Perspective

Innovative approaches to exploit host plant metabolites in malaria control

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Abstract

Malaria is the most important vector-borne disease in sub-Saharan Africa (SSA). Recent reports indicate that the levels of malaria-associated mortality and morbidity in SSA have remained the same. Malaria vectors have modified their feeding behavior in response to the selective pressure from indoor-based interventions, and there is emerging malaria parasite resistance to artemisinin-based combination therapies. These challenges have created an altered malaria landscape, especially within local scales in some malaria-endemic countries in SSA. To address these challenges, complementary new strategies are urgently required for malaria control. This paper argues that to develop the next generation of vector and chemotherapeutic tools for malaria control, especially based on natural products with novel modes of action, a better understanding of mosquito bioecology and, more importantly, plant sugar feeding is needed.
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Keywords: malaria; mosquitoes; parasite; sugar feeding; natural products; phytochemicals

1 INTRODUCTION

Approximately 17% of the global infectious disease burden is vector-borne and this accounts for an estimated 700 000 deaths in humans annually.[1] These diseases impact significantly on the economies and public and veterinary health systems of affected countries. Malaria remains the most significant vector-borne disease, especially in tropical Africa. The recent World Malaria Report in 2018 estimates that globally there were 219 million malaria cases in 2017.[2] Over 90% of the global malaria burden disproportionately affects sub-Saharan Africa (SSA), causing over 400 000 deaths annually, mainly in children under 5 years of age.[2] While substantial gains have been achieved in the last two decades in malaria control and consequent reduction in malaria deaths, progress has generally plateaued in the last 3 years (2015, 2016 and 2017), with some countries in Africa reporting more malaria cases than the previous year.[2] The World Health Organization (WHO) Global Technical Strategy for Malaria 2016–2030[3] proposes to reduce malaria deaths by at least 40% by 2020 and malaria incidence and mortality rates by at least 90% by 2030, with eradication by 2040. Given the current malaria situation, it appears that these milestones are unlikely to be achieved unless new complementary strategies are developed to address the persistent malaria problem.

Human malaria is a mosquito-borne parasitic disease caused by infection of any of six species of the protozoan parasites within the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale wallickeri*, *P. ovale curtisi*, *P. malariae* and *P. knowlesi*.[4] Humans become infected with the malaria parasites through the bite of an infective female *Anopheles* sp. mosquito vector during a blood meal. As such, to complete its life cycle, the *Plasmodium* parasite must undergo various developmental stages in both human (asexual) and mosquito (sexual) hosts. Success in malaria elimination can be achieved using an integrated arsenal of interventions relating to the parasite and vector among others (human behavior and environment). However, a myriad of factors influences the success of control efforts, some of which will be highlighted in subsequent sections. In SSA, the most dominant and efficient malaria vectors are the four mosquito species *Anopheles gambiae*, *Anopheles coluzzi*, *Anopheles arabiensis* and *Anopheles funestus*.[5,6] Their characteristic features are that they are susceptible to infection with the malaria parasites, are long-lived and bite humans, although *An. arabiensis* is known to show plasticity in its biting tendencies and is largely zoophilic. Their adult stages are adapted to living close to human dwellings and their juvenile stages develop in both natural and man-made aquatic environments. Additionally, of the malaria parasites that occur in SSA (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*), *P. falciparum* is the most prevalent and accounts for most malaria deaths.[7] In this paper, the efforts that have contributed to the reduction in malaria cases and deaths are highlighted. Subsequently, some of the contributing factors leading to the levelling off in the gains made in malaria control are examined. Finally, the way in which knowledge about the chemical ecology of parasite-vector-host plant interactions can contribute to development of the next generation of vector and chemotherapeutic control tools is discussed.

2 THE NEED FOR NEXT-GENERATION MALARIA CONTROL TOOLS

Pathogen and vector control using proven interventions over a long period often alter the behavior, physiology and genotype of the target recipient. For instance, to date malaria control has focused on a combination of interventions. Control of vectors

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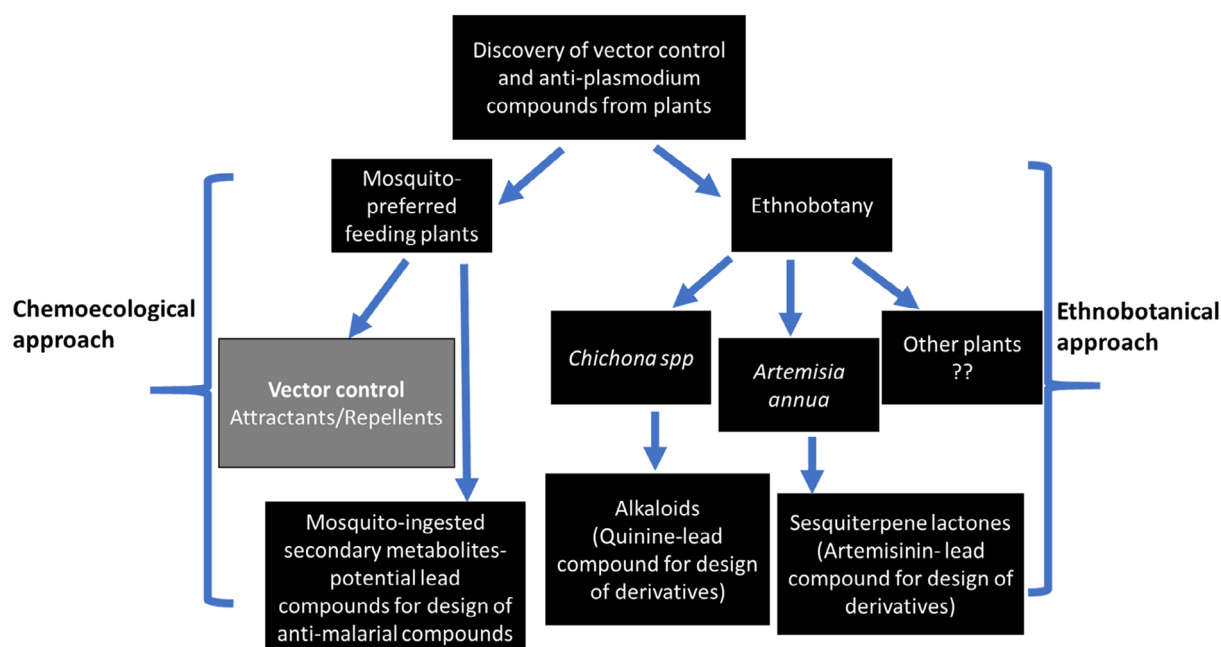
Figure 1. Schematic view of discovery of malaria vector and parasite control compounds on ethnobotany and chemical ecology.

mainly relies on the widespread use of insecticide-treated nets (ITNs), indoor residual spraying (IRS) and sometimes larval source management, while for the parasite, case management involving prompt diagnosis with rapid diagnostic tests and treatment with artemisinin-based combination therapies (ACTS) is used. In the last 20 years, application of these interventions, catalyzed by significant investments from the international donor community, provided a trajectory in the access and use of ITNs and ultimate reduction in malaria morbidity and mortality in SSA to about 50%.[3] According to WHO, despite these gains, a child still dies from this preventable disease every two minutes in SSA, and it recommends that additional financial investments and strategies are needed to sustain the gains made in malaria control.[2]

The current vector control tools (ITNs and IRS) only target indoor resting and biting mosquitoes, precluding transmission that occurs in the early evening outdoors. Moreover, malaria vectors have also been reported to adapt and modify their feeding behavior in response to the selective pressure from these indoor-based interventions, shifting from indoor nocturnal feeding to crepuscular outdoor feeding.[8,9] Together, this has created an altered vectorial landscape, especially within local scales. In other cases, previously not so important species otherwise termed as 'secondary vectors' have gained prominence in malaria transmission. For parasite control, as shown in Fig. 1, previously ethnobotanical information provided some of the best leads to the discovery of antimalarial compounds. For example, from the plant barks of *Chichona* spp. chichona alkaloids (e.g. quinine) were isolated, and from the foliar parts of the plant *Artemisia annua* the sesquiterpene lactone artemisinin was isolated. The semi-synthetic derivatives of artemisinin, for example, artemether and artesunate, are currently used for malaria control as ACTs. ACTs are highly effective in treating symptomatic malaria fever,[10] but they cannot curtail transmission due to their moderate impact on infectious stages of the parasites.[11] Another challenge is emerging resistance to ACTs already evident in the Greater Mekong Subregion,[12] with potential threat of spread to other malarious areas of the world, especially in SSA.

Given these scenarios, an important focus of research towards malaria eradication is the need for interventions that break the cycle of disease transmission through the mosquito vector. This may require novel molecules with alternative modes of actions, which can be achieved with a better understanding of mosquito bio-ecology. While several studies in the past have isolated natural products from plants with anti-mosquito activity either as larvicides or adulticides, as well as anti-plasmodial effects, most of these plants were not selected based on knowledge of mosquito bio- and chemo-ecology. Mosquito bio-ecology essentially embodies reproduction, host seeking in females for a blood meal, oviposition and sugar feeding. Of these different life traits, the mechanism of sugar feeding, mainly from plants, is poorly understood. While both mosquito sexes indulge in sugar feeding, important knowledge gaps exist about this trait that may provide leads in developing novel plant natural products for malaria control. For instance, how do mosquitoes find their sugar sources? What mechanisms underlie the host plant seeking process? Does host plant feeding differentially affect parasite-infected and parasite non-infected mosquitoes and what cues are involved? Are plant secondary metabolites ingested by mosquitoes when they sugar feed? Do parasite-infected mosquitoes self-medicate? This paper draws on previous findings carried out elsewhere and by our group at the International Centre of Insect Physiology and Ecology (*icipe*) on malaria mosquito plant sugar feeding and argue for future research directions in these highlighted areas.

3 CHEMICAL ECOLOGY CONSIDERATIONS FOR DEVELOPING NEXT-GENERATION MALARIA CONTROL TOOLS

Sugar feeding is central to various bio-ecological processes in mosquitoes. As shown in Fig. 2, it contributes to development and dispersal, mating, host seeking for a blood meal and oviposition in mosquitoes. More than two decades ago Foster[13] provided the most comprehensive analysis of mosquito sugar
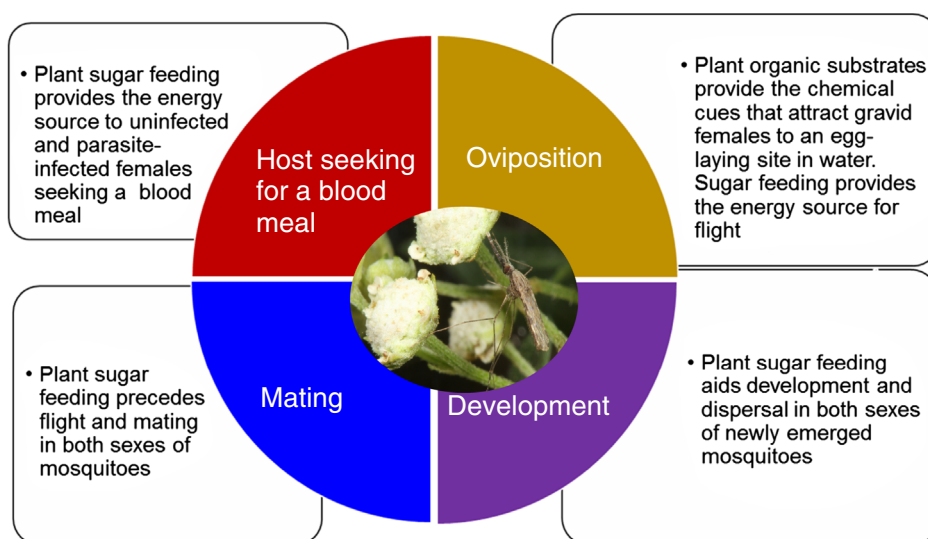
Figure 2. Biological processes driven by plant sugar feeding in malaria vectors and mediated by plant volatiles.

feeding and reproductive energetics. Since then, several studies have provided insights into the role of sugar feeding on malaria transmission potential and vectorial capacity.[14,15] After emergence, both sexes of malaria mosquitoes sugar feed to facilitate their development, dispersal and reproductive competitiveness. According to Foster,[13] the sugar source for both mosquito sexes is mainly floral nectar, extrafloral nectar and honeydew. Plant volatile organic compounds facilitate attraction of both sexes to these sugar sources. Field and semi-field studies with randomly selected plants/fruits/seed pods in selected malaria endemic areas of SSA have confirmed that finding a sugar source in both sexes of *An. gambiae* is selective rather than opportunistic.[16–19] However, whether females share similar or different host plant preferences as males, is yet to be determined. From a chemical ecology perspective, characterizing the chemical cues that guide mosquitoes to their sugar sources can lead to development of efficient monitoring tools for malaria vectors.[20–22] In principle, the advantages of using plant-derived compounds as lures for vector population monitoring over mammalian-based ones include the ability to (i) target diverse species of both sexes, and females in all gonotrophic states, (ii) provide precise information on total population density and composition, and (iii) minimize or even eliminate the reliance on CO_2 for trapping, if well formulated.[22] For example, *An. gambiae* females respond to a blend of chemicals identified from the volatiles of the invasive host plant *Parthenium hysterophorus*.[21] Recent work has shown that both sexes of *An. gambiae* and *An. funestus* can be monitored effectively in the field using this blend of compounds, but, more importantly, attraction can be attributed to one of the blend components, linalool oxide, a monoterpene, which when combined with carbon dioxide enhances mosquito attraction and is as effective as human skin odors presented as worn socks.[23] Moreover, optimization of the efficacy of linalool oxide with the other monoterpenoids β-pinene, β-ocimene and L-limonene increased trap captures of the secondary vector of malaria *An. pharoensis,* but not that of *An. arabiensis* and *An. funestus*.[24] Plant material degrading in soil in aquatic habitats has been found to produce the sequiterpene cedrol to attract gravid females of *An. gambaie* to an egg-laying site.[25] It would be interesting to investigate whether cedrol-producing plants and related sequiterpenes mediate gravid female attraction to a sugar

source for flight purposes. Nonetheless, these findings suggest variation in the sensitivies of different mosquito species to plant volatiles and the need to identify blends of compounds that can target a wide range of malaria vectors.

On the other hand, parasite infection increases the response of female *An. gambiae* to plant volatiles and their survival,[26] possibly facilitating completion of sporogonic cycles of malaria parasites. Additionally, mosquitoes have been shown to ingest plant secondary metabolites during plant feeding, some of which reduce parasite load in the vector. For example, in laboratory assays, *An. gambiae* females which fed on host plants ingested and tolerated the secondary metabolites parthenin, a sesquiterpene lactone found in *P. hysterophorus* and the alkyne 1-phenylhepta-1,3,5-triyne found in *Bidens pilosa*, but not the alkaloid ricinine found in *Ricinus communis*.[27] In additional experiments, the natural products parthenin and its analogue parthenolide, found in the plant feverfew, *Tanacetum parthenium*, were found to equally reduce the parasite load in *An. gambiae*, which was comparable to artemisinin, the main active ingredient in the current antimalarial drug.[28] Given the fact that ethnobotanical information led to the discovery of the plants *Chichona* spp. and *Artemisia annua* for the treatment of malaria, it would be interesting to establish whether these plants are also fed upon by malaria mosquitoes. Also of interest would be whether they ingest secondary metabolites from the plants, including the anti-plasmodium alkaloids and sesquiterpene lactones. Taken together, these findings suggest that chemical communication mediating plant–mosquito interactions are complex. They also confirm that plants are important sources of identifying novel monitoring tools to target different mosquito species and sexes at different developmental and physiological stages, and as potential chemotherapeutic leads.

4 CONCLUSIONS AND FUTURE DIRECTIONS

Since plants are the major sources of sugar feeding in mosquitoes, it is important to know precisely the plants used for this behavioral trait by mosquitoes. During sugar feeding, mosquitoes ingest minute amounts of plant tissue.[29] The reason for mosquitoes ingesting plant tissue needs further exploration. To identify the

sugar and plant tissue sources ingested in a crop of mosquitoes, very sensitive and robust techniques are needed. Research has shown that by using DNA barcoding, the precise plants fed upon by mosquitoes[29,30] and other blood-feeding insects, such as sandflies,[31] in their natural habitats can be identified. The first report of plants fed upon by wild-caught *An. gambiae* based on DNA barcoding was by Nyasembe *et al.*[29] Several plant species were identified from a malaria endemic area in Kenya, including *Senna alata* and *Senna tora* (Fabaceae), *Ricinus communis* (Euphorbiaceae), *Parthenium hysterophorus* and *Leonotis nepetifolia* (Lamiaceae). Additionally, research has shown that use of sensitive chemical techniques such as gas chromatography linked to electroantennographic detection (GC-EAD) can allow for isolation of headspace volatile components detected by antennae of *An. gambiae*. The identities of GC-EAD-active components can be tentatively established by GC-mass spectrometric analyses (e.g. mass spectral data, retention times, retention indices) on both non-polar and polar GC columns, and confirmed by comparison with synthetic standards. Furthermore, behavioral assays both in the laboratory and field can establish the roles of blends and individual chemicals as potential host location cues. Some of the chemicals identified from the volatiles of these plants have previously been reported as attractants for *An. gambiae*.[21] Thus, accurate identification of plants fed upon by mosquitoes using molecular techniques can provide opportunities to identify the key chemicals utilized for host plant location and for development of odor-bait technologies. It could also guide researchers to uncover plants avoided by mosquitoes, yet abundant in a given environment, for development of repellents. To aid in uncovering this knowledge, controlled laboratory mosquito plant-feeding experiments would be needed. Secondary metabolites that are ingested by mosquitoes while feeding on plant tissue and which influence various biological processes can be identified using liquid chromatography coupled to mass spectrometry (LC-MS) and other spectroscopic techniques.[27] Once the identity of an ingested secondary metabolite has been established by spectroscopy, its mode of action can be studied. Such studies would provide opportunities to identify metabolites with therapeutic potential for harnessing into druggable targets with a novel mode of action for malaria control. Despite the diversity of plants found in malaria endemic areas, selective feeding by mosquitoes occurs only on certain plant species. Therefore, future studies should focus on establishing precise knowledge of the plants used by mosquito disease vectors to guide chemo-ecological investigations for development of bait techonologies[19] and identification of metabolites with therapeutic potential for malaria control. Furthermore because plant feeding is common in other disease vectors (e.g. sandflies), similar studies should be extended to other vector-pathogen systems.

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REFERENCES

1 Vector-borne diseases. Fact sheet. World Health Organization, Geneva, Switzerland. Available: https://www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases [2017].[Accessed 7 December 2018].

2 World Health Organisation. *World malaria report*. Geneva: World Health Organization ISBN 978–92–4-156565-3 (2018).

3 World Health Organisation. *Global technical strategy for malaria 2016–2030*. World Health Organization, United Kingdom, ISBN 978-92-4-156499-1 (2015).

4 Milner DA Jr, Malaria pathogenesis. *Cold Spring Harb Perspect Med* **8** (2017). https://doi.org/10.1101/cshperspect.a025569.

5 Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW *et al.*, The dominant Anopheles vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. *Parasit Vectors* **3**:72 (2010).

6 Ogola E, Fillinger U, Ondiba IM, Villinger J, Masiga DK, Torto B *et al.*, Insights into malaria transmission among *Anopheles funestus* mosquitoes, Kenya. *Parasit Vectors* **11**:577 (2018).

7 Snow RW and Omumbo JA, Malaria, in *Disease and Mortality in Sub-Saharan Africa*, ed. by, ed. by Jamison DT . The World Bank, Washington DC, USA pp. 195–213 (2006).*et al.*

8 Moiroux N, Gomez MB, Pennetier C, Elanga E, Djènontin A, Chandre F *et al.*, Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. *J Infect Dis* **206**:1622–1629 (2012).

9 Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP and Killeen GF, Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J* **10**:80 (2011).

10 Abdulla S, Sagara I, Borrmann S, D'Alessandro U, González R, Hamel M *et al.*, Efficacy and safety of artemether-lumefanthrine dispersible tablets compared with crushed commercial tablets in African infants and children with uncomplicated malaria: a randomized, single-blind, multicenter trial. *Lancet* **372**:1819–1827 (2008).

11 Bousema JT, Schneider P, Gouagna LC, Drakeley CJ, Tostmann A, Houben R *et al.*, Moderate effect of artemisinin-based combination therapy on transmission of *Plasmodium falciparum*. *J Infect Dis* **193**:1151–1159 (2006).

12 Hii J, Vythilingam I and Roca-Feltrer A, Human and simian malaria in the Greater Mekong Subregion and challenges for elimination, in *Towards Malaria Elimination – A Leap Forward*,IntechOpen Limited, London, United Kingdom, pp. 95–127 (2018). https://doi.org/10.5772/intechopen.76901.

13 Foster WA, Mosquito sugar feeding and reproductive energetics. *Annu Rev Entomol* **40**:443–474 (1995).

14 Gu W, Müller G, Schlein Y, Novak RJ and Beier JC, Natural plant sugar sources of Anopheles mosquitoes strongly impact malaria transmission potential. *PLoS One* **6**:e15996 (2011).

15 Stone, C.M. and Foster, W.A., Plant-sugar feeding and vectorial capacity, in *Ecology of Parasite–Vector Interactions. Ecology and Control of Vector Borne Diseases*, ed. by Takken W and Koenraadt CJM. pp. 35–79 (2013).

16 Manda H, Gouagna LC, Nyandat E, Kabiru E, Jackson R, Foster W *et al.*, Discriminative feeding behaviour of *Anopheles gambiae s.s.* on endemic plants in western Kenya. *Med Vet Entomol* **21**:103–111 (2007).

17 Gougana L, Poueme RS, Kounbobr RD, Ouédraogo J, Fontenille D and Simard F, Patterns of sugar feeding and host preferences in adult males of *An. gambiae* (Diptera: Culicidae). *J Vector Ecol* **35**:267–276 (2010).

18 Impoinvil D, Kongere J, Foster W, Njiru B, Killeen G, Githure J *et al.*, Feeding and survival of the malaria vector *Anopheles*

gambiae on plants growing in Kenya. *Med Vet Entomol* **18**:108–115 (2004).

19 Müller GC *et al.*, Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. *Malar J* **9**:1–11 (2010).

20 Foster WA, Phytochemicals as population sampling lures. *J Am Mosq Control Assoc* **24**:138–146 (2008).

21 Nyasembe VO, Teal PEA, Mukabana WR, Tumlinson JH and Torto B, Behavioural response of the malaria vector *Anopheles gambiae* to host plant volatiles and synthetic blends. *Parasit Vectors* **5**:234 (2012).

22 Nyasembe VO and Torto B, Volatile phytochemicals as mosquito semio-chemicals. *Phytochem Lett* **8**:196–201 (2014).

23 Nyasembe VO, Tchouassi DP, Mbogo CM, Sole CL, Pirk CWW and Torto B, Linalool oxide: generalist plant-based lure for mosquito disease vectors. *Parasit Vectors* **8**:581 (2015).

24 Jacob JW, Tchouassi DP, Lagat ZO, Mathenge EM, Mweresa CK and Torto B, Independent and interactive effect of plant- and mammalian-based odors on the response of the malaria vector, *Anopheles gambiae*. *Acta Trop* **185**:98–106 (2018).

25 Lindh JM, Okal MN, Herrera-Varela M, Borg-Karlson A, Torto B, Lindsay SW *et al.*, Discovery of an oviposition attractant for gravid malaria vectors of the *Anopheles gambiae* species complex. *Malar J* **14**:119 (2015).

26 Nyasembe VO, Teal PEA, Sawa P, Tumlinson JH, Borgemeister C and Torto B, *Plasmodium falciparum* infection increases *Anopheles gambiae* attraction to nectar sources and sugar uptake. *Curr Biol* **24**:217–221 (2014).

27 Nyasembe VO, Cheseto X, Kaplan F, Foster WA, Teal PEA, Tumlinson JH *et al.*, The invasive American weed *Parthenium hysterophorus* can negatively impact malaria control in Africa. *PLoS One* **10**:e0137836 (2015).

28 Balaich JN, Mathias DK, Torto B, Jackson BT, Tao D, Ebrahimi B *et al.*, The nonartemisinin sesquiterpene lactones parthenin and parthenolide block *Plasmodium falciparum* sexual stage transmission. *Antimicrob Agents Chemother* **60**:2108–2117 (2016).

29 Nyasembe VO, Tchouassi DP, Pirk CWW, Sole CL and Torto B, Host plant forensics and olfactory-based detection in Afro-tropical mosquito disease vectors. *PLoS Negl Trop Dis* **12**:e0006185 (2018). https://doi.org/10.1371/journal.pntd.0006185.

30 Junnila A, Müller G and Schlein Y, Species identification of plant tissues from the gut of *An. sergentii* by DNA analysis. *Acta Trop* **115**:227–233 (2010).

31 Lima LHDM, Mesquita MR, Skrip L, de Souza Freitas MT, Silva VC, Kirstein OD *et al.*, DNA barcode for the identification of the sand fly *Lutzomyia longipalpis* plant feeding preferences in a tropical urban environment. *Sci Rep* **6**:29742 (2016).

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