Feeding behavioural patterns of *Imbrassia belina* larvae that minimizes the production of tannins in *Colophospermum mopane* leaves

Madeline Sibula and Shadreck Dube

Department of Applied Biology and Biochemistry, National University of Science and Technology, Bulawayo, Zimbabwe

Email: madeline.sibula@nust.ac.zw

ABSTRACT

Mopane (*Colophospermum mopane*) trees respond to herbivory by producing secondary metabolites known as tannins. In order to exploit such trees, insects will adopt feeding patterns that will best reduce production of such feeding deterrents. To elucidate this, the behavioural feeding patterns of *Imbrasia belina* larvae were studied on Mopane trees and artificial damage on the same was done by punching small holes on a large number of leaves over a period of 25 days. Similarly, a control was used to simulate feeding behaviour of *I. belina* larvae by removing a large number of full leaves on each day over a period of 25 days. In each instance leaves were collected and tested for tannin content using the Folin-Ciocalteau method. The feeding behavioural patterns showed that *I. belina* larvae will feed on a full leaf at a time and will not leave a leaf unfinished. The surface area of leaf eaten by these larvae increases exponentially from the first instar to the last instar until the entire tree foliage is finished. The results obtained for tannin content showed that removing full leaves as in the control resulted in no increase in amount of tannin produced whereas artificial damage resulted in an increase in amount of tannin produced. From these results it can then be concluded that the feeding behaviour of *I. belina* will minimises production of tannins on Mopane trees.

1. INTRODUCTION

Colophospermum mopane forms a large portion of the vegetation of Southern Africa and Zimbabwe [(Makhado et al., 2012); (Nyarega *et al.*, 2021)]. It is drought resistant and few herbivores forage on it except larva *Imbrassia belina* which solely feed on it and other insects like *Arytaina mopani* and *Plebina denoita (Mojeremane and Lumbile, 2005).* A four hundred hectare land can support approximately 19 million *I. belina* larvae (Bartlett, 1996). *Imbasia belina* larvae are a delicacy and source of income for part of the population of Southern Zimbabwe. It has been suggested that *I. belina* be domesticated like other Lepidoptera such as *Bombix mori* the silk worm, which feeds on mulberry trees (Gardiner, 2003). This makes it mandatory to study its feeding habits as well.. Leaves of certain plant species produce more tannins when overgrazed and at the same time produce signals that prompt the neighbours of the same species to produce similar levels of tannins as the overgrazed ones (Cooper and Owen-Smith, 1985; de Vries *et al.*, 2019; Hu *et a*l., 2021; Hu, 2022).

Colophospermum mopane in Zimbabwe starts producing leaves in mid-October and by mid-November, *I.*

belina moths start laying their eggs which hatch in 10 days. It is from this time onwards that the *I. belina* and *C. mopane* begin interacting. For a few plants that have been studied, leaf damage by whatever means results in production of defense mechanism by the plant. These could be anatomical defenses (thorns), leaf trichome formation, resistance at the plant cuticle, wound-periderm formation, traumatic resin ducts and polyphenolic parenchyma cells, production of secondary metabolites (tannins), induced terpenoid defences, phenylpropanoid metabolism induced by wounding, defense by pyrrolizidine alkaloids, anti-nutritional enzymes and proteins, plant protease inhibitors, polyphenol oxidases, and plant lectins among others(War *et al.*, 2012; Lev-Yadun, S., 2021).

Tannins are the most abundant secondary metabolites made by plants, ranging from 5% to 10% dry mass of tree leaves. They can defend leaves against insect herbivores by deterrence and/or toxicity (War *et al.*, 2012; Raymond *et al.*, 2011). They are especially prone to oxidation in insects with high pH in the gut, forming semiquinone radicals and quinones, as well as other reactive oxygen species which are the basis of toxicity in insects. The ability of insects to tolerate ingested tannins comes from a variety of biochemical and physical defenses in their guts, including surfactants, high pH, antioxidants, and a protective peritrophic envelope that lines the midgut (Raymond *et al.*, 2011). These damages include protein coagulation, destruction of intestinal flora which aid digestion and rendering digestive enzymes dysfunctional. It has been pointed out that the growth rate of 5th instar larvae of *Antheraea mylitta,* a Lepidoptera fed on previously damaged foliage reduced by 87.1% (Abraham *et al.*, 2004).There is a possibility that deer browsing increases leaf hardness, which exerts an indirect negative effect on the herbivorous insects utilizing a common host plant (Shimazaki and Miyashita, 2002).Palatability of a leaf is related to the amount of condensed tannins and it has been shown that all plants containing more than 5% condensed tannins are rejected by some herbivores as food during the wet season (Cooper and Owen-Smith, 1985). Large mammalian herbivores are influenced by condensed tannins due to their dependence upon microbial fermentation of plant cell walls for part of their energy needs whereas insect herbivores have a different pattern (Cooper and Owen-Smith, 1985).

The aim of this study was to investigate the behaviour of *I. belina* larvae feeding and determine levels of tannins produced by simulating *I. belina* larvae herbivory on *C. mopane* leaves. This is a preliminary study in understanding the response of *C. Mopane* to herbivory.

2. MATERIALS AND METHODS

For this study, two sites were chosen, both of which have a high density of *C. Mopane* in Bulawayo, Zimbabwe. The locations of the study sites were Richmond (20º 7' 2" South and 28º 34' 45" with an altitude of 1286) and the National University of Science and Technology (NUST) (20º 9' 28" South and 28º 38' 33" East with an altitude of 1336). To observe the behaviour and feeding habits of *I. belina* in all larval stages, one of the study sites, Richmond, which was heavily infested with *I. belina* eggs was chosen. Observations, were then made from the time hatching occurred up to the fifth and final instar and the average leaf size consumed at each larval stage was quantified and recorded. The number of days for feeding were also noted. The other site, NUST, which did not have any or history of *I. belina* infestation was for simulating *I. belina* herbivory on Mopane trees. To study the effects of *I. belina* larvae damage on leaves with no signs of previous herbivore damage, the leaves were artificially damaged by either cutting or punching. Cutting was conducted by removing a portion of a leaf using a paper punch 5-mm-

diameter. A total of twenty five leaves were subjected to this punching progressively. On subsequent days additional punch holes 20mm^2 were made. The damage method was done to mimic as much as possible the most common natural damage form found on the plants by *I. belina*. In total, the following treatments to plants were conducted as follows:

(1) Punching 20mm^2 holes on leaves and increasing the area of the holes to double every two days in a way that mimicks the feeding of *I. belina*;

(2) Collecting the previously damaged and undamaged leaves on the same tree for tannin testing each.

Each treatment was applied to thirteen plants, and thirteen undamaged plants served as controls. The Mopane leaves from each of the 13 trees purposively selected were the subject of this study. The trees were 1.5 -2.5m high and all were at least 50meters from the next plant selected for experimenting on. Two branches were purposefully selected and each had between 50 and 100 leaves. For the first branch the leaves were not subjected to leaf damage except that one leaf was removed to test the tannin levels on a daily basis for 25 days. From the second branch 25 leaves were subjected to leaf damage accomplished by adding one punch hole every day. One of the leaves was taken out to test for tannin levels. One leaf which was not damaged from the second branch was tested for tannins daily for 25 days. For determination of the total polyphenolic compound concentrations, the Folin-Ciocalteau method [8] was used. The dried mopane leaves were finely ground and 200mg were weighed into a glass beaker. Extraction was done by adding 10ml of 70% acetone into the beaker and suspending in an ultrasonic water bath for 20 minutes. The contents of the beakers were then transferred to centrifuge tubes and centrifugation was done for 20 minutes at 4°C at a speed of 3 000g. The supernatant was collected and the Folin-Ciocalteu method was used for determination of tannins. In this method, 0.5ml of the extract was added to a tube containing 2ml distilled water, 1.25ml Folin reagent (0.1N) and 6.25ml Sodium Carbonate. This was vortexed and absorbance was read at 725nm after 40min. A calibration curve was prepared using standard tannic acid solution (0.1mg/ml) to make different concentrations. The test for each extract was done in triplicates. The total phenolic compound content was calculated from the absorbance values obtained and was expressed as µg Tannic Acid (TA). Leaf damage by the *I. belina* was observed as they were feeding on trees. The speed of grazing was estimated by measuring the amount of leaf material consumed per unit time and assuming that grazing was a non-stop process except during molting.

3. RESULTS

The feeding behavioural patterns showed the following trends that could minimise tannin production: Eggs numbering 120 -230 are laid in clusters on one leaf (Fig. 1A and Fig. 2B). Hatching takes place in 12-15 hours and no feeding takes place until all the eggs are hatched. Hatchlings start feeding on one leaf synchronously which ensures their molting is also synchronized as shown on Fig. 1A and 1B. In the first three instars the larva are gregarious (Fig. 1A, B, C). Once a leaf is selected, the larva feed on it until it is completely consumed and then approach another one as shown in Fig. 1C, D, E and F. This prevents invoking tannin production as confirmed by the results of the control. In the third instar, each worm can now consume a complete leaf in less than two hours. They feed day and night on the fleshy part of the leaves (Fig. 3). Oviposition takes place on trees that are not less than 30 meters from where one moth oviposited. Larva from an overgrazed tree will not climb a tree in which a moth has currently oviposited and only mopane leaves are used as food for the larva. The larvae continue to increase

surface area of leaf consumed with time and as they grow (Fig. 4).

Mopane has increased tannin levels with progressive leaf damage for the first three days until a threshold is reached where the tannin content no longer increases (Fig 5). Only the tree with the damaged leaves responds by producing tannins expressed in both the damaged and undamaged leaves. Damage of leaves on a tree has effect on other undamaged leaves on the same tree (Fig 5). From the first day of feeding the worms line up along the edges and feed progressively until the whole leaf is consumed. If the leaf is too old the midribs of the leaves are not eaten. This collective feeding continues to the third instar. Worms which chose to feed solo before the end of first instar died. The larva rarely left an unfinished leaf to go to the next one for feeding. At instar three each larva is able to feed on at least one complete leaf in two hours. At this point the gregarious behaviour stops and each larva moves as far away as possible from the rest and select a branch that has about one hundred leaves which it consumes one by one until it has finished all the healthy leaves on the branch. The larva will release greenish saliva if disturbed and this turns brown in about 30 minutes if it falls on a white cloth or paper and is not readily washable. The feaces are shaped like segments of an orange fruit each made of neatly piled leaf fragments choped as the larva fed. The leaf fragments are the same shape throughout the alimentary suggesting only juice is digested and squeezed from them. The feaces are almost dry. Towards the end of the larval stage the worms choose leaves independently (Fig. 2A and B) and no longer congregate. In their 5th instar, *I. belina* larvae reduce their feeding and the larvae doubles in size (Figure 2C and 2D). A single worm at this stage consumes 0.75 cm^2 leaf area per minute (Fig. 4).The fourth ecdysis takes place 31±2 days after hatching. On day 35±2, feeding stops. The worms pass brown droppings and spend their day with either the anterior or posterior body end hanging downwards. They loosely grip onto the tree such that a slight shaking of the tree will dislodge them to the ground.
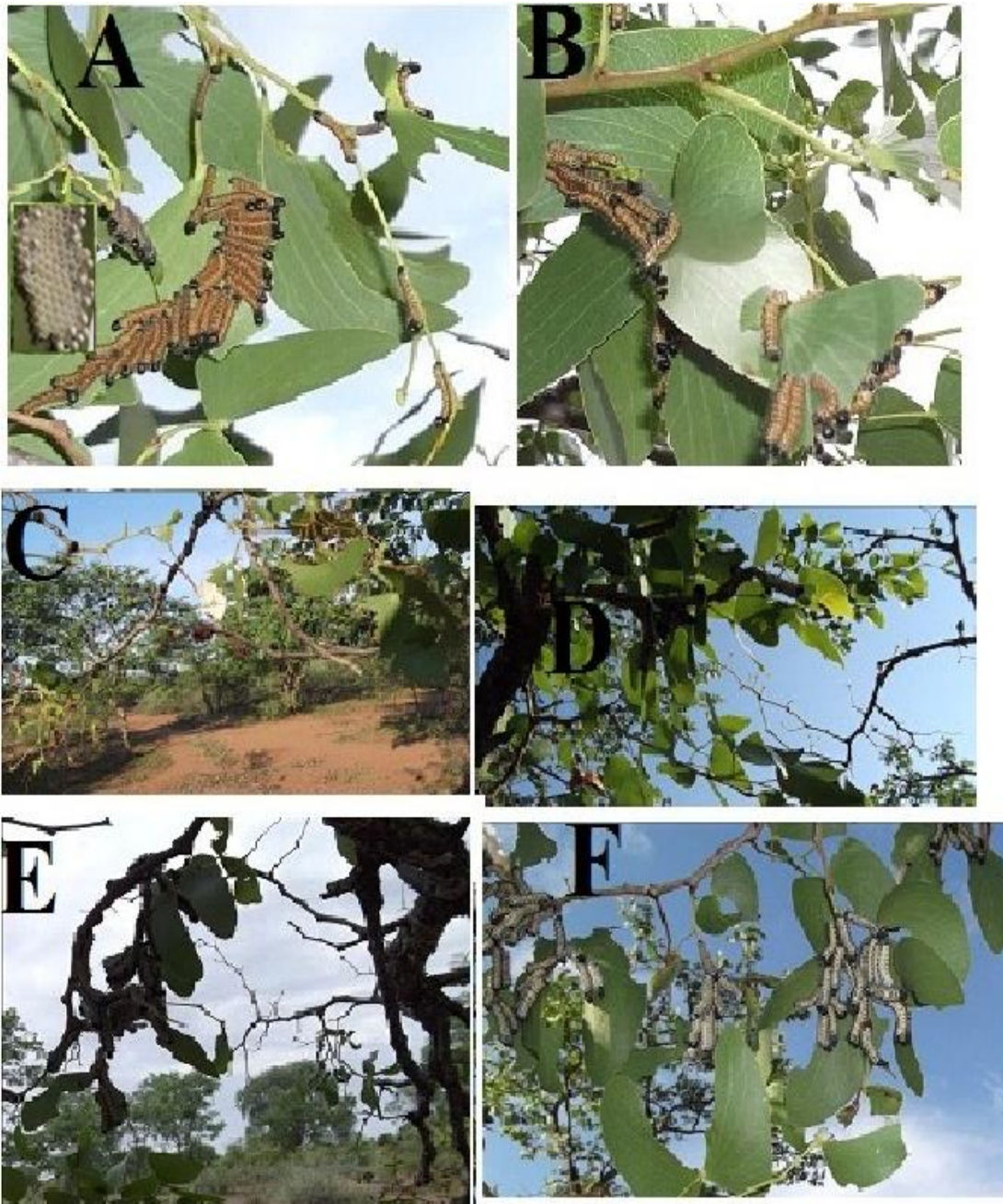
Fig. 1: The different stages of growth of the *I. belina* larvae. **A**: Instar 1- larvae cluster to feed on a single leaf and eggs of Imbrasia belina before hatching shown on insert. **B**: Instar 2- larvae have grown but still clustering together to feed. **C**: Instar 3- a lot of the leaves have been completely eaten as larvae move to other leaves. **D, E, F**: Instar 3- growing larvae still feeding in clusters.
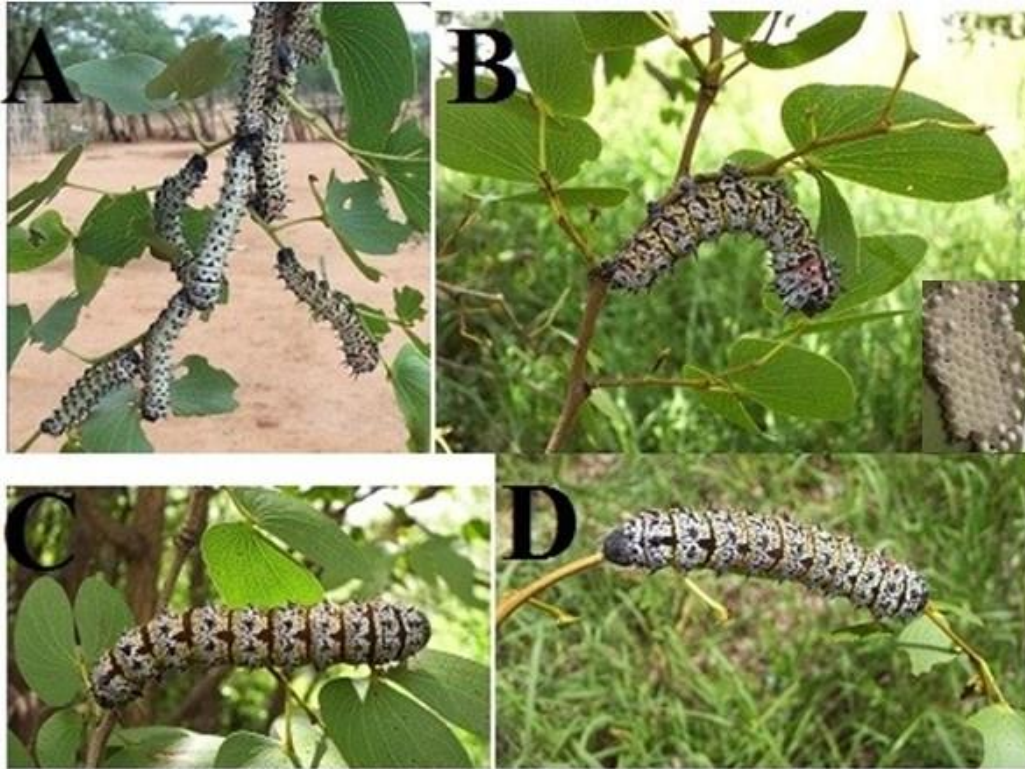
Figure 2: The different stages of growth of the *I. belina* larvae. **A**: Instar 4- larvae start to feed individually on whole leaves. **B**: Full grown larvae at instar 5 and eggs of Imbrasia belina before hatching shown on insert. **C- D**: Instar 5- full grown larvae feeding on mopane leaves.
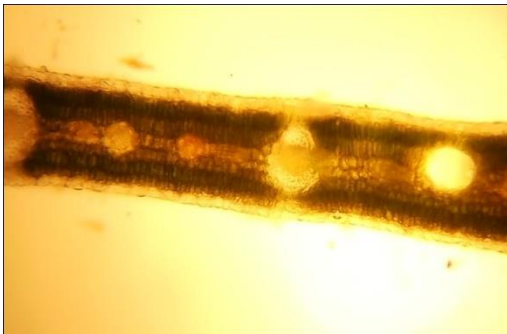

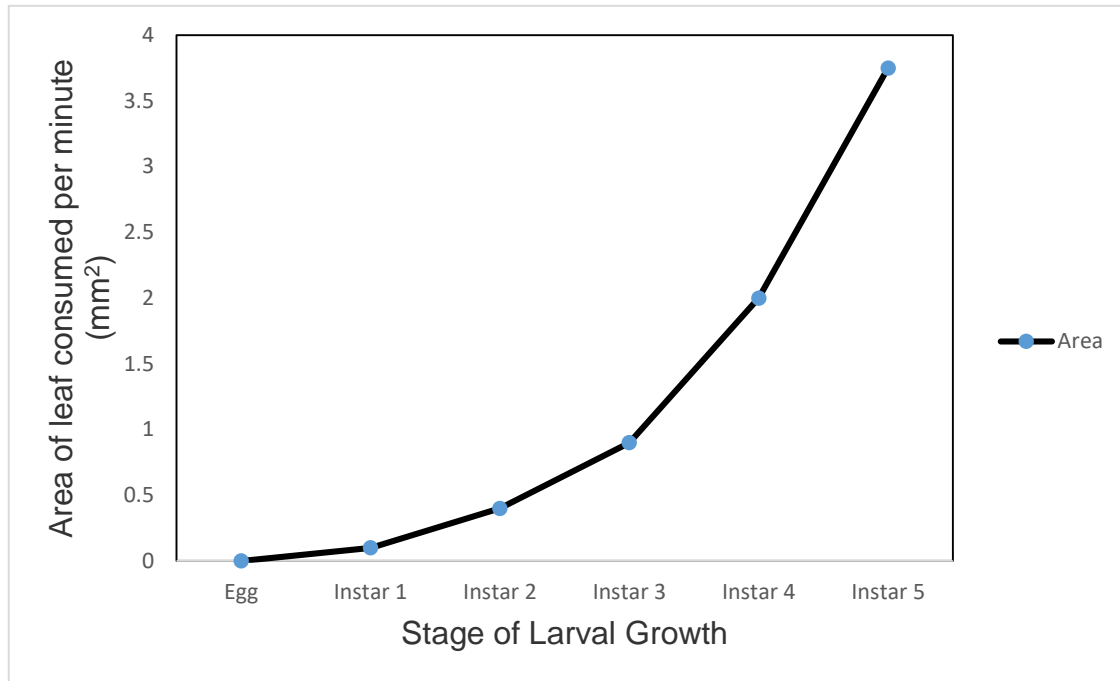**Figure 3**: Cross sectional area of a typical Mopane leaf

Figure 4: Average surface area of leaf consumed by the larvae at different stages.
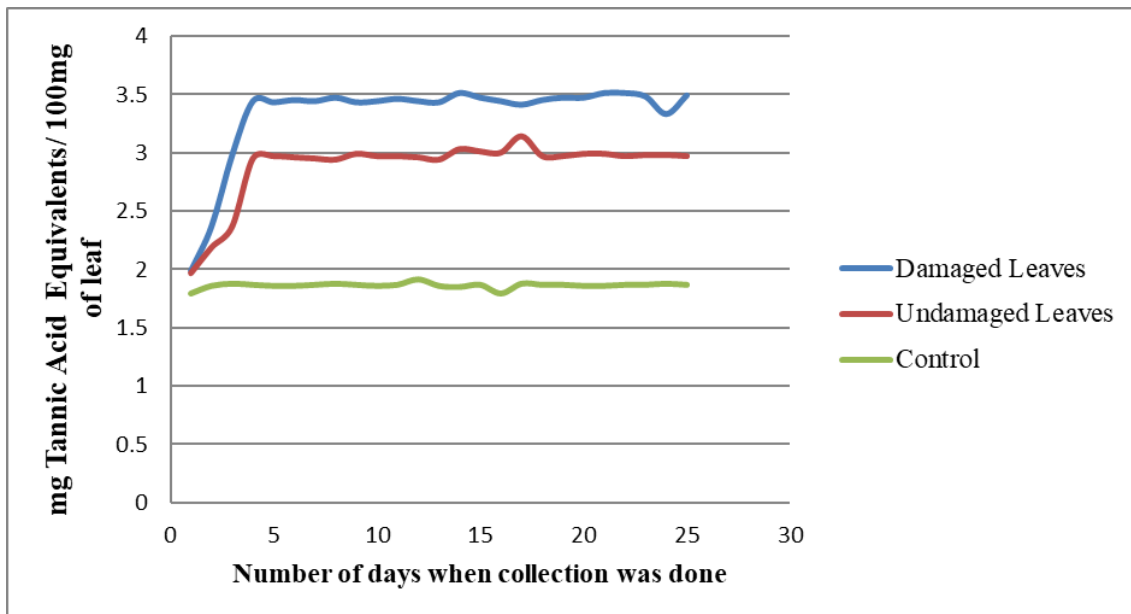

Figure 5: Amount of Tannins produced on leaves that were damaged and those undamaged collected from the same tree.

Mopane leaves progressively removed whole don't seem to provoke production of tanins even after 25 leaves have been plucked off in 25 days as shown in Fig.2. Mopane progressively damaged $20mm^2$ at a time provoke production of tannins progressively in the leaves. Leaves on the same branch as the damaged ones are influenced to produce tannins to a lesser extent. The production of tannins firstly increases sharply and after day 4 remained at relatively

constant levels for the remainder of the treatment.

4. DISCUSSION

Our results on artificially damaged leaves showed an increase in tannin levels and a mild influence on the neighbouring leaves and this is consistent with other studies for some plants (Faeth, 1985). Similarly, the differences in tannin content of the leaves (intact and damaged) are maintained throughout the treatments. Removing the leaves completely one at a time daily did not induce tannin production at least for 25 days. A leaf completely removed from the plant will not invoke tannin production unless a large number of leaves have been removed. Notably, *I. belina* removed leaves completely during feeding which would have resulted in tannin production being minimised. Behaviourally, *I. belina* when small will collectively feed on one leaf until it is finished which takes a day, yet if each of the 200 worms went for a single leaf by its self it would take a week to go through the first leaf by that time the leaf would have developed high tannin levels.

Defoliation intensity is an important factor that determines production of tannins (Kohi *et al.*, 2009). In a study done by Kohi *et al.* (2009) the results showed that at both low defoliation (less than 50%) and high defoliation intensity (Above 75%) low tannin provocation is achieved. This could be due to the fact that tannin production is a costly pathway that utilises carbon, making it non-available for growth. At such intensities, use of carbon for growth is most preferred compared to the secondary metabolite pathway for tannin production. From the way *I. belina* larvae feed, it is evident that they have adapted to the best methods of exploiting the Mopane foliage. The feeding behaviour of *I. belina* larvae is such that during the first instar, tannin production is minimised as feeding is done in clusters on one leaf. The surface area of the leaf consumed increases exponentially, resulting mostly in extensive defoliation at instar 5. At this stage more than 75% of the foliage on the tree will have been consumed. On the contrary, in a study done by Makhado *et al.* (2018) on the effects of pruning on *C. Mopane*, it was concluded that the amount of secondary metabolites in Mopane leaves is not dependent on less than 10% pruning. This shows that Mopane leaves will produce different amounts of secondary metabolites depending on types of herbivory on *C. Mopane* trees.

It has also been argued that some lepidoptera to which *I. belina* belongs have tolerance for high tannin levels (Karowe, 1989). Even if that be the case *I. belina* seem to have behaviourally adapted to feeding when tannin levels are at levels of the ungrazed state. Our study showed that no two *I. belina* moths will lay eggs on the same tree. If that happened it could mean that the second lot could be half way their development when the plant reaches a point of producing high levels of tannins a situation that could be unfavourable to the development of the worms. Previous investigations on the growth rate of 5th instar larvae of *Antheraea mylitta a* Lepidoptera fed on previously damaged foliage showed a growth reduction by 87.1% (Abraham *et al.*, 2004) whereas

other lepidoptera were observed to tolerate high levels of tannins with no negative effects (Barbehenn and Martin, 1992). Such findings make it imparative to investigate each insect species as results from one insect may not be applicable to other insects. *I. belina* concentrate on one plant and they seem to have adapted to it in a number of ways. Adaptations could involve a number of mechanisms including (i) gut pH modifications (Barbehenn *et al.*, 2006) (ii) the role of the ascorbate recycling system in the midgut lumen (Barbehenn *et al.*, 2001) (iii) fenton-type reactions in the midgut fluids of tree-feeding caterpillars (Barbehenn *et al.*, 2006). Mechanisms by which some Lepidoptera such as *Malacosoma disstria, Orgyia leucostigma, Lymantria dispar, Orgyia leucostigma, Manduca sexta, Choristoneura fumiferana, Operophtera brumata* and *Helicoverpa zea* react to tannins in their feed have been studied and shown to be varied [(Barbehenn and Martin, 1992); (Barbehenn *et al.*, 2006); (Feeny, 1968); (de Veau and Schultz, 1992); (Summers and Felton, 1994); (Hemming and Lindroth, 1995); (Hwang and Lindroth, 1995); (Barbehenn and Martin, 1998); (Osier *et al.*, 2000); (Kopper *et al.*, 1985) ;(Haukioja *et al.*, 2002); (Barbehenn *et al.*, 2005b); (Barbehenn *et al.*, 2008b); (Barbenhenn *et al.*, 2009a); (Barbehenn *et al.*, 2009a); (Cardinal-Aucoin *et al.*,

2009)]. Other plants on which herbivory responses result in changes in tannin levels that have been studied include the red oak , myrtle oak sugar maple, hybrid poplar, cotton, mountain birch, *Populus tremuloides*, *Eucalyptus globules*, *Terminalia arjuna, Chamaecrista linearifolia* (Fabaceae) [(Abraham *et al.*, 2004); ; (Barbehenn *et al.*, 2005a) ; ((Feeny, 1968); (Osier *et al.*, 2000); (Barbehenn *et al.*,2009b); (Fox and Macauley, 1977); (Klocke and Chan, 1982); (Rossiter et al., 1988); (Madeira *et al.*, 1998); (Ossipov *et al.*, 2001); (Rossi *et al.*, 2004); (Rapley *et al.*, 2007); (Marquis and Lill, 2010)]. These plants occur mostly in temperate regions. There is paucity of studies on tropical plants. The *C.mopane* not withstanding its high tannin content under normal conditions is the sole selected plant by *I. belina* larva. Behaviourally these worms do activities that minimise increasing tannin levels as they feed on the plant leaves. This is a preliminary study on the behaviour of *I. belina* and also on the response of *C. Mopane* to herbivory needing further study.

ACKNOWLEDGEMENTS:

REFERENCES

Abraham, G., Thomas, G., and Babu, C.R. 2004. Induction of Biomolecules in Mature Leaves of *Terminalia arjuna* due to Feeding of *Antheraea mylitta* Drury. *The Scientific World Journal* 4: 887–889.

Bartlett, E. 1996. Hold the Turkey (the mopane worm as a food source). *New Scientist* 152: 58-59.

Barbehenn and Constabel 2011. Tannins in plant–herbivore interactions. *Phytochemistry* 72 :1551–1565.

Barbehenn, R.V., Martin, M.M. (1992). The protective role of the peritrophic membrane in the tannin-tolerant larvae of Orgyia leucostigma (Lepidoptera). *J. Insect Physiol.* 12 : 973–980.

Barbehenn, R.V., Jones, C.P., Hagerman, A.E., Karonen, M., Salminen, J.-P. 2006a. Ellagitannins have greater oxidative activities than gallotannins and

condensed tannins at high pH: potential impact on caterpillars. *J. Chem. Ecol.* 32: 2253–2267.

Barbehenn, R.V., Bumgarner, S.L., Roosen, E., Martin, M.M. 2001. Antioxidant defenses in caterpillars: role of the ascorbate recycling system in the midgut lumen. *J. Insect Physiol.* 47: 349–357.

Barbehenn, R.V., Cheek, S., Gasperut, A., Lister, E., Maben, R. 2005. Phenolic compounds in red oak and sugar maple leaves have prooxidant activities in the midguts of Malacosoma disstria and Orgyia leucostigma caterpillars. *J. Chem. Ecol.* 31: 969–988.

Barbehenn, R.V., Martin, M.M., 1998. The formation of insoluble and colloidally dispersed tannic acid complexes in the midgut fluid of Manduca sexta (Lepidoptera: Sphingidae): an explanation for the failure of tannic acid to cross the peritrophic nvelopes of lepidopteran larvae. *Arch. Insect Biochem. Physiol.* 39: 109 –117.

Barbehenn, R.V., Dodick, T., Poopat, U., Spencer, B. 2005b. Fenton-type reactions and iron concentrations in the midgut fluids of tree-feeding caterpillars. *Arch. Insect Biochem. Physiol.* 60: 32–43.

Barbehenn, R.V., Weir, Q., Salminen, J.-P. 2008b. Oxidation of ingested phenolics in the tree-feeding caterpillar Orgyia leucostigma depends on foliar chemical composition. *J. Chem. Ecol.* 34: 748–756.**159**

Barbehenn, R.V., Jaros, A., Lee, G., Mozola, C., Weir, Q., Salminen, J.-P. 2009a. Tree resistance to Lymantria dispar caterpillars: importance and limitations of foliar tannin composition. *Oecologia* 159: 777–788.

Barbehenn, R.V., Jaros, A., Lee, G., Mozola, C., Weir, Q., Salminen, J.-P. 2009b. Hydrolyzable tannins as "quantitative defenses": limited impact against Lymantria dispar caterpillars on hybrid poplar. *J. Insect Physiol.* 55: 297–304.

Cardinal-Aucoin, M., Bauce, E., Albert, P.J. 2009. Preingestive detection of tannins by Choristoneura fumiferana (Lepidoptera: Tortricidae). *Ann. Entomol. Soc. Am.* 102: 717–726.

Cooper, S. M. and Owen-Smith N. 1985. Condensed tannins deter feeding by browsing ruminants in a South African savanna. *Oecologia* 67 (1):142-146.

de Veau, E.J.I., Schultz, J.C. 1992. Reassessment of the interaction between gut detergents and tannins in Lepidoptera and significance for gypsy moth larvae. *J. Chem. Ecol.*18: 1437–1453.

de Vries, J., Evers, J.B., Dicke, M. and Poelman, E.H., 2019. Ecological interactions shape the adaptive value of plant defence: Herbivore attack versus competition for light. *Functional Ecology* **33** (1):129-138.

Faeth, S.H. 1985. Quantitative defense theory and patterns of feeding by oak insects. *Oecologia* 6 (8): 34-40.

Feeny, P.P. 1968. Effect of oak leaf tannins on larval growth of the winter moth Operophtera brumata. *J. Insect Physiol.*14: 805–817.

Fox, L.R.and Macauley, B.J., 1977. Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. *Oecologia.* 29: 145–162.

Gardiner, A.J. 2003. Internal Final Report: Mopane Woodlands and the Mopane Worm: Enhancing Rural Livelihoods and Resource Sustainability, DFID Project No. R7822. The Domestication of Mopane Worms (Imbrasia belina). Veld Products Research & Development, Gaborone, Botswana.

Haukioja, E., Ossipov, V., Lempa, K. 2002. Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth. *Entomol. Exp. Appl.*104: 125–136.

Hemming, J.D.C., Lindroth, R.L. 1995. Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* 103: 79–88.
Hwang, S.Y., Lindroth, R.L. 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. *Oecologia* 111 : 99– 108.

Hu, L., Zhang, K., Wu, Z., Xu, J. and Erb, M., 2021. Plant volatiles as regulators of plant defense and herbivore immunity: Molecular mechanisms and unanswered questions. *Current Opinion in Insect Science* **:**82-88.

Hu, L., 2022. Integration of multiple volatile cues into plant defense responses. *New Phytologist* 233(2):618-623.

João Augusto Madeira, Katia Torres Ribeiro, G. Wilson Fernandes. 1998 Herbivory, tannins and sclerophylly in Chamaecrista linearifolia (Fabaceae) along an altitudinal gradient. *Brazilian Journal of Ecology* 2(**1**).

Karowe, D. N. 1989. Differential effect of tannic acid on two tree-feeding Lepidoptera:implications for theories of plant anti-herbivore chemistry. *Oecologia* 80: 507-512.

Klocke, J.A., Chan, B.G., 1982. Effects of cotton condensed tannin on feeding and digestion in the cotton pest, Heliothis zea. *J. Insect. Physiol.* 28: 911–916.

Kohi, E.M., de Boer, W.F., Slot, M., van Wieren, S.E., Ferwerda, J.G., Grant,R.C., Heitko¨nig, I.M.A., de Knegt, H.J., Knox, N., van Langevelde, F., Peel, M., Slotow., R,van der Waal., C., and Prins, H.H.T. 2009 Effects of simulated browsing on growth and leaf chemical properties in Colophospermum mopane saplings. *Afr. J. Ecol.* 48: 190–196.

Kopper, B.J., Jakobi, V.N., Osier, T.L., Lindroth, R.L. 2002. Effects of paper birch condensed tannin on whitemarked tussock moth (Lepidoptera: Lymantriidae) performance. *Environ. Entomol.* **31**: 10–14.

Lev-Yadun, S., 2021. Avoiding rather than resisting herbivore attacks is often the first line of plant defence. *Biological Journal of the Linnean Society* 134(4):775-802.

Makkar, H.P.S., Bluemmel, M., Borowy, N.K., Becker, K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric.* 61**:** 161–165.

Makhado, R. A., Mapaure, I., Potgieter, M.J., Luus-Powell, W. J., Saidi, A.T. 2014. Factors influencing the adaptation and distribution of *Colophospermum mopane* in southern Africa's mopane savannas - A review. *Bothalia – African Biodiversity and Conservation* 44(1), 1-9.

Makhado, R. A., Potgieter, M.J., Wessels, D. C. J., Saidi, A.T., Masehela, K.K. 2012. Use of Mopane Woodland Resouces and Associated Woodland Management Challenges in Rural areas of South Africa. *Ethnobotany Research and Application* :369-380.

Makhado, R. A., Potgieter M. J. and Luus-Powell W.J. 2018: Effects of pruning on the concentration of secondary metabolites in Colophospermum mopane leaves, *Southern Forests: a Journal of Forest Science.* 1-6.

Marquis, R.J. and Lill, J.T. 2010. Impact of plant architecture versus leaf quality on attack by leaf-tying caterpillars of five oak species. *Oecologia* 163: 203–213.

Mojeremane, W and Lumbile, A.U. 2005. The characteristics and Economic Values of Colospermum mopane (Kirk ex Benth.) J. Leonard in Botswana. *Pakistan Journal of Biological Science 8*(5): 781-784.

Ngarega, B.K., Masocha, V.F. and Schneider, H. 2021. Forecasting the effects of bioclimatic characteristics and climate change on the potential distribution of Colophospermum mopane in southern Africa using Maximum Entropy (Maxent). *Ecological Informatics* 65:1014- 1019.

Osier, T.L., Hwang, S.Y. and Lindroth, R.L. 2000. Effects of phytochemical variation in quaking aspen Populus tremuloides clones on gypsy moth Lymantria dispar performance in the field and laboratory. *Ecol. Entomol.* 25: 197–207.

Ossipov, V., Haukioja, E., Ossipova, S., Hanhimaki, S. and Pihlaja, K. 2001. Phenolic and phenolic-related factors as determinants of suitability of mountain birch leaves to an herbivorous insect. *Biochem. Syst. Ecol.* 29: 223–240.

Rapley, L.P., Allen, G.R., Potts, B.M., Davies, N.W. 2007. Constitutive or induced defences – how does Eucalyptus globulus defend itself from larval feeding? *Chemoecology* 17: 235–243.

Rossi, A.M., Stiling, P., Moon, D.C., Cattell, M.V., Drake, B.G. 2004. Induced defensive response of myrtle oak to foliar insect herbivory in ambient and elevated CO2. *J. Chem. Ecol.* 30: 1143–1151.

Rossiter, M., Schultz, J.C. and Baldwin, I.T. 1988. Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. *Ecology* 69: 267–277.

Shimazaki, A. and Miyashita, T. 2002, Deer browsing reduces leaf damage by herbivorous insects through an induced response of the host plant. *Ecological Research* 17: 527–533.

Summers, C.B. and Felton, G.W. 1994. Prooxidant effects of phenolic acids on the generalist herbivore Helicoverpa zea (Lepidoptera: Noctuiidae): potential mode of action for phenolic compounds in plant anti-herbivore chemistry. *Insect Biochem. Mol. Biol.* 24: 943–953.

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain,B., Ignacimuthu, S., and Sharma, H.C. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signal Behav.* 7 (10): 1306-1320.