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Mini-Review

Test systems for tick repellents

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Abstract

There is an interest in the development of repellents for personal protection of humans and animals against ticks. Evaluation of new substances or formulations needs adequate test procedures to show efficacy of the compounds. A variety of repellent assays for ticks are described in the literature. Available biotests can be grouped in three categories (i) use of live hosts, (ii) use of some kind of tick attractant associated with hosts, or (iii) no use of attractants at all. The latter are often better to standardize and are cheap, but suffer from a poor ability to filter out weak repellents. The former two are usually more predictive in terms of forecasting the efficacy of the product under practical conditions, although sometimes difficult to standardize, particularly in the field, but usually expensive and time consuming. Therefore, recent developments concentrated on laboratory assays like the Moving-object bioassay or the human volunteer test, allowing the tick to display its host-seeking behaviour as close as possible to that shown in nature, yet offering a standardized procedure.

Key words: Tick – ixodidae – repellent – attractant

Introduction

Prevention of tick-borne disease (TBD) can be achieved by a variety of measures on an individual or community basis (see (Stafford and Kitron, 2002)). Most of the measures performed are ultimately aimed at reducing the contact frequency between vector ticks and human or animal hosts. In humans, the use of repellents for personal protection against ticks is widely accepted and can thus constitute an important prophylactic component of a TBD management strategy. Ideally, a repellent should prevent every tick from biting. As a repellent would seldom be applied to all parts of the human body, there is always a risk that a tick might encounter an untreated body part and bite there. Therefore, it would be even more desirable if a repellent could prevent a tick from contacting the host at all. In addition, an ideal repellent has to fulfil, further requirements, e.g. easy to apply, non-toxic for vertebrates, and so on. It is apparent that at present no such perfect tick repellent exists, and therefore, there is an ongoing interest in the development of more efficacious and safe products. Evaluation of new repellents, however, needs testing of the candidates in different phases of product development. During such development, different kinds of questions have to be answered, requiring appropriate experimental set-up. The present paper discusses some advantages and disadvantages of

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existing repellent assays for ticks, distinguishing between laboratory tests without any hosts or attractant stimuli, biotests involving host or host associated stimuli, and assays using live hosts performed inside or outside the laboratory.

Repellent assays without hosts and attractant stimuli

In early stages of screening for repellents one might wish to know whether a compound shows any repellent effect at all. When great numbers of substances are to be tested, the assay must be simple, fast and cheap. However, there is no published report available of a genuine mass screening system involving thousands of candidate substances. Such a system would require automation which is particularly difficult with respect to tick handling and is a significant limitation of existing systems.

Petri dish assays

The simplest test used with ticks are therefore handmade, involving a tick-walking arena with treated and untreated zones. Repellence is detected by a significantly reduced number of ticks entering the treated zone compared to a control (Dremova and Smirnova, 1970). Such kinds of assays have the advantage of being easy to perform, cheap and can provide controlled conditions enabling the comparison of substances. Additionally, every tick species can be used. However, the demands on a repellent in such an assay are not very high.

Generally speaking, a repellent has to counteract the motivation of the tick to approach/cling to a host and/or to bite the host skin. In nature, exophilic ticks that are activated and exhibit host-seeking behaviour have probably only a few opportunities to encounter a host. Such ticks might therefore have a very strong motivation to contact the host, or, once staying on the host, to remain on it. In a Petri dish, however, the motivation of a tick to walk into a particular zone will very probably be small. Therefore even very weak repellents might work there. As Schreck (1977) has pointed out in his excellent review, such an assay will thus reveal "untold millions of repellents" as large numbers of substances will show a repellent effect if only the test concentration is high enough. In order to limit the number of such unnecessary tests, it could be wise to use an upper limit of test concentrations of substances or to incorporate some means that increase the movement of the tick in a particular direction. This was done by Dusbabek et al. (1997), who used a synthetic analogue of the assembly pheromone of the soft tick *Argas persicus*. Although the assembly pheromone is certainly not related to host-seeking of this species, it indeed diminished the natural repellent effect of different acaricides tested.

However, a general disadvantage of such Petri-dish tests remains: the experimenter generally does not know whether or not a particular tick is in a host-seeking mode. This would be desirable in order to show repellent activity of a compound against a tick displaying appetence behaviour.

Tick climbing assays

To overcome this problem, tests using vertically placed rods have been developed (Ndungu et al., 1995; Mwangi et al., 1995; Lwande et al., 1999; Ndungu et al., 1999). Similar tests were performed with vertical tubes or strips of fabric (Kaaya et al., 1995; Carroll et al., 1989). Ticks showing the so-called ambush strategy (Sonenshine, 1993) climb a vantage point, e.g. a grass stem or other vegetation, waiting there for a passing host. Ticks climbing up rods in a laboratory assay probably display this kind of behaviour, which becomes particularly convincing, when ticks then come to rest on the rod, showing the questing pose. Repellents placed on the rod prevent ticks from climbing up, and a repellent effect is deduced by a significantly reduced number of ticks climbing the treated rods compared to an untreated control. Although ticks in such an assay are probably in a host-seeking mode, this does not necessarily imply that there is a high motivation in the tick to climb up a particular rod. Therefore, this kind of assay suffers from the same weakness as the Petri dish assay, i.e. the low ability to filter out weak repellents, though there is less random movement. In addition, the assay is extremely time consuming if ticks are monitored continuously. This can only be overcome by automated observation. Alternatively the position of ticks may be observed after fixed time intervals, but contacts between tick and repellent may be easily missed. Attempts have been made to increase the attractiveness of the tip of the rods by means of wet cotton wool creating a humid atmosphere that should induce the tick to rest there (Ndungu et al., 1999). This, however, is quite contrary to the natural situation where the humid conditions are at the base of the stem rather than at its tip. In this situation it is even questionable whether the tick is still in the host-seeking mode or engaged in water vapour absorption (Gaede and

Knülle, 1997; Kahl and Alidousti, 1997). However, it could be promising to investigate the use of animal associated kairomones in this kind of assay (Carroll, 2001).

Repellent assays with host-associated attractant stimuli

Moving-object bioassay

In order to increase the motivation of the tick to move while displaying host-seeking behaviour, we developed the Moving-object (MO) bioassay (Dautel et al., 1999). This assay uses warmth and a moving as attractive stimuli associated with hosts and allows the ticks to display their natural behaviour of clinging to a passing host in the laboratory. Briefly, a slowly rotating vertical drum is heated to a surface temperature of 35 – 36 °C. The temperature of the drum surface can be measured remotely with an infrared thermometer. On the drum there is an elevated surface of limited size serving as a tick attachment site. Ticks approach the drum on a horizontally placed glass rod, ending shortly in front of the drum. The distance between drum and tip of the rod is adjusted in such a way that the tick cannot reach the drum surface but only the elevated attachment site. As the drum rotates, the elevated attachment site periodically passes by and the tick is able to attach to this moving object as if it was a passing animal (see Fig. 1). For repellent tests, the test substance is applied to the attachment site, and records are made of whether or not the tick approaches the drum (distance effect), attaches to the drum, and, once on the drum, remains on the treated surface or drops off (contact repellence). In addition, the duration of each behavioural step can be measured which can reveal more subtle repellent effects.

The moving object was found to be very attractive to *Ixodes ricinus* nymphs, as about 90% of them attached to it in the absence of any repellent. Furthermore, even when N-N-diethyl-m-toluamide (DEET) was applied to the attachment site at 0.1 mg/cm^2 on filter paper, more than 50% attached to the repellent-treated surface (Dautel et al., 1999). This rate is not very different from that using humans as the tick target host. In a trial where DEET-containing products were applied to human volunteers at similar or higher levels of active ingredient more than 50% of *I. ricinus* nymphs initially entered the treated skin in spite of the repellent (Dautel, 2002). In contrast, when DEET was applied to filter paper on a vertical rod without any attractive

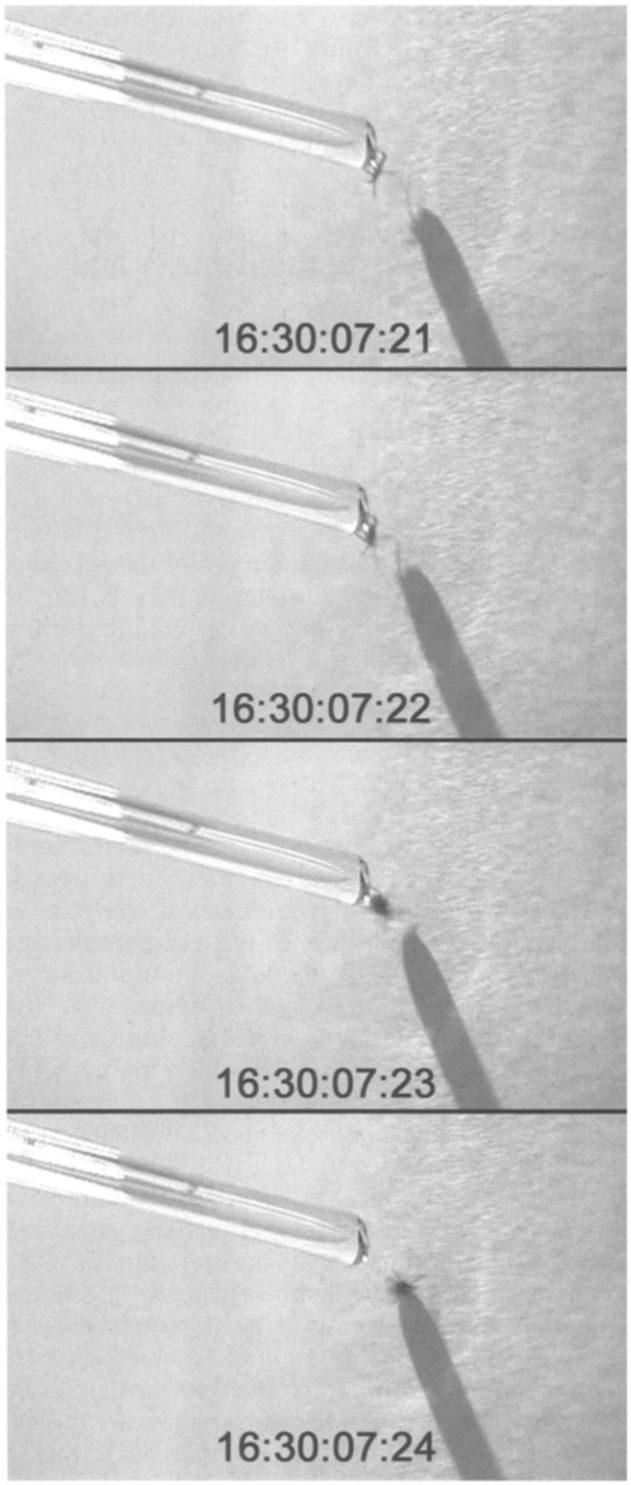

Fig. 1. Change of the tick from the glass rod to the elevated drum surface covered by filter paper. Four consecutive video frames (time intervals of 40 milliseconds) show the tick shortly before contacting the surface (uppermost frame), at the moment of contact (second frame from below) and after change to the drum (bottom frame).

stimulus, none of the ticks entered or even touched it. Instead, all were repelled over a distance of a few millimetres in front of it (Dautel et al., 1999). So the MO bioassay should predict more precisely whether or not a test substance can work on a host.

The MO bioassay thus provides a fast test procedure and was used by us intensively to screen a number of pure substances as well as to compare current repellent products against *Ixodes* spp. ticks (*I. ricinus, I. scapularis, I. persulcatus*) and *Rhipicephalus sanguineus* (unpubl. results). A strength of the assay is that compounds, different formulations or even products can be compared under standardized conditions. Furthermore, the system allows discrimination of repellents acting at a distance, from contact repellents, because several behavioural steps, including the approach of the tick to the heat source, and its walking behaviour on the treated surface can be investigated. The system has the disadvantage, however, that an experimenter cannot run several tests in parallel, so mass screening is currently not possible. Additionally, the test system is inherently confined to tick species and developmental stages displaying the aforementioned ambush strategy, rendering it unsuitable for soft ticks and certain hard ticks.

Olfactometer assay

Ticks that actively search and approach a host by walking, can in principle also be investigated in an air stream using an olfactometer. It is well known that ticks react to airborne stimuli (Guerin et al. 2000), but research has predominantly concentrated on tick attractants. Such olfactometer tests can be rather simple Y-tube assays (Yoder et al., 1998) or highly sophisticated tracking systems using e.g. a locomotion compensator (McMahon and Guerin, 2002). The latter are primarily used to investigate walking patterns of arthropods e.g. in the presence of attractant stimuli. When such search patterns are altered or inhibited by a repellent, this unequivocally shows, that the repellent acts via the gas phase. Such olfactometer assays are in principle a refinement of the Petri dish assay using kairomones. The directional movement of the tick that the repellent would have to counteract in such an olfactometer largely depends on the attractiveness of the stimulus presented in the air stream.

Y-tube olfactometers, however, are probably not suitable for repellent tests, as the tick can make its choice between the repellent treated and the untreated arm only after a walk through the stem, where, of course, the repellent is also present in the gas phase.

Tick feeding assay

Several attempts have been made to feed ticks artificially (Waladde et al., 1991; Kuhnert et al., 1995; Waladde et al., 1996; Kuhnert et al., 1998). When such a feeding system works well, it might also be used as a repellent assay, i.e. by covering the feeding membrane with repellent. Whereas artificial feeding of some fast-feeding soft ticks is rather simple (Kirch et al., 1991; Schwan et al., 1991; Ben-Yakir and Galun, 1993), systems for slow-feeding ticks (hard ticks and some soft tick larvae) are much more complicated because fresh blood must constantly be supplied and maintained at appropriate conditions for several days, and the growth of fungi and bacteria must be inhibited without interference with tick physiology. However, for the purpose of repellent testing, only the first phase of feeding is important, i.e. the insertion of the mouthparts of the tick into the membrane. The assay would require an attractive membrane, preferably a standardized artificial one, together with a reliable means to detect penetration of the membrane by the mouthparts of the tick. The apparent simplicity of these requirements suggests that the development of such an assay would be worthwhile.

Repellent assays using hosts

Assays using live hosts provide ticks with the full range of attractive stimuli and are thus very useful in terms of predicting the efficacy of the substance under field conditions.

Laboratory animal hosts

Often standard laboratory animals like rabbits, guinea pigs or mice are used (Bar-Zeev and Gothilf, 1973, 1974; Mehr et al., 1986; Kumar et al., 1992; Salafsky et al., 2000). These animals, however, are not necessarily the preferred hosts of the ticks under study. Therefore, extrapolation of the results might result in inaccurate estimation of natural efficacy. The same applies for the tick specimens used (in any kind of assay). If laboratory-reared ticks are used, it must be ensured that such ticks are in a proper physiological state, i.e. show all features of appetence behaviour. Secondly, as repellents are not equally effective against different tick species, the results with one species might not predict the efficacy against another species. It is therefore advisable to use the target tick species and animal to be protected whenever possible for such assays (Painter, 1967; Schreck, 1977). This is the more important as such

assays are comparatively time consuming and expensive.

Field tests

Properly designed and performed field-tests with animals or humans will in principle produce the most relevant results to the natural situation. However, many difficulties arise from the very variable and uncontrolled conditions that occur in the field. It must be ensured that the tests are performed at the right season and in an area with sufficient tick questing activity. Habitats may be heterogeneous or tick density may be clustered for other reasons, rendering it difficult to choose comparable test areas. Additionally, weather conditions can alter tick questing activity in the short term (Randolph and Storey, 1999; Perret et al., 2003). It is also difficult to observe tick behaviour towards the repellent in the field.

Another important feature of a repellent test with exophilic ticks in the field is the behaviour of the test animal or human volunteer, as it is usually the host who picks up the tick and not the tick that approaches its host. This means that standardized walking behaviour of the target host is desirable, which might be difficult to achieve with an animal host like a dog, particularly when several dogs are used together. When such dogs move freely together in a group, it is quite important, which dog tends to walk in front and which one walks behind. In addition, different dog breeds differ distinctly with respect to their behaviour, particularly locomotory and investigative behaviour, as well as in their size and hair length. Beagles that are typically used for such studies have short hair which are less suitable to pick up ticks than in some other breeds. Additionally the dogs might be unfamiliar with field conditions when reared in the laboratory and might thus show less intensive locomotor activity outside.

Such problems are less evident with human volunteers. Here, however, it must be decided whether the repellents are applied on clothing (Schreck et al., 1980; Lane, 1989; Evans, 1990) or on skin (Solberg et al., 1995). As tick populations under study might carry one or more human pathogenic agents, there is a potential infection risk for the volunteers. Therefore, researchers might prefer volunteers to use tight clothes and consequently to apply the repellent on the clothes in order to minimize infection risk. The results, however, might be quite different from a test where the same repellent is applied on the skin (unpubl. results), as in the latter the repellent has to counteract not only short-distance attractants like warmth and skin volatiles potentially penetrating the fabrics, but also contact attractants of the skin.

Laboratory test with human volunteers

In order to provide more standardized test conditions than usually possible in the field, a laboratory assay with human volunteers might be preferable, particularly for a comparison of repellent products. Such an assay is described by the Environmental Protection Agency of the U.S.A. and recommended for U.S. regulatory affairs (see www.epa.gov). Briefly, the procedure involves application of a repellent on a subject's arm positioned vertically, leaving an area at the bottom of the arm untreated. Ticks are placed 2 cm below the repellent border and are monitored for entering/not entering the treated zone. Similar tests using a finger instead of an arm were performed by Schreck at al. (1995) and Pretorius et al. (2003). The test relies on the propensity of the ticks to walk upwards after contacting the host. In a recent study (Dautel 2002), 83% of the *I. ricinus* ticks (n = 518) walked upwards, a behaviour that could be reversed by a repellent. However, the strength of the motivation to walk upwards might not be very high because the tick in this assay is already on untreated skin. So the decision the tick has to make at the repellent border is either to stay on untreated skin or to walk up onto the repellent-treated area. Since the motivation of the tick to move onto treated skin might be higher, when it has not such an attractive alternative, the test procedure was modified as described in Dautel (2002). Briefly, human forearms or lower legs were treated with repellent according to consumer labels. Then a small copper plate was placed on the treated area and a tick placed on this plate. Here the tick has to make the decision between staying on an unattractive 'nonskin' area or entering an area of repellent-treated skin. It is then observed whether or not the tick enters the treated skin, and once on the skin, whether or not it walks a distance of at least 5 cm. If traversing a circle drawn 5 cm around the plate, the tick is considered not repelled. Tests are repeated until the repellence of the product diminishes. Additionally, the walking direction of the tick (up, down, or to the side) determines whether there is at least a small repellent effect or none at all. This kind of assay was performed for a German consumer care organisation and permitted comparison of the repellence of a number of commercially available tick repellents for humans. The rigorous nature of the test is shown by the fact, that even the best repellents tested could prevent only 50% or less of the ticks from entering the repellent-treated skin.

This particular assay permits the detailed comparison of potential tick repellents in terms of absolute repellence in addition to duration of the effect.

References

Bar-Zeev, M., Gothilf, S.: Laboratory evaluation of tick repellents. J. Med. Entomol. 10, 71–74 (1973).

Bar-Zeev, M., Gothilf, S.: Field evaluation of repellents against the tick *Ornithodoros tholozani* Labou & Megn in Israel. J. Med. Entomol. 11, 389–392 (1974).

Ben-Yakir, D., Galun, R.: Comparative study of two argasid tick species: feeding response to phagostimulants. Israel J. Zool. 39, 169–176 (1993).

Carroll, J. F.: Interdigital gland substances of White-Tailed Deer and the response of host-seeking ticks (Acari: Ixodidae). J. Med. Entomol. 38, 114–117 (2001).

Carroll, J. F., Maradufu, A., Warthen Jr., J. D.: An extract of *Commiphora erythraea*: a repellent and toxicant against ticks. Entomol. Exp. Appl. 53, 111–116 (1989).

Dautel, H.: In vivo test method for repellents against the hard tick, *Ixodes ricinus* (Acari: Ixodidae), main vector of Lyme borreliosis and TBE in Europe. In: Proceedings of the 4th Conference on Urban Pests (Jones, S. C., Zhai, J., Robinson, W. H., eds.), pp: 387–398. Pocahonta Press, Blacksburg, USA, 2002.

Dautel, H., Kahl, O., Siems, K., Oppenrieder, M., Müller-Kuhrt, L., Hilker, M.: A novel test system for detection of tick repellents. Entomol. Exp. Appl. 91, 431–441 (1999).

Dremova, V. P., Smirnova, S. N.: Effects of repellents on hard (Ixodidae) and soft (Argasidae) ticks. Int. Pest Control 12, 10–14 (1970).

Dusbábek, F., Rupeš, V., Šimek, P., Zahradníčková, H.: Enhancement of permethrin efficacy in acaricide-attractant mixtures for control of the fowl tick *Argas persicus* (Acari: Argasidae). Exp. Appl. Acarol. 21, 293–305 (1997).

Evans, S. R., Korch, G. W., and Lawson, M. A. Comparative field evaluation of permethrin and DEET-treated military uniforms for personal protection against ticks (Acari). J. Med. Entomol. 27, 829–834 (1990).

Gaede, K., Knülle, W.: On the mechanism of water vapour sorption from unsaturated atmospheres by ticks. J. Exp. Biol. 200, 1491–1498 (1997).

Guerin, P. M., Kröber, T., McMahon, C., Guerenstein, P., Grenacher, S., Vlimant, M., Diehl, P.-A., Steullet, P., Syed, Z.: Chemosensory and behavioural adaptations of ectoparasitic arthropods. Nova Acta Leopoldina NF 83, 197–213 (2000).

Kaaya, G. P., Mwangi, E. N., Malonza, M. M.: Acaricidal activity of *Margaritaria discoidea* (Euphorbiaceae) plant extracts against the ticks *Rhipicephalus appendiculatus* and *Amblyomma variegatum* (Ixodidae). Int. J. Acarol. 21, 123–129 (1995).

Kahl, O., Alidousti, I.: Bodies of liquid water as a source of water gain for *Ixodes ricinus* (Acari: Ixodidae). Exp. Appl. Acarol. 21, 731–746 (1997).

Kirch, H. J., Teel, P. D., Kloft, W. J., Deloach, J. R.: Artificial feeding of *Ornithodoros concanensis* (Acari: Argasidae) nymphs on bovine blood and morphological changes in erythrocytes undergoing hemolysis in the tick midgut. J. Med. Entomol. 28, 450–455 (1991).

Kuhnert, F., Issmer, A. E., Grunewald, J.: Teilautomatisierte in vitro Fütterung adulter Schildzecken (*Amblyomma hebraeum*). ALTEX 15, 67–72 (1998).

Kuhnert, F., Diehl, P. A., Guerin, P. M.: The life cycle of the Bont Tick *Amblyomma hebraeum* in vitro. Int. J. Parasitol. 25, 887–896 (1995).

Kumar, S., Prakash, S., Kaushik, M. P., Rao, K. M.: Comparative actvity of three repellents against the ticks *Rhipicephalus sanguineus* and *Argas persicus*. Med. Vet. Entomol. 6, 47–50 (1992).

Lane, R. S.: Treatment of clothing with a Permethrin spray for personal protection against the Western Black-Legged tick, *Ixodes pacificus* (Acari: Ixodidae). Exp. Appl. Acarol. 6, 343–352 (1989).

Lwande, W., Ndakala, A. J., Hassalani, A., Moreka, L., Nyandat, E., Ndungu, M., Amiani, H., Gitu, P. M., Malonza, M. M., Punyua, D. K.: *Gynandropis gynandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry 50, 401–405 (1999).

McMahon, C., Guerin, P. M.: Attraction of the tropical bont tick, *Amblyomma variegatum*, to human breath and to the breath components acetone, NO and CO_2. Naturwissenschaften 89, 311–315 (2002).

Mehr, Z., Amorales, E. L., Inase, J. L.: Laboratory evaluation of commercial and experimental repellents against *Ornithodoros parkeri* (Acari: Argasidae). J. Med. Entomol. 23, 136–140 (1986).

Mwangi, E. N., Essuman, S., Kaaya, G. P., Nyandat, E., Munyinyi, D., Kimondo, M. G.: Repellence of the tick *Rhipicephalus appendiculatus* by the grass *Melinis minutifloria*. Trop. Anim. Health Prod. 27, 211–216 (1995).

Ndungu, M., Lwande, W., Hassanali, A., Moreka, L., Chabra, S. C.: *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellents. Entomol. Exp. Appl. 76, 217–222 (1995).

Ndungu, M. W., Chabra, S. C., Lwande, W.: *Cleome hirta* essential oil as livestock tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellent. Fitoterapia 70, 514–516 (1999).

Painter, R. R.: Repellents. In: Pest Control (Killgore, W. W., Doutt, R. L. eds.), pp. 267–285. Academic Press, New York, London, 1967.

Perret, J. L., Guerin, P. M., Diehl, P. A., Vlimant, M., Gern, L.: Darkness induces mobility, and saturation deficit limits questing duration, in the tick *Ixodes ricinus*. J. Exp. Biol. 206, 1809–1815 (2003).

Pretorius, A.-M., Jensenius, M., Clarke, F., Ringertz, S. H.: Repellent efficacy of DEET and KBR 3023 against *Amblyomma hebraeum* (Acari: Ixodidae). J. Med. Entomol. 40, 245–248 (2003).

Randolph, S. E., and Storey, K.: Impact of microclimate on imature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. J. Med. Entomol. 36, 741–748 (1999).

Salafsky, B., Xun, H. Y., Jing, L., Takeshi, S., Kalyanasundaram, R.: Study on the efficacy of a new long-lasting formulation of N-N-diethyl-m-toluamide (DEET) for the prevention of tick attachment. Am. J. Trop. Med. Hyg. 62, 169–172 (2000).

Schreck, C. E.: Techniques for the evaluation of insect repellents: a critical review. Annu. Rev. Entomol. 22, 101–119 (1977).

Schreck, C. E., Snody, E. L., Mount, G. A.: Permethrin and repellents as clothing impregnants for protection from the Lone Star Tick. J. Econ. Entomol. 73, 436–439 (1980).

Schreck, C. E., Fish, D., McGovern, T. P.: Activity of repellents applied to skin for protection against *Amblyomma americanum* and *Ixodes scapularis* ticks (Acari: Ixodidae). J. Am. Mosq. Contr. Assoc. 11, 136–140 (1995).

Solberg, V. B., Klein, T. A., McPherson, K. R., Bradford, B. A., Burge, J. R., Wirtz, R. A.: Field evaluation of DEET and a piperidine repellent (AI3–37220) against *Amblyomma americanum* (Acari: Ixodidae). J. Med. Entomol. 32, 870–875 (1995).

Sonenshine, D. E.: Biology of Ticks. Vol. 2. Oxford Univ. Press, New York, 1993.

Stafford, K. C., Kitron, U.: Environmental management for Lyme Borreliosis control. In: Lyme Borreliosis Biology, Epidemiology and Control (Gray, J. S., Kahl, O., Lane, R. S., Stanek, G. eds.), pp. 301–334. CABI Publishing, New York, 2002.

Schwan, E. V., Hutton, D., Shields, K. J. B.: Artificial feeding and successful reproduction in *Ornithodoros moubata moubata* (Murray, 1877) (Acarina: Argasidae). Exp. Appl. Acarol. 13, 107–115 (1991).

Waladde, S. M., Ochieng, S. A., Gichuhi, P. M.: Artificial-membrane feeding of the ixodid tick, *Rhipicephalus appendiculatus*, to repletion. Exp. Appl. Acarol. 11, 297–306 (1991).

Waladde, S. M., Young, A. S., Morzaria, S. P.: Artificial feeding of ixodid ticks. Parasitol. Today 12, 272–278 (1996).

Yoder, J. A., Atwood, A. D., Stevens, B. W.: Attraction of squalene by ticks (Acari: Ixodidae): first demonstration of a host-derived attractant. Internat. J. Acarol. 24, 143–147 (1998).