, wouldn’t be expected to. For Sf9 there is a whole genome sequence available and suspension culture is possible3,4. Genome assembly for *Trichoplusia ni* Tni-FNL insect cell line available and was originally selected for suspension growth5. Suspension important for growth measurements using a spectrophotometer. Even this isn’t a very strong idea (see below).

Explore the differences between formulations and active ingredients in yeast. If active formulations inhibit growth more than the active ingredients alone then there are some off-target effects driven by the additives. I could also perform a study like this in bees, which would be my preference if I’m honest. There is evidence that formulations are more toxic to bees than pure insecticides6–8. Investigating this at the transcriptomic level would uncover the additional effects, driven by “inert” additives/co-formulants, responsible for the toxicity gap.

Acute field realistic exposure compared to my concentrations

Alicja’s experiment is using an acute dose of 25ppb. I have found that Thiacloprid causes small but significant growth inhibition in yeast at 5mM. 5mM = 1263600 ppb. So, I am testing approximately x50000 the concentration of the acute, field realistic dose. Therefore, if intracellular concentration was 1% of the media concentration, it would still be x500 the acute, field realistic dose. This isn’t environmentally relevant. One study has already looked at the effects of insecticides on an insecticide cell line, Sf9. They reported a IC20 of 61.6µM for acetamiprid9. This is equal to 13710ppb. A 20% growth inhibition required a concentration x548.4 greater than the acute dose in Alicja’s experiment. Taken together, the results imply that the off-target effects of neonicotinoids are negligible at field realistic exposures and aren’t worthy of further investigation.

Response to Yannick Wanting to Use Yeast for Further Expts

Yeast was useful for separating off target effects from those mediated through the primary molecular target. For any further experiments this would no longer be the aim. Instead, if the effects of co-formulants was explored in more detail (formulations vs active ingredients), using yeast would be counterintuitive because not all the effects in the species of interest would be detected. To study the effects of formulations and how they differ from active ingredients in isolation, the aim is to characterise the difference in overall effects between the two, including those mediated through nAchR (or other primary molecular target). Therefore, testing on yeast would yield an incomplete picture. In fact, the very reason yeast was selected in the first place (lack of nAchR) is what makes it unsuitable for any subsequent studies that are interested in effects as a whole, not just off target effects. My previous experiment suggests there are essentially no insecticide effects in yeast, making this model organism unsuitable for further insecticide testing.

References

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