The aphid-repellent effects of *Pittosporum* leaf extracts: assessment of inter-plant variability and comparison with common garden plants and essential oils

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Introduction

As concerns grow regarding the environmental impacts of conventional pesticides, growers and researchers are seeking alternative ways to manage and control insect pests. These alternative methods include biocontrol (Walter 2003), the use of physical barriers (Merfield et al. 2017), and application of compounds that stimulate and enhance natural plant defence systems (Hodge et al. 2005). Many plants contain natural secondary metabolites that are insecticidal and/or insect repellent, and much research has been performed into the potential of botanical extracts for use as crop protection agents (e.g. Maia & Moore 2011; Pavela 2016; Lengai et al. 2020). These botanically-derived crop protection agents are generally considered less environmentally harmful than synthetic biocides, with reduced longevity of effects, lower risk to users, and fewer non-target effects (Regnault-Roger et al. 2012; Pavela & Benelli 2016; Isman 2020).

Many native New Zealand plants contain highly bioactive compounds, and several species have been evaluated for their insecticidal, insect repellent, and anti-feedant properties (Brooker et al. 1987; Barrington et al. 1993; Russell & Lane 1993; Gerard et al. 1997; Hodge 2020). New Zealand has around 20 native *Pittosporum* (Pittosporaceae; Wardle 2011), and plants in this genus have been investigated for their biochemical properties for over 70 years (e.g. Carter and Heazlewood 1949). *Pittosporum* contain several groups of bioactive chemicals, such as phytoalexins. alkanes, and terpenoids (Weston 2004; Shanshi 2013). Many lemon-scented compounds, such as citronella, are repellent to insects, although Weston (2004) suggested that the citrus-scent of *Pittosporum eugenioides* (Tarata; ‘lemon wood’) is not primarily due to limonene, as in many citrus fruits, but from a mixture of compounds including the aliphatic ester octyl acetate. In terms of bioactivity, Calder et al. (1986) explored the anti-biotic potential of New Zealand *Pittosporum* and Kellam et al. (1992) investigated their enzyme inhibiting properties. The phyto-activity of *Pittosporum* is of much current interest, and globally species are being evaluated for anti-bacterial, anti-inflammatory, anti-oxidant, and cancer-controlling abilities (Huang et al. 2023; Riyas & Sukumaran 2023; Sha et al. 2023). *Pittosporum* have also been shown to be toxic to larval and adults mosquitoes (Maharaj et al. 2011; Ratsimiebo et al. 2015) and are repellent to the tomato-potato psyllid (S Hodge *unpublished data*).

Aphids are significant agricultural pests that impact development and yield of many important crops. Historically, aphids have been controlled by a suite of powerful synthetic insecticides, including organophosphates, pyrethrins, and neonicotinoids. Impetus to develop novel pest control strategies has arisen because of several instances of aphids evolving resistance to different classes of insecticidal compounds and because many synthetic aphicides are no longer certified for general use. Previously Hodge (2020) described the repellent effects of kawakawa (*Piper excelsum* G.Forst.) leaf extracts against pea aphids, although this work also highlighted that there could be considerable variation in the potency of different batches of leaves.

The primary aim of this study was to explore the aphid-repellent properties of *Pittosporum* leaf extracts. To meet the main objective, it was desirable to establish the consistency of any effects by testing several independent batches of leaves. Additionally, as it was necessary to compare any repellent effects obtained with *Pittosporum* with those obtained by commercially-available plant protection products, the study was also extended to include leaf extracts of several readily-available garden plants and emulsions of essential oils suggested as having insect repellent properties.

Methods

Pea aphids, *Acyrthosiphon p*isum (Harris), were collected from common bean plants (*Phaseoulus vulgaris* L.) at University College Dublin, and a colony subsequently maintained under room conditions on tic beans (*Vicia faba* L.). In settling assays, a mixture of nymph ages was used but very small individual (< 1 d old) and alate and apterous adults were avoided.

The aphid settling assay used was based on previous methods developed to examine both aphid attraction and avoidance (Hodge and Powell 2008; Hodge 2020). A filter paper [Ahlstrom Munksjö Munktell] was placed in the lid of a 9 cm plastic Petri dish and dampened with tap water. A 12 mm diameter stainless steel cork-borer was used to cut discs from bean leaves (*Vicia faba*) and both sides of the discs were sprayed with water or a test solution using a plastic spray bottle. Six leaf discs were placed equidistantly around the edge of each Petri dish: three water-sprayed discs at 0^o, 120^o and 240^o and three treated discs at 60^o, 180^o and 300^o. Ten aphids were placed into the centre of each Petri dish and allowed 24 h to settle, after which the total numbers of aphids on the control and treated discs in each Petri dish were recorded.

Plant extracts were made by combining chopped plant material with water in a ratio of 1:10 weight:volume and heating in a conical flask to approximately 75^oC for 10 minutes. The extract was allowed to cool and separated from solid material by pouring through a fine metal sieve. To gain insight into the generality of the repellent effects of *Pittosporum*, leaves were collected from eight different gardens in south Dublin, Ireland. Because the plants were ornamental forms, identification to species was problematic, although we are confident that the plants used were either *P. eugenioides* (tarata) or *P. tenuifolium* (kohuhu).

In addition to *Pittosporum*, ten other plant extracts were tested for their aphid repellent properties: lavender (*Lavandula angustifolia*), lemon balm (*Melissa officinalis*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), catnip (*Nepeta meyeri*), *Pectranthus* spp. (all Lamiaceae), *Eucalyptus* (Myrtaceae), tobacco (*Nicotinia* sp.; Solanaceae), lemon grass (*Cymbopogon citratus*; Poaceae), and mandarin orange (*Citrus reticulata*;

Rutaceae). Generally, the plant material used was freshly collected foliage obtained from domestic gardens or the University College Dublin campus. The two exceptions were fresh lemon balm, where stem was purchased from a grocery store, and mandarin orange, where fresh peel was used.

Seven essential oils were tested for their aphid repellent properties: citronella [Calmer Solutions, UK], lemon grass, sweet orange, peppermint, lavender, eucalyptus and tea tree [Pure Aroma Oil,]. To prepare the oil emulsions, 1 ml of essential oil was added to 99 ml of tap water in a conical flask and heated on an electric hotplate with a magnetic stirrer for 10 minutes (~ 75^{o}C) until an emulsion was formed.

The commercial sprays 'BugClear' and 'BugFree' were used as positive controls to ascertain if the leaf disc protocol could demonstrate an aphid avoidance response to known chemical repellents. BugClear contains 1, 2-benzisothiazolin-3-one (BIT) and 0.5g/L of acetamiprid and BugFree contains 0.045 g/L of pyrethrin combined with 8.25 g/L of rapeseed oil.

The numbers of aphids settled on the control and treated discs were compared using a paired t-test with the data from each Petri dish as the independent experimental unit. To examine variability among the *Pittosporum* trials, the counts of aphids on control and treated discs in each of the eight trials were assessed with a chi-square test of heterogeneity (Zar 1984). Additionally, for the *Pittosporum* trials, the proportion of aphids settled on the treated discs was calculated along with a 95% confidence interval, and tested for a significant deviation from the null proportion of 0.5.

Results

The suite of assays involved 496 Petri dishes and 4960 aphids, of which 2423 (48.9%) had settled on a leaf disc after 24 h. As evidence the leaf disc protocol could demonstrate an avoidance response, both commercial insecticides resulted in a significant reduction in aphid settling (Table 1).

Table 1. Summary of leaf disc trials performed to examine the repellent effects of commercial insecticides, aqueous plant extracts, and emulsions made from essential oils. Ten pea aphids were placed into each Petri dish and their position recorded after 24 h. P-values obtained by paired t-test.

Product class	Product	Petri dishes	Aphids settled (%)	Settled aphids on treated (%)	t	P
Commercial	BugFree	30	38.7	24.1	4.99	**<0.001**
	BugClear	30	21.3	21.9	5.41	**<0.001**
Foliage	*Pittosporum*	96	48.1	31.4	7.21	**<0.001**
	Lavender	20	50.0	12.0	6.02	**<0.001**
	Lemon balm	20	69.5	30.9	3.02	**0.007**
	Peppermint	20	52.5	31.4	2.75	**0.013**
	Spearmint	20	54.0	28.7	3.59	**0.002**
	Catnip	20	63.0	12.7	7.19	**<0.001**
	Plectranthus	20	43.0	50.0	0.00	1.000
	Eucalyptus	20	36.5	16.4	5.24	**<0.001**
	Nicotinia	20	38.0	32.9	2.35	**0.030**
Stem	Lemongrass	20	55.0	35.5	2.10	**0.049**
Peel	Mandarin	20	51.5	12.6	5.92	**<0.001**
Essential oils	Citronella	20	48.5	43.3	1.66	0.114
	Lavender	20	49.5	38.4	2.17	**0.043**
	Peppermint	20	50.5	49.5	0.09	0.930
	Eucalyptus	20	39.0	44.9	0.59	0.561
	Tea tree	20	64.5	39.5	1.71	0.103
	Lemongrass	20	56.5	55.8	-0.60	0.554
	Sweet orange	20	69.0	30.4	4.13	**0.001**

The proportion of aphids settled on the leaf discs sprayed with the eight *Pittosporum* extracts ranged from 24% to 38%, and the heterogeneity test indicated there was no statistically significant difference among the results of the eight trials ($\chi^2 = 3.3$, for 7 d.f., $P = 0.85$; Table 2; Figure 1). Based on the data from all 96 Petri dishes, only 31.4% of the aphids that had settled did so on the *Pittosporum* treated leaf discs (Table 2; Figure 1).

The paired t-tests performed using the data within each *Pittosporum* assay indicated that in only five cases from eight was the distribution of aphids statistically significant from that expected by chance (Table 2). Of the three assays (#1, #5, #7) identified as being non-significant by the t-tests, only one of these (#5) was also identified as non-significant using the binomial test (Figure 1). Conversely, the distribution of aphids in Assay #6 was identified as showing a significant avoidance of *Pittosporum* by the t-tests ($P = 0.046$), but was borderline non-significant when using the binomial test ($P = 0.054$ (Table 1; Figure 1).

Table 2. Results of two-way choice assays investigating settling of pea aphids on leaf discs treated with eight different batches of aqueous *Pittosporum* leaf extracts. Ten pea aphids were placed into each Petri dish and their position recorded after 24 h. P-values obtained by paired t-test.

Trial	Petri dishes	Aphids settled (%)	Aphids on treated discs (%)	t	P
#1	12	54.2	35.4	1.38	0.194
#2	12	52.5	27.0	3.75	**0.003**
#3	12	59.2	29.6	3.25	**0.008**
#4	12	55.0	31.8	3.13	**0.010**
#5	12	39.2	38.3	1.89	0.085
#6	12	38.3	34.8	2.24	**0.046**
#7	12	38.3	32.6	2.15	0.054
#8	12	48.3	24.1	3.74	**0.003**
Total	96	48.10	31.4	7.21	**< 0.001**

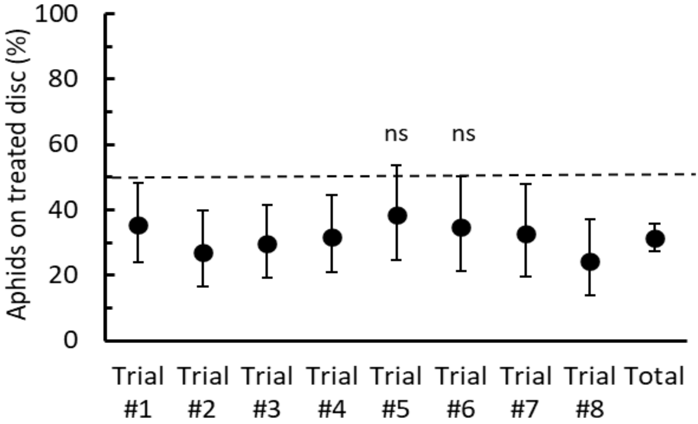

Figure 1. Proportion (%; ±95% CIs) of pea aphids settled on leaf discs coated with eight different batches of aqueous *Pittosporum* extracts in 2-way Petri dish choice assays. (ns; nonsignificant avoidance as indicated by binomial exact test)

Except *Plectranthus*, all of the foliage extracts caused a significant reduction in aphid settling, with the strongest effects seen in the assays using lavender (12.0% of aphids on treated discs), catnip (12.7%) and *Eucalyptus* (16.4%; Table 1). Additionally, spraying the leaf discs with extracts of lemon grass stem and mandarin peel also reduced settling by pea aphids (Table 1). However, only two of the essential oils, lavender and sweet orange, produced a statistically significant avoidance response (Table 1).

Discussion

Although there was some minor variation among the eight trials, the *Pittosporum* leaf extracts produced fairly consistent effects with respect to pea aphid settling behaviour. Overall, approximately 30% of aphids settled on treated discs, with no statistically significant variation among the batches of extract used, or any increasing or decreasing trends in repellency as the sequence of trials progressed. The deterrent effect produced by the *Pittosporum* extracts was not as strong as that observed for the two commercial insecticides (both < 25%) but was comparable, or greater, than that obtained by many of the other plants, such as *Plectranthus*, *Nicotinia*, peppermint, lemon balm, and lemon grass. Many lemon or citrus scented compounds, such as citronella, have strong insect-repellent properties. In this study, we did not necessarily find that to be the case, and the citronella oil did not result in a significant deterrent effect. Nevertheless, the lemon-scented extracts of lemon balm, lemon grass, and *Pittosporum* did produce significant avoidance responses, with around a third of aphids settling on the treated discs in each case.

That only two of the eight essential oils tested deterred aphid settling may point to one of the potential weaknesses of our study; we only tested a single concentration of each extract, and this may have been too dilute to produce such an effect. Nevertheless, two essential oils and most leaf extracts were able to induce an avoidance response at the concentrations used. So, as a screening process, this exercise was successful and highlighted a number of avenues for further research; for example, the repellent effect produced by the mandarin peel was very strong, and was only surpassed by that obtained with the lavender leaf extract. In line with the current surge in circular economy principals, the use of a waste product, orange peel, to create a new commodity, a botanically-derived insect repellent, is appealing, and further research and testing of citrus fruit extracts is warranted.

The use of leaf-disc assays provides a rapid screening technique to examine the avoidance (or attraction) responses of plant-feeding insects in both choice and no choice scenarios. Nevertheless, we accept there are several issues with this method. For example, the arenas used are highly simplistic and unrealistic, and often only a small fraction of the aphids used in the trials actually settle on the leaf discs. This latter problem commonly occurs in leaf disc trials, especially with older aphids (e.g. Hodge et al. 2008; Hodge 2020), so reducing the realized sample size of insects, which in turn lowers the power of subsequent statistical analysis.

Our results also highlight that some caution may be needed when analysing the data obtained from such assays in terms of null hypothesis significant testing. Some inconsistencies were observed when declaring significant avoidance responses when using the binomial tests, based on pooled data, compared with the paired t-test, which considers the data from each unit as an independent paired replicate. Whereas the former tests are based on the overall distribution of individuals between the two leaf disc treatments, the results of the t-test are dependent upon several factors: the magnitude of the average difference, the consistency of the set of differences, and the number of independent replicates (i.e. degrees of freedom). It might be argued that, given a set number of aphids are available for each assay, more independent replicates (Petri dishes in this case) each with fewer aphids might enhance the statistical power of the t-test, whereas for the tests using pooled data this may be of no consequence. These aspects of experimental design for two-way insect choice tests, and the subsequent statistical analysis of the data obtained, should be further explored (see Roberts et al. 2023).

As alluded to above, we concede our study has several weakness: we used only a single, relatively unrealistic laboratory assay, tested only one species of aphid, and examined only one performance measure over a relatively short time scale. Nevertheless, even given the above considerations, the leaf-disc assays we used comprehensively demonstrated that *Pittosporum* leaf extracts produced a consistent deterrent effect towards pea aphids. Additionally, the aphid-repellent effects obtained with *Pittosporum* were similar or better than those produced by several other plant extracts and most of the essential oils we tested. Consequently, we consider that *Pittosporum* shows good potential for development of botanically-derived plant protection agents and therefore advocate further investigation into the efficacy of aqueous (and other) extracts, identification of the specific compounds causing aphid avoidance responses, and comparison of extracts from garden/ ornamental varieties with naturally-occurring plants.

Acknowledgements

The thank Laura Gallego-Lorenzo, Dave Brogan, and Gordon Kavanagh for technical support, Ballyroan gardening group for supplying plant material, and Martin and Fiona Giffney for support throughout this research.

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