Insecticidal and synergistic activity of two monoterpenes against diamondback moth (Lepidoptera: Plutellidae)

A.E. Webster, P. Manning,[1] J.M. Sproule, N. Faraone, G.C. Cutler

Abstract—We examined the insecticidal activity of linalool and thymol, against diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), and whether they would synergise the activity of spinosad against this pest. Both linalool and thymol were toxic to diamondback moth larvae by topical and oral exposure, but orders of magnitude less so than spinosad. We found that low concentrations of linalool weakly synergised spinosad, increasing its toxicity more than twofold. An interaction between spinosad, and acetone meant it was not possible to identify any potential synergisms between thymol and spinosad. Our results demonstrate limited potential for thymol and linalool to act as biopesticides or synergists for managing diamondback moth.

Introduction

Insecticides are generally considered critically important tools for pest management in most cropping systems, but their applications can be problematic, with potential harmful effects on non-target organisms (Tuck *et al*. 2014), prolonged environmental persistence (Arias-Estévez *et al*. 2008), and eventually reduced effectiveness through development of insecticide resistance (Brattsten *et al*. 1986). An important emerging alternative to conventional products are biopesticides, natural compounds derived from plants, animals, or microorganisms that have bioactivity against pests (Chandler *et al*. 2011).

Plant-derived essential oils are biopesticides that contain complex mixtures of secondary metabolites including phenols, monoterpenes, and sesquiterpenes that help deter attack from herbivores or pathogens (Bennett and Wallsgrove 1994). Both commercially available formulations and rudimentary essential oil products have shown promise in controlling crop pests. For instance, EcoTrol (a rosemary oil-based pesticide)

has shown to be highly effective in controlling twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) under greenhouse conditions (Miresmailli and Isman 2006). Likewise, in less developed economies where preharvest crop losses are high (Oerke 2006) and access to synthetic products is limited – unrefined products, formed of suspensions of dried and crushed plant material have been successfully used to control herbivorous pests in agricultural small-holdings, reducing crop losses (Mkindi *et al*. 2017; Stevenson *et al*. 2017).

Despite numerous studies demonstrating insecticidal activity of essential oils, they are not as widely used or available as conventional synthetic insecticides. This is often due to reduced efficacy and persistence relative to synthetic compounds, and variable efficacy of the oils, resulting from the inherent variability in secondary metabolite profiles occurring amongst different phenotypes, parts of the plant, and environmental conditions (Isman 2000; Angioni *et al*. 2006).

Instead of using whole essential oils for pest management, it may be adequate to use key

A.E. Webster, P. Manning,[1] J.M. Sproule, G.C. Cutler, Department of Plant, Food, and Environmental Sciences, Agricultural Campus, Dalhousie University, PO Box 550, Truro, Nova Scotia, B2N 5E3, Canada
N. Faraone, Department of Plant, Food, and Environmental Sciences, Agricultural Campus, Dalhousie University, PO Box 550, Truro, Nova Scotia, B2N 5E3, Canada; and Department of Biology, Acadia University, 33 Westwood Ave, Wolfville, Nova Scotia, B4P 2R6, Canada

[1]Corresponding author (e-mail: paul.manning@dal.ca).
Subject editor: Cécile Le Lann
doi:10.4039/tce.2017.63

constituent components of essential oils that have insecticidal properties (Miresmailli *et al*. 2006). In some cases, the most dominant secondary metabolites exhibit toxicity equal to that of the parent essential oils (Faraone *et al*. 2015; Tak *et al*. 2016). Many secondary metabolites have relatively simple organic structures (Bennett and Wallsgrove 1994), and can be inexpensively synthesised (Thomas 2007). In addition, secondary metabolites of essential oils may also act as synergists (Barile 2013); a phenomenon where the combined toxicity of two compounds is larger than its predicted sum.

In this study, we examined the insecticidal activity of the monoterpenes linalool and thymol, the major constituents of essential oils of thyme, *Thymus vulgaris* Linnaeus (Lamiaceae), and lavender *Lavandula angustifolia* Linnaeus (Lamiaceae), respectively, against diamondback moth (*Plutella xylostella* (Linnaeus), Lepidoptera: Plutellidae). Diamondback moth is one of the most destructive pests of cruciferous crops worldwide (Furlong and Wright 2013), with annual crop damage and pest management costs estimated to be US$4–5 billion (Zalucki *et al*. 2012). Diamondback moth is notorious for its ability to develop resistance to multiple classes of insecticide (Furlong and Wright 2013). Identifying natural compounds with insecticidal or synergistic properties could be important in reducing reliance on conventional insecticides for control, while also addressing concerns of insecticide resistance and environmental pollution. We conducted experiments that examined the toxicity of linalool and thymol to diamondback moth through contact and oral exposure, and the ability of low concentrations of each monoterpene to synergise the biopesticide spinosad.

Materials and methods

Plant and insect material

Larvae used in experiments were from a laboratory colony of diamondback moth maintained on the Agricultural Campus, Dalhousie University (Truro, Nova Scotia, Canada). Insects were reared on cabbage plants, *Brassica oleracea* Linnaeus (Brassicaceae), in BugDorm (Bioquip Products, Compton, California, United States of America) rearing cages ($47.5 \times 47.5 \times 93$ cm) inside a walk-in environmental chamber (22 ± 2 °C, 16:8 light:dark hours, $65 \pm 5\%$ relative humidity). Second instars were used in experiments. Cabbage plants (Copenhagen market variety) were grown from seed in Pro-Mix (Premier Tech Home & Garden, Mississauga, Ontario, Canada) potting medium in an insecticide-free greenhouse and were watered as needed. Four to six-week-old plants were used for the experiments and for sustaining the diamondback moth colony. Foliage was rinsed with distilled water before use in experiments.

Treatment solutions

Serial dilutions of spinosad were prepared from formulated Entrust 80 WP insecticide (spinosad 800 g/L; Dow AgroSciences, Calgary, Alberta, Canada). Linalool (97%) and thymol (98%) were purchased from Alfa Aesar (Ward Hill, Massachusetts, United States of America). Test solutions were prepared using distilled water as a solvent and Polysorbate 80 (Tween 80; Sigma-Aldrich, Saint Louis, Missouri, United States of America) as a surfactant (0.5% v/v). As thymol was partially insoluble in water, all tests involving thymol included acetone (1.0% v/v) to allow solubilisation. When testing the synergistic effect of thymol, spinosad solutions additionally included 1.0% v/v acetone to serve as a positive control. When testing the toxicity of secondary metabolites, we used concentrations ranging from 10 000 to 100 000 ppm and 3000 to 100 000 ppm for linalool and thymol, respectively. When testing for synergism, spinosad concentrations ranged from 1 to 10 000 ppm. Concentrations of linalool and thymol were included as synergists at maximum no observable effect concentrations of 5000 and 1000 ppm, respectively. For further detail on test concentrations see Supplementary Material Table 1.

Bioassays

For direct contact exposure with individual compounds, cohorts of five second instars were placed in 90 mm diameter Petri dishes. A 2-mL application of test solution was administered to each cohort using a Potter tower (Burkard Scientific, Uxbridge, United Kingdom) at 78 kPa. Immediately following treatment, larvae were transferred to a 15 mm diameter untreated cabbage leaf disc held in a 55 mm diameter Petri dish lined with filter paper. For oral exposure, treatments were applied to leaf discs using the Potter tower, as

described above. Leaf discs were left to dry for 10–15 min and then transferred to clean plastic Petri dishes as above. Cohorts of five untreated larvae were then randomly selected and placed on each disc using a fine paint brush. For both direct contact and oral toxicity bioassays, larval mortality was assessed with a dissecting microscope at 10× magnification, 24 hours after exposure. Larvae that did not respond to gentle prodding with a blunt probe were considered dead.

In synergism experiments, the concentration of synergist used should be a maximum dose that causes no observable toxicity (Scott 1990). A range of spinosad concentrations that caused ~10–90% mortality was used. Insects or leaf discs were treated with spinosad + linalool/thymol mixtures using a Potter tower, and mortality was assessed after 24 hours as described above.

Each experiment was conducted as a complete randomised block design. Each block was comprised of four concentrations of a compound plus a control, replicated in triplicate. Fresh treatment solutions were prepared for each bioassay replicate. Experiments were repeated in time, beginning on three separate days, so that every concentration of compound was replicated $n = 9$ times. This resulted in a total of 225 larvae per compound used for determining acute toxicity.

Data analysis

We used generalised linear models with a binomial error structure and a probit link function to model the relationship between larval survival and the $\log_{10}$ concentration of each compound. When model residuals indicated overdispersion, a quasibinomial error structure was used instead (Crawley 2012). The slopes and intercepts of these models were then used to estimate the median lethal concentration (LC50) and its 95% confidence limits. We determined whether linalool or thymol enhanced the toxicity of spinosad using a ratio test (Wheeler *et al*. 2006). This test calculates the ratio between the toxicity of a compound with and without a synergist. If the confidence limits of the ratio are above, and do not include a value of 1, the synergism is statistically significant (Wheeler *et al*. 2006). All analyses were conducted using R 3.3.2 (R Core Team 2012). The LC50 for each compound was estimated using the package "MASS" (Venables and Ripley 2002), and the ratio tests were conducted with the package "drc" (Ritz *et al*. 2015).

Results

Both linalool and thymol were toxic to diamondback moth larvae, with similar toxicity for each compound regardless of exposure route (Table 1). The toxicity of spinosad alone was three orders of magnitude greater than either linalool or thymol (Table 1). Through the ratio test, we found that linalool significantly synergised the toxicity of spinosad (Table 1) through contact exposure (Fig. 1: A, 95% confidence limits = 1.02–1.98) and oral exposure (Fig. 1: B, 95% confidence limits = 1.03–2.04). The contact

Table 1. Median lethal concentration (LC50), and 95% confidence limits (95% CL) of two monoterpenes and spinosad, with and without synergists to second instar *Plutella xylostella*.

Compound	Exposure route	Synergist	LC50 (95% CL) (ppm)	Slope	Synergism ratio
Linalool	Contact	–	3.37E4 (2.56E4–4.44E4)	1.65	–
	Oral	–	4.83E4 (3.89E4–6.00E4)	2.19	–
Thymol	Contact	–	1.80E4 (1.34E4–2.43E4)	2.08	–
	Oral	–	2.67E4 (1.78E4–4.02E4)	1.16	–
Spinosad	Contact	–	0.18 (0.09–0.32)	1.34	–
		Linalool	0.09 (0.06–0.14)	1.02	2.0
	Oral	–	0.18 (0.13–0.25)	0.94	–
		Linalool	0.08 (0.06–0.09)	1.10	2.25
	Contact	–	0.07 (0.04–0.11)	1.79	–
		Thymol	0.06 (0.04–0.08)	1.18	0.85
	Oral	–	0.10 (0.06–0.15)	1.13	–
		Thymol	0.08 (0.05–0.11)	1.29	0.80

Note: Larvae were treated either by contact or oral exposure.

Fig. 1. Toxicity of spinosad to second instar diamondback moth with or without a secondary metabolite synergist. Values shown are the LC50s for spinosad with 95% confidence intervals. Asterisks identify assays where the monoterpene synergised spinosad, as determined using the ratio test.
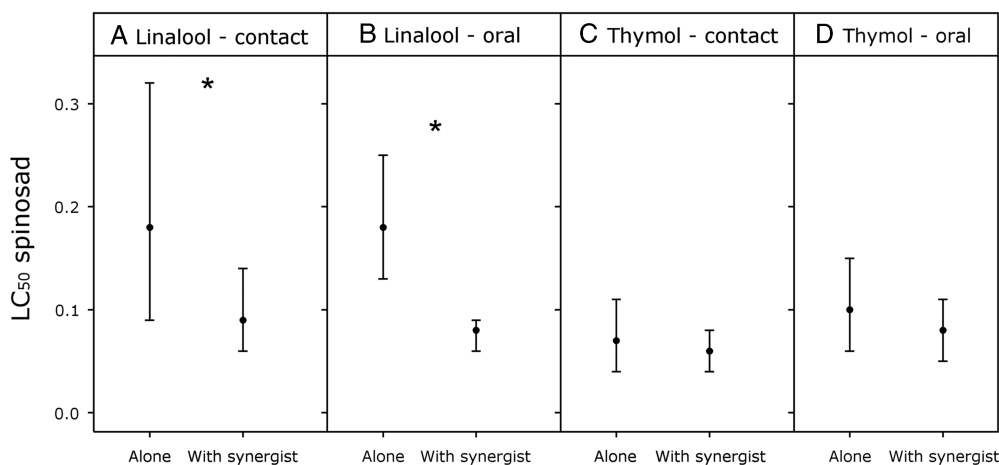
toxicity of spinosad was enhanced 2.0-fold by the addition of linalool, and the oral toxicity of spinosad was enhanced 2.3-fold by the addition of linalool (Table 1). Through the ratio test, we found thymol did not synergise the toxicity of spinosad through contact exposure (Fig. 1: C, 95% confidence limits = 0.81–1.36) or oral exposure (Fig. 1: D, 95% confidence limits = 0.87–1.41). The highest control mortality (11.1%) occurred in the combined spinosad + 0.5% linalool topical bioassay. In all other bioassays control mortality was below 9.0%.

Discussion

We found that both linalool and thymol were toxic to diamondback moth larvae. A study using *Musca domestica* Linnaeus (Diptera: Muscidae) showed that linalool inhibits the neurotransmitter acetyl cholinesterase (López and Pascual-Villalobos 2010). A second study using *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), showed that thymol obtains its toxicity by interfering with post-synaptic gamma-aminobutyric acid receptors (Priestley *et al.* 2003). Using contact exposure assays with diamondback moth larvae, Kumrungsee *et al.* (2014) reported an LD50 of 0.22 µg for thymol, but found that linalool did not on its own induce mortality rates of 50%, even when topical application exceeded 15 µg per

larva. Although we found linalool and thymol to be toxic to diamondback moth in our experiments, their toxicity was more than three orders of magnitude lower than that of spinosad, and other widely used synthetic insecticides used to control diamondback moth (Hill and Foster 2000). Moreover, given their low levels of persistence (Ngamo *et al.* 2007; Hu and Coats 2008), it is unlikely that either of these compounds will serve as stand-alone insecticides in the field.

The known variability of plant essential oils (Angioni *et al.* 2006), suggests that identifying and subsequently purifying or synthesising key constituents that give essential oils their insecticidal properties might offer greater promise for commercialisation. In some cases, dominant secondary metabolites have similar toxicity as their parent essential oils. For example, Faraone *et al.* (2015) found that essential oils from lavender and thyme respectively consisted of 54.3% linalool and 72.7% thymol. When tested against the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), the contact exposure toxicity of both secondary metabolites was essentially equal to that of their respective parent oils. Similarly, work by Tak *et al.* (2016) found the toxicity of lemongrass essential oil to third instar *Trichoplasia ni* (Hübner) (Lepidoptera: Geometridae), was equivalent to citral, its dominant secondary metabolite. However

in contrast, other studies have shown that parent oils have greater toxicity than their dominant constituent parts (Jiang *et al*. 2009).

We found that oral and topical exposure of linalool synergised the toxicity of spinosad, a pesticide that works through targeting binding sites on nicotinic acetylcholine receptors at a unique site to other insecticides (Orr *et al*. 2009). We were unable to detect any synergistic properties of thymol, due to an interaction between acetone and spinosad (Table 1). The synergistic properties of plant essential oils are usually achieved by the inhibition of cytochrome P450 monooxygenases and carboxylesterases, which are enzymes responsible for metabolising insecticides (Bernard and Philogène 1993). The potential of essential oils or secondary metabolites to act as synergists seems to be dependent on the compounds being studied (Tong and Bloomquist 2013; Faraone *et al*. 2015). For instance, Tong and Bloomquist (2013) identified six essential oils that synergised carbaryl, but found these same six essential oils, and eight others, antagonised the toxicity of permethrin.

If dominant secondary metabolites retain properties of their parent essential oils, their potential for pest management might extend beyond their insecticidal and synergistic properties. For example, a recent study of eight essential oils for *P. xylostella* management showed that treating cabbage plants with black pepper, *Piper nigrum* Linnaeus (Piperaceae), essential oil increased larval mortality, it also deterred larval colonisation, and reduced oviposition (Sangha *et al*. 2017).

Relative to conventional insecticides, linalool and thymol have low toxicity to diamondback moth larvae. Owing to the low toxicity (Kumrungsee *et al*. 2014) and short persistence (Ngamo *et al*. 2007; Hu and Coats 2008) of these secondary metabolites, it is unlikely that either could serve as standalone products for control of diamondback moth. Linalool provided only low levels of synergism in our laboratory bioassays, and it is unlikely any appreciable synergism would occur in field or greenhouse situations. Although higher levels of synergism might be achieved with whole essential oils (*e.g.*, Faraone *et al*. 2015), compositional inconsistencies of these natural compounds is a significant hurdle to regulatory approval in North America and Europe (Isman 2006). An alternative approach might be to build a synthetic blend of secondary metabolites, modelled after a particular essential oil to contain all bioactive compounds (*e.g.*, Miresmailli *et al*. 2006). Such a mixture could be tested and formulated with different adjuvants to improve persistence and efficacy, or tested as a synergist of conventional insecticides, the latter of which might be useful in resistance management.

Acknowledgements

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.4039/tce.2017.63

References

Angioni, A., Barra, A., Coroneo, V., Dessi, S., and Cabras, P. 2006. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. *stoechas* essential oils from stem/leaves and flowers. Journal of Agricultural and Food Chemistry, **54**: 4364–4370.

Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.-C., and García-Río, L. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agriculture, Ecosystems & Environment, **123**: 247–260.

Barile, F.A. 2013. Principles of toxicology testing. CRC Press, Boca Raton, Florida, United States of America.

Bennett, R.N. and Wallsgrove, R.M. 1994. Secondary metabolites in plant defence mechanisms. New Phytologist, **127**: 617–633.

Bernard, C.B. and Philogène, B.J.R. 1993. Insecticide synergists: role, importance, and perspectives. Journal of Toxicology and Environmental Health, **38**: 199–223.

Brattsten, L.B., Holyoke, C.W., Leeper, J.R., and Raffa, K.F. 1986. Insecticide resistance: challenge to pest management and basic research. Science, **231**: 1255–1261.

Chandler, D., Bailey, A.S., Tatchell, G.M., Davidson, G., Greaves, J., and Grant, W.P. 2011. The development, regulation and use of biopesticides for integrated pest management. Philosophical Transactions of the Royal Society of London B: Biological Sciences, **366**: 1987–1998.

Crawley, M.J. 2012. The R book. John Wiley & Sons, Chichester, United Kingdom.

Faraone, N., Hillier, N.K., and Cutler, G.C. 2015. Plant essential oils synergize and antagonize toxicity of different conventional insecticides against *Myzus persicae* (Hemiptera: Aphididae). Public Library of Science One, **10**: e0127774.

Furlong, M. and Wright, D. 2013. Diamondback moth ecology and management: problems, progress, and prospects. Annual Review of Entomology, **58**: 517–541.

Hill, T.A. and Foster, R.E. 2000. Effect of insecticides on the diamondback moth (Lepidoptera: Plutellidae) and its parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). Journal of Economic Entomology, **93**: 763–768.

Hu, D. and Coats, J. 2008. Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory. Pest Management Science, **64**: 775–779.

Isman, M.B. 2000. Plant essential oils for pest and disease management. Crop Protection, **19**: 603–608.

Isman, M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, **51**: 45–66.

Jiang, Z., Akhtar, Y., Bradbury, R., Zhang, X., and Isman, M.B. 2009. Comparative toxicity of essential oils of *Litsea pungens* and *Litsea cubeba* and blends of their major constituents against the cabbage looper, *Trichoplusia ni*. Journal of Agricultural and Food Chemistry, **57**: 4833–4837.

Kumrungsee, N., Pluempanupat, W., Koul, O., and Bullangpoti, V. 2014. Toxicity of essential oil compounds against diamondback moth, *Plutella xylostella*, and their impact on detoxification enzyme activities. Journal of Pest Science, **87**: 721–729.

López, M.D. and Pascual-Villalobos, M.J. 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Products, **31**: 284–288.

Miresmailli, S., Bradbury, R., and Isman, M.B. 2006. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Management Science, 62: 366–371.

Miresmailli, S. and Isman, M.B. 2006. Efficacy and persistence of rosemary oil as an acaricide against twospotted spider mite (Acari: Tetranychidae) on greenhouse tomato. Journal of Economic Entomology, **99**: 2015–2023.

Mkindi, A., Mpumi, N., Tembo, Y., Stevenson, P.C., Ndakidemi, P.A., Mtei, K., *et al.* 2017. Invasive weeds with pesticidal properties as potential new crops. Industrial Crops and Products, **110**: 113–122.

Ngamo, T.S.L., Ngatanko, I., Ngassoum, M.B., Mapongmestsem, P.M., and Hance, T. 2007. Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests. African Journal of Agricultural Research, **2**: 173–177.

Oerke, E.-C. 2006. Crop losses to pests. The Journal of Agricultural Science, **144**: 31–43.

Orr, N., Shaffner, A.J., Richey, K., and Crouse, G.D. 2009. Novel mode of action of spinosad: receptor binding studies demonstrating lack of interaction with known insecticidal target sites. Pesticide Biochemistry and Physiology, **95**: 1–5.

Priestley, C.M., Williamson, E.M., Wafford, K.A., and Sattelle, D.B. 2003. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA A receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. British Journal of Pharmacology, **140**: 1363–1372.

R Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. R program version 3.3.2. Available from http://www.R-project.org [accessed 6 March 2017].

Ritz, C., Baty, F., Streibig, J.C., and Gerhard, D. 2015. Dose-response analysis using R. Public Library of Science One, **10**: e0146021.

Sangha, J.S., Astatkie, T., and Cutler, G.C. 2017. Ovicidal, larvicidal, and behavioural effects of some plant essential oils on diamondback moth (Lepidoptera: Plutellidae). The Canadian Entomologist, **10**: 1–10.

Scott, J. 1990. Investigating mechanisms of insecticide resistance: methods, strategies, and pitfalls. *In* Pesticide resistance in arthropods. *Edited by* R. Roush and B. Tabashnik. Springer, New York, New York, United States of America. Pp. 39–57.

Stevenson, P.C., Isman, M.B., and Belmain, S.R. 2017. Pesticidal plants in Africa: a global vision of new biological control products from local uses. Industrial Crops and Products, https://doi.org/10.1016/j.indcrop.2017.08.034.

Tak, J.-H., Jovel, E., and Isman, M.B. 2016. Contact, fumigant, and cytotoxic activities of thyme and lemongrass essential oils against larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni*. Journal of Pest Science, **89**: 183–193.

Thomas, A.F. 2007. The synthesis of monoterpenes. *In* The total synthesis of natural products. *Edited by* J. ApSimon. John Wiley & Sons, Hoboken, New Jersey, United States of America. Pp. 1–196.

Tong, F. and Bloomquist, J.R. 2013. Plant essential oils affect the toxicities of carbaryl and permethrin against *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology, **50**: 826–832.

Tuck, S.L., Winqvist, C., Mota, F., Ahnström, J., Turnbull, L.A., and Bengtsson, J. 2014. Land-use intensity and the effects of organic farming on biodiversity: a hierarchical meta-analysis. The Journal of Applied Ecology, **51**: 746–755.

Venables, W.N. and Ripley, B.D. 2002. Modern applied statistics with S. 4th edition. Springer, New York, New York, United States of America.

Wheeler, M.W., Park, R.M., and Bailer, A.J. 2006. Comparing median lethal concentration values using confidence interval overlap or ratio tests. Environmental Toxicology and Chemistry, **25**: 1441–1444.

Zalucki, M.P., Shabbir, A., Silva, R., Adamson, D., Shu-Sheng, L., and Furlong, M.J. 2012. Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): just how long is a piece of string? Journal of Economic Entomology, **105**: 1115–1129.