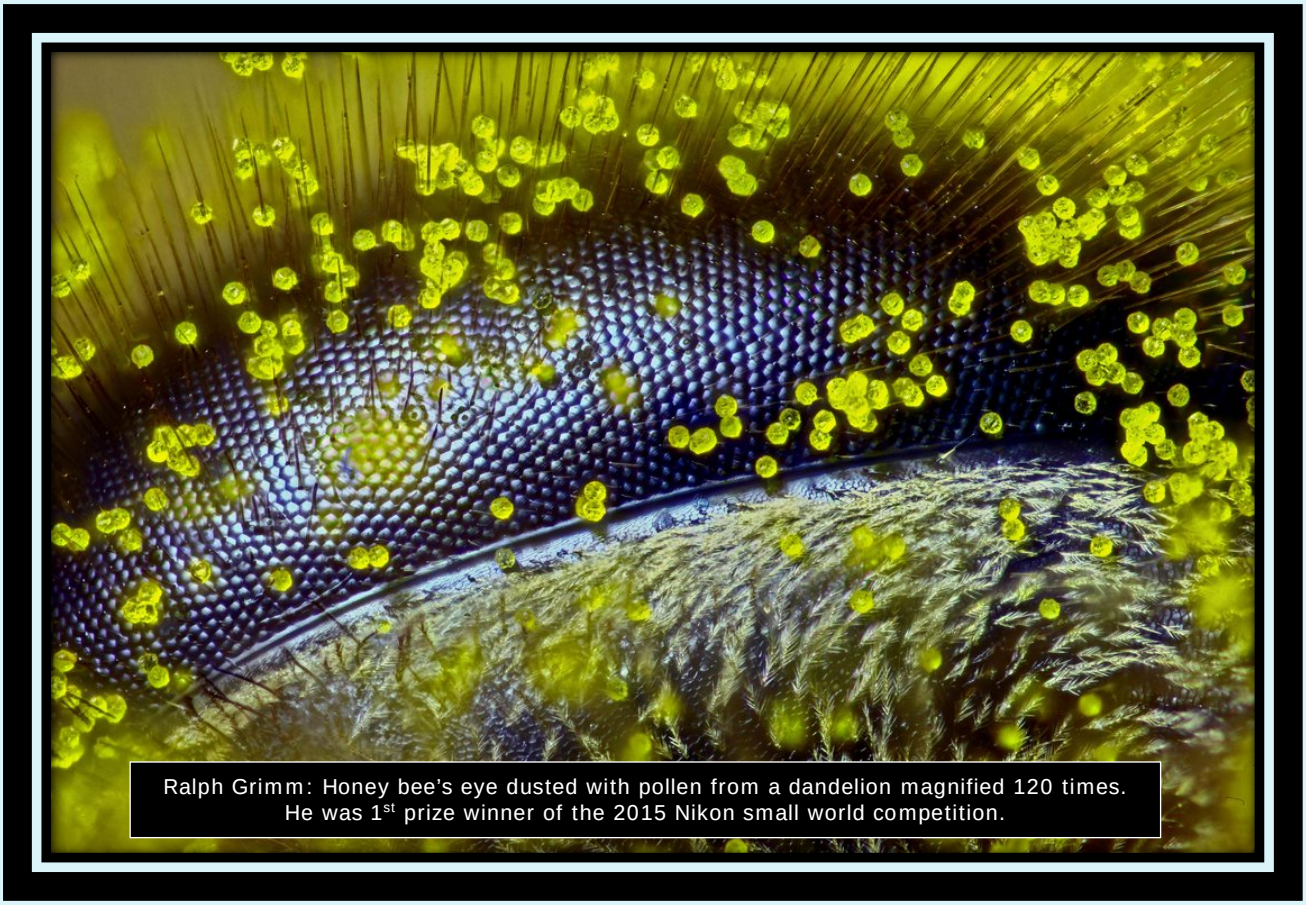
BEELINES

Newsletter of The Beekeepers Club Inc.

February 2017



Ralph Grimm: Honey bee's eye dusted with pollen from a dandelion magnified 120 times. He was 1st prize winner of the 2015 Nikon small world competition.

Next Club Meeting: Thursday 16th February 2017

Inventors Fair - Beekeeping inventions & gadgets

Beginners Q&A

NEW VENUE

Venue: Performing Arts Centre (PAC)
Doncaster Secondary College
123 Church Rd Doncaster
Melway 33 G 12

********** Guests and Visitors Welcome **********

Enquiries and information: editor@beekeepers.org.au

UPCOMING EVENTS

February 18th and 19th 2017

STEERING Visits

Mentoring visits to members' home hives at a variety of locations.

Join an inspection, build confidence and enjoy a convivial cuppa with fellow members.

See website for details and registration: http://www.beekeepers.org.au/events

JUNIORS MEETING

Next meeting

February 18th 2017

10.00 -11.30 am

33 Saxon St, Brunswick

February 1st, 8th, 15th 2017

BEGINNERS COURSE

7.30–10.00 pm

Venue: Senior Citizens Clubrooms, 895-901 Doncaster Rd, Doncaster

April 8th & 9th April 2017

WEEKEND BEGINNERS COURSE

at

Saxon St, Brunswick.

Registrations and full details on club web site under events.

Watch website for more upcoming events

Sunday 26th Feb 2017

HIVE OPENING SOCIAL DAY

with Sausage sizzle and light refreshments

at

the club apiary site Lookover Rd Donvale

11.00 am – 3.00 pm

.

This is a good chance to open some hives with experienced beekeepers. Both experienced and newbees are welcome.

Entry fee is a gold coin donation.

Go to club website to register under events for catering purposes.

Bee Facts

One percent of all middle- aged **bees** become undertakers. A genetic brain pattern compels them to remove dead **bees** from the hive.

But **most** amazingly, regular **honeybees,** which perform multiple jobs in their lifetime, will change their brain chemistry before taking up a new gig.

To reinforce their hives, bees use a resin from poplar and evergreen trees called propolis. It's basically beehive glue. Although bees use it as caulk, humans use it to fight off bacteria, viruses, and fungi. Research shows that propolis taken from a beehive may relieve cold sores, canker sores, herpes, sore throat, cavities, and even eczema.

THEY CAN SOLVE HAIRY MATHEMATICAL PROBLEMS

Pretend it's the weekend, and it's time to do errands. You have to visit six stores and they're all at six separate locations. What's the shortest distance you can travel while visiting all six? Mathematicians call this "traveling salesman problem," and it can even stump some computers. But for bumblebees, it's a snap. Researchers at Royal Holloway University in London found that bumblebees fly the shortest route possible between flowers. So far, they're the only animals known to solve the problem.

THEIR BRAINS DEFY TIME

When aging, bees do jobs usually reserved for younger members, their brain stops aging.

In fact, their brain ages in reverse. Imagine if riding a tricycle didn't just make you feel young—it actually made your brain tick like a younger person's.

An investigation into the cytotoxicity of Manuka honey, coconut oil, cinnamon, aloe vera, and olive leaf extract using Bacteria Luminescence Toxicity Screening with bioluminescent bacteria, *Photobacterium leiognathi.*

By Caroline Bert

ABSTRACT

The increasing prevalence of bacterial resistance to antimicrobial agents has severe global implications and alternative antimicrobial strategies are becoming increasingly sought-after. This situation has driven a re-evaluation of the medicinal properties of plant-based substances as a long-term solution to antibacterial resistance (Mandal 2011, p. 154).

The effectiveness, in terms of cytotoxicity, of Manuka honey, coconut oil, cinnamon, aloe vera, and olive leaf extract, using Bacteria Luminescence Toxicity Screening with bioluminescent bacteria, *Photobacterium leiognathi* was investigated in two toxicity assay setups. The first plate setup (containing all five natural substances) showed Manuka honey and olive leaf extract were most effective in terms of cytotoxicity, followed by cinnamon. The aloe vera and coconut oil displayed insignificant antibacterial activity. Thus, a second plate set-up containing Manuka honey and olive leaf extract at different concentrations was assessed at 30 minutes, 20 hours and 24 hours. Both substances exhibited potent antibacterial activity, with olive leaf extract slightly more effective. The cytotoxic activity of the olive leaf extract was attributed to the antibacterial properties of oleuropein and caffeic acid. High levels of methylglyoxal (MGO) may explain Manuka honey's cytotoxic activity, in addition to *bee defensin-1*, an antimicrobial peptide that interacts with the bacterial membrane. Thus, the conclusion reached provides valuable insight into the effective antibacterial properties of Manuka honey and olive leaf extract. These findings are significant as they contribute to the growing body of research on the development of natural, more effective antibiotics that do not promote antibacterial resistance, and also may have applications in the treatment of gastric cancers, and microbial control in food preservation.

BACKGROUND INFORMATION

A significant medical advance of the last century has been the development of effective antibiotics. Most antibiotics modify or interfere with the essential functions or structures of bacteria (Collignon 2002, p. 325). The administration of antibiotics kills or inhibits susceptible bacteria, resulting in a selective pressure for the survival of resistant strains of bacteria. Bacteria can synthesise and use antibiotics against other bacteria, which has led to a low level of natural selection for resistance to antibiotics. However, the present higher levels of

antibiotic-resistant bacteria are believed to be a consequence of the overuse of antibiotics (Alliance for the Prudent Use of Antibiotics, 2014). Mandal (2011, p. 154) states that as resistant pathogens develop and spread, the effectiveness of the antibiotics is weakened.

Many compounds sourced from plants are constituted of numerous complex compounds acting synergistically, meaning it is difficult for bacteria to develop a resistance. The cytotoxicity of a substance refers to its toxicity to cells (The Royal Children's Hospital Melbourne, 2015). Therefore, this essay will examine the cytotoxic potential of several natural substances in order to explore the significance of their antibacterial activity.

Manuka honey

Honey has an extensive medicinal history and, for a significant period of time, has been known to possess effective antimicrobial properties (Mandal 2011, p. 154). The chemical properties of honey are dependent on the floral source of the nectar, growing conditions and environmental stress (Melcare, n.d). Studies have confirmed that natural, unheated honey has some broad-spectrum antibacterial activity when tested against bacteria (Mandal 2011, p. 154). Research has shown that Manuka honey, more specifically, *Leptospermum scoparium* (*L. scoparium*) honey, has an inhibitory effect on approximately 60 species of bacteria (Molan, 1992).

The antimicrobial activity in honeys is due to the enzymatic production of hydrogen peroxide. When honey comes in contact with water, glucose oxidase synthesises hydrogen peroxide. Furthermore, honey's hygroscopic properties mean it can absorb moisture from the environment and dehydrate bacteria, and its acidity and high sugar content has been shown to inhibit bacterial growth (Mandal 2011, p. 154).

However, Manuka honey displays significant antibacterial effects even after the hydrogen peroxide activity is blocked. Medical grade Manuka honey has been found to have powerful *in vitro* bactericidal activity against antibiotic-resistant bacteria and in the treatment of chronic wound infections that do not respond to antibiotic therapy (Mandal 2011, p. 158). The emerging antimicrobial resistance trends in burn wound bacterial pathogens mean confirmation of Manuka honey's effective antimicrobial properties against antibiotic-resistant organisms is much anticipated (Mandal 2011, p. 159).

Olive leaf extract

Research conducted by Markin et al on the antimicrobial action of olive leaf extract (Markin, Duek, & Berdicevsky, 2003, p. 132-6) showed complete inhibition of bacterial activity and complete destruction of *Escherichia coli* cells, suggesting there is powerful antimicrobial potential for olive leaves (Rochway, 2012). Researchers have identified an active component called elenolic acid in mature olive leaves, thought to be a natural defence mechanism

against pathogens. Studies have shown this compound has strong inhibitory effects on the growth of viruses, bacteria, fungi and parasites (Hoffman, 2014).

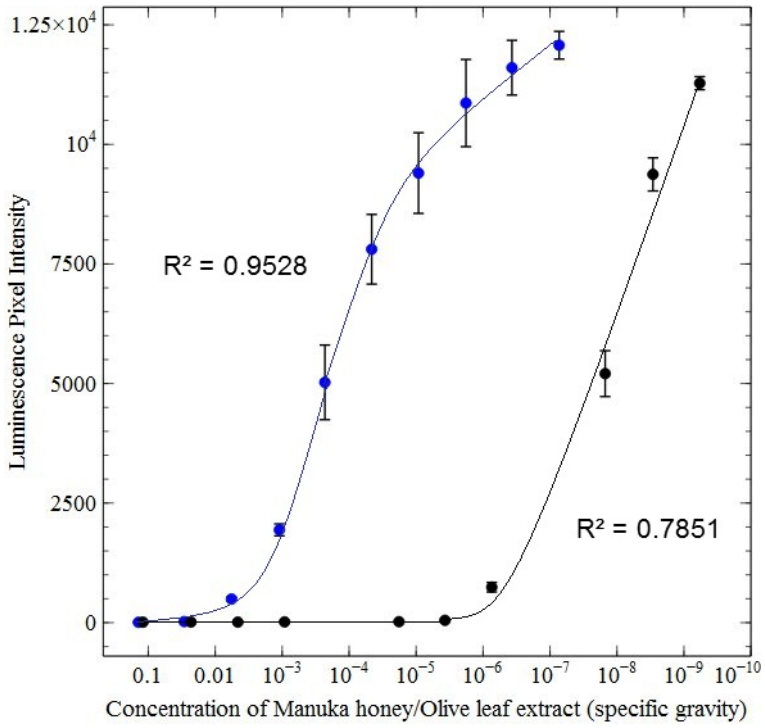
Bacteria Luminescence Toxicity Screening

The Bacteria Luminescence Toxicity Screening allowed the inhibition of light emitted by bioluminescent bacteria, *Photobacterium leiognathi*, to be measured (as *Luminescence Pixel Intensity*). Toxicity is assessed by measuring the extent to which light production by the bacteria is inhibited (percentage inhibition determined and analysed). When the bioluminescent bacteria are exposed to a "toxic" substance, a decrease in luminescence is observed as the number of viable bacteria decreases. The mechanisms underlying the toxicity of substances may involve interactions with cell surface receptors, disruption of cell membrane function, chemical reaction with cellular components, or inhibition/competition of enzyme systems (Jennings, Rayner-Brandes, & Bird, 2001, p. 3448). A low Luminescence Pixel Intensity therefore indicates the substance being tested has stronger antibacterial properties (has a higher cytotoxicity).

A pH buffer was used as the ionisation of other compounds may produce highly acidic or alkaline solutions where pH is the primary cause of toxicity (Jennings et al, 2001). A growth medium was used in the overnight plates to sustain the bacteria for the duration of the test (Jennings et al, 2001).

EXPERIMENTAL RESULTS FOR MANUKA HONEY

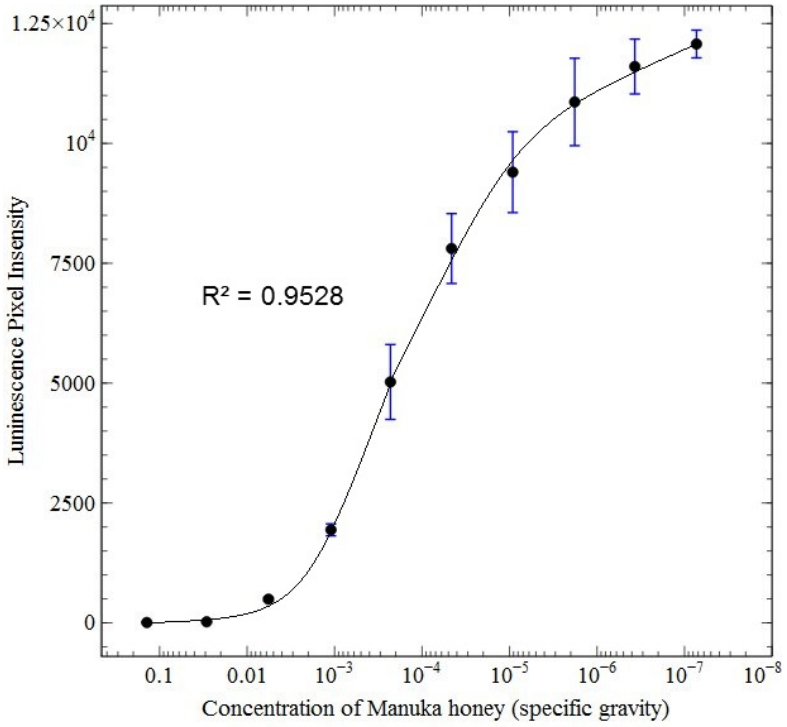
Graph 1: Line graph comparing Luminescence Pixel Intensity of **Manuka honey** (blue) and **olive leaf extract** (black) in relation to concentration (specific gravity).



A logarithm scale was used as the x-axis and the concentrations (x-axis) are displayed in decreasing order for ease of interpretation.

In both conditions, as the concentration decreases, Luminescence Pixel Intensity (LPI) increases, which is evidence of the antibacterial properties of both Manuka honey and olive leaf extract (light emittance, and thus luminescence intensity, is proportional to the number of *Photobacterium leiognathi).* The olive leaf extract displays equally effective antibacterial activity at all concentrations above $5.5x10^{-6}$. From this graph, an LC50 value (50% mortality of *Photobacterium leiognathi*) of $10^{-3.8}$ ($1.6x10^{-4}$) was obtained for Manuka honey, compared to $10^{-7.8}$ ($1.6x10^{-8}$) for olive leaf extract. Analysis of the results indicates that although olive leaf extract displays potent cytotoxic effects at lower concentrations than Manuka honey, Manuka honey, nevertheless displays significant antibacterial activity against *Photobacterium leiognathi.*

Graph 2: **Manuka honey**: Line graph displaying Luminescence Pixel Intensity in relation to concentration (specific gravity) (30 minute plate setup, BLT-Screen)



EXPLANATION OF RESULTS

The antibacterial activity of olive leaf extract may be attributed to the presence of oleuropein and caffeic acid which are thought to inhibit microbial action. Research by Pereira et al (2007) indicates the phenolic compounds identified in olive leaf extract (caffeic acid, verbascosie, oleuropein, luteolin 7-O-glucosise, rutin, apigenin7-O-glucoside and luteolin 4'-O-glucoside) display an unusual combined antibacterial and antifungal action (Pereira, Ferreira, Marcelino, Valentão, Andrade, Seabra, Estevinho, Bento & Pereira, 2007) and (Hoffman, 2014). The mechanisms underlying oleuropein's cytotoxicity are thought be linked to this compound's ability to inactivate cellular enzymes crucial for bacterial replication, or altering

the permeability of the cell membrane, consequently causing leakage of cytoplasmic constituents (Tassou, Nychas & Board, 1991). The antimicrobial action of *olive oil* polyphenols, has also been demonstrated on *Helicobacter pylori*, exerting strong bactericidal activity against eight strains of *H. pylori*, three of which were resistant to some antibiotics (Medina, de Castro, Romero, Ramírez & Brenes, 2013). The resistance of the *H. pylori* against antibiotics has increasing prevalence, thus these findings provide insight into the value of olive oil in the treatment of, for example, gastric ulcers and gastric cancer.

Manuka honey also displayed effective antibacterial action. Honeys display relatively unstable antibacterial activity referred to as Peroxide Activity (PA) which involves the activity of enzyme, glucose oxidase, which oxidises glucose to gluconic acid, making the honey highly acidic (a by-product being hydrogen peroxide). However, Manuka honey also displays Non-Peroxide Activity (NPA) meaning it has a strong, stable activity shown to be effective even after the elimination of peroxide activity. Other honeys display NPA, but to a lesser extent (Cramp, 2015). High levels of the antibacterial compound, methylglyoxal (MGO) (an effective protein-glycerating agent) are also thought to contribute to Manuka honey's antibacterial properties (GreenMedInfo, 2014). MGO is thought to possess the ability to modify the structure and function of target molecules (Majtan, 2011). In addition, *bee defensin-1*, an antimicrobial peptide has been found in honey (Kwakman, te Velde, de Boer, Speijer, Vandenbroucke-Grauls, Zaat 2010, p. 2576). Defensins often interact with the bacterial membrane, facilitating mechanisms that damage its structure (Byrne, 2012).

APPLICATIONS

Extended use of antibiotics results in a weakened immune system, making it more susceptible to the invasion of antibiotic-resistant pathological organisms (Hansen et al., 2002). Thus natural substances, such as olive leaf extract or Manuka honey, may offer a solution to the damaging effects of concentrated, synthetic drugs which build up immune system resistance. The complexity of the molecular interactions in these substances, and their synergistic effect in the human body, should be considered when evaluating their potential in the development of antibiotics.

The results of this investigation provide valuable insight into the potential use of Manuka honey for medicinal purposes. In the future, it may be of interest to examine the cytotoxicity of honeys from varying floral backgrounds, or the cytotoxic properties of honeybee venom. Honeybee venom contains apidaecins which bind to, and inactivate, DnaK (a protein essential in the bacteria's response to stress), resulting in bacterial mortality. Developing a more comprehensive understanding of this ability to induce mortality via intracellular mechanisms is of great interest in fields of drug development and may lead to the development of new antibiotics (Byrne, 2012).

REFERENCES

Alliance for the Prudent Use of Antibiotics. 2014. *General Background: About Antibiotic Resistance*. [Online]. Available at: http://www.tufts.edu/med/apua/about_issue/about_antibioticres.shtml. [Accessed 22 December 2014].

Collignon, P, 2002. 11: Antibiotic resistance. *The Medical Journal of Australia*. [Online]. 177 (6), 325-329. Available at: https://www.mja.com.au/journal/2002/177/6/11-antibiotic-resistance [Accessed 22 December 2014].

Cramp, D, 2015. Manuka Honey Explained. *Bee World.* [Online]. 90(2), 40. Available at: http://www.tandfonline.com/doi/abs/10.1080/0005772X.2013.11417533#preview [Accessed 28 July 2015].

Hansen, M, Verity, A, 2002. *Olive Leaf Extract*. 1st ed. Australia: Goldgolf Pty. Ltd.

James Byrne. 2012. *Scientific American*. [Online]. Available at: http://blogs.scientificamerican.com/disease-prone/you-should-rub-honey-on-your-everywhere/. [Accessed 25 August 2015].

Jennings, V, Rayner-Brandes, M, & Bird, D, 2001. Assessing Chemical Toxicity With the Bioluminescent Photobacterium (*Vibrio Fischeri*): A Comparison of Three Commercial Systems. *Water Research.* [Online]. 35(14), 3448. Available at: http://www.researchgate.net/publication/11799312_Assessing_chemical_toxicity_with_the_bioluminescent_photobacterium_%28Vibrio_fischeri%29_a_comparison_of_three_commercial_systems [Accessed 27 April 2015].

Kwakman, P, te Velde, A, de Boer, L, Speijer, D, Vandenbroucke-Grauls, C, & Zaat, S, 2010. How honey kills bacteria. *The Journal of the Federation of American Societies for Experimental Biology*, [Online]. 24(7), 2576-2582. Available at: http://www.fasebj.org/content/24/7/2576.full.pdf+html [Accessed 06 January 2015].

Majtan, J, 2011. Methylglyoxal—A Potential Risk Factor of Manuka Honey in Healing of Diabetic Ulcers.*Evidence-Based Complementary and Alternative Medicine*, [Online]. 1-2. Available at: http://www.hindawi.com/journals/ecam/2011/295494/ [Accessed 28 July 2015].

Mandal, M & S, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, [Online]. 1(2), 154-160. Available at:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609166/pdf/apjtb-01-02-154.pdf [Accessed 22 December 2014].

Markin, D, Duek, L, & Berdicevsky, I, 2003.In vitro antimicrobial activity of olive leaves. *Mycoses.*[Online]. 46(3-4), 132-6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12870202 [Accessed 17 July 2015].

Medina, E, de Castro, A, Romero, C, Ramírez E, & Brenes, M, 2013. Effect of antimicrobial compounds from olive products on microorganisms related to health, food and agriculture. *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education*. [Online]. 1089-90. Available at: http://www.formatex.info/microbiology4/vol2/1087-1094.pdf [Accessed 28 July 2015].

Melcare, n.d. *Leptospermum sp Honey (Manuka) - a brief introduction*. [Online]. Available at: http://melcare.com/clinical1.html. [Accessed 06 January 2015].

Molan, PC, 1992. The antibacterial nature of honey. The nature of the antibacterial activity. *Bee World.* [Online]. 73/1, 5-28. Available at:http://researchcommons.waikato.ac.nz/bitstream/handle/10289/2094/antibacterial%20activity.ocr.pdf?sequence=1&isAllowed=y [Accessed 06 January 2015].

Omar, S, 2010. Oleuropein in Olive and its Pharmacological Effects. *Scientia Pharmaceutica*, [Online]. 78, 133–154. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3002804/pdf/scipharm.2010.78.133.pdf [Accessed 28 July 2015].

Pereira AP, Ferreira IC, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, & Pereira JA, 2007. Phenolic compounds and antimicrobial activity of olive (Olea europaea L. Cv. Cobrançosa) leaves. *Molecules.* [Online]. 12(5), 1153-62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17873849 [Accessed 17 July 2015].

Rochway. 2012. *Natural Anti-bacterial effects of Olive Leaf Extract*. [Online]. Available at: http://www.rochway.com.au/blog/natural-anti-bacterial-effects-of-olive-leaf-extract/. [Accessed 19 January 2015].

Ronald Hoffman. 2014. *Olive leaf extract*. [Online]. Available at: http://drhoffman.com/article/olive-leaf-extract-2/. [Accessed 19 January 2015].

Tassou C, Nychas GJ, & Board R, 1991. Effect of Phenolic Compounds and Oleuropein on the Germination of Bacillus cereus T Spores.*Biotechnology and Applied Biochemistry*.13, 231-37.

The Royal Children's Hospital Melbourne. 2015. *Cytotoxic Drugs - The Management Of*. [Online]. Available at: http://www.rch.org.au/rchcpg/hospital_clinical_guideline_index/Cytotoxic_Drugs_The_Management_Of/. [Accessed 08 July 2015].

Editor's Note: There has been a bit of nepotism going on here. The author, Caroline Bert. is my granddaughter. This essay was written by her, 2015, for which she was awarded runner-up prize Queensland Young scientist of the year. It is an excerpt from the much larger study report she undertook.

Flow Hive page

The Brood Box:

Check your brood box regularly at this time of the year at least every 4 weeks for FEDSS. **F**ood, **E**ggs, **D**isease, **S**pace, **S**warming. I have heard of swarming occurrences in the last few weeks, so it appears in this strange season we are having you should not discount a swarm even now. A monthly inspection is too long to control a swarm event so if you see a sign on one inspection inspect again in 10 days.

When you do your inspection it is a good idea to ensure your flow hive has a slope of 25 - 50 mm towards your honey outlets. The Flow hive does have a slope built in to the bottom board, so if placed on a level surface it should be ok.

Harvesting:

Check each Flow frame you are harvesting is ready and capped. Look for 90% of cells capped. The best way to get to know is to open the hive and inspect the flow frames. Look in the end frame view. If the cells are mostly full and capped with wax, it's a good indication that it is ready for harvest.

A suggestion is - you open the hive (with appropriate protection) and pull out the inner flow frames to become familiar with them and recognise what the end window is indicating. This is a great time to check for at least 90% capping.

Make sure the baseboard corflute slider is in the top slot. If in the top slot, the corflute is pushed up against the mesh so any dribbles of honey that may occur will stay within the reach of the bees.

The FLOW company has been very supportive of The Beekeepers Club, with Stuart Anderson presenting at our beginners courses and by donating 2 FLOW hives. They are now generously offering club members a discount for a limited time. The discount code for club members when purchasing from Flow Hive is**: DONCASTER15%.** This code is only effective for 72 hours after receipt of this Beelines newsletter so you will need to act fast.