Repellency of the Mosquito Repellent MyggA® (*N,N*-diethyl-3-methyl-benzamide) to the Common Tick *Ixodes ricinus* (L.) (Acari: Ixodidae) in the Laboratory and Field

THOMAS G.T. JAENSON, ANDERS LINDSTRÖM AND KATINKA PÅLSSON

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In Sweden, MyggA® is one of the most commonly used commercial repellents against mosquitoes and other blood-feeding Diptera. This product contains 19.0% deet (*N,N*-diethyl-3-methyl-benzamide = *N,N*-diethyl-*m*-toluamide); perfume, i.e., terpene fractions of essential oils of, e.g., lavender, geranium and roses; and "inactive" ingredients, which are all well known ingredients of cosmetic products. In the field in south-central Sweden, we tested by randomised, standardized methodology the potential anti-tick activity (repellency to *Ixodes ricinus* nymphs) of MyggA applied to a cotton flannel cloth dragged over the ground vegetation or applied to the legs of trousers of two persons walking through the vegetation. In the laboratory the different components of MyggA were tested for repellency to ticks subjected to the stimuli emitted from a human host. A total of 528 *I. ricinus* nymphs were collected by the cloth dragging method, 90.3% of which were on the untreated control blanket ($p<0.0001$; repellency=89.3%). A total of 70 nymphs were collected from the MyggA-treated legs and 145 nymphs from the untreated legs ($p<0.0001$; repellency=51.7%). In the laboratory one or five drops of MyggA repelled 64.6% and 99.0%, respectively of nymphs. The diluted (1%) and pure (98.2%) perfume, containing essential oils of plants, repelled 94.6% and 100.0%, respectively of nymphs. Concentrated (98.2%) and diluted (19%) deet had a repellency of 100.0% and 95.2%, respectively. This study shows that MyggA and its components, i.e., deet and the oils of, e.g., lavender, geranium, and roses, have significant repellent activities against *I. ricinus* nymphs.

T.G.T. Jaenson, Medical Entomology Unit, Department of Systematic Zoology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden. E-mail: Thomas.Jaenson@ebc.uu.se

Introduction

Ticks are the most important arthropod vectors of diseases to man and farm animals in the northern hemisphere, including northern Europe. Here, the most common and the medically most important tick species is *Ixodes ricinus* (L.) (Fig. 1). In Sweden alone, each year an estimated 10 000 people become infected with Lyme borreliosis spirochetes (*Borrelia burgdorferi s.l.*). Additionally, ~100 people become each year seriously ill due to infection with the virus causing tick-borne encephalitis (TBE). Other pathogens transmitted by *I. ricinus* to man and domestic animals are the agents of anaplasmosis/ehrlichiosis [*Anaplasma* (formerly *Ehrlichia*) *phagocytophilum*], Q-fever (*Coxiella burnetii*), tularaemia (*Francisella tula-*

*Figure 1. Nymph of the common tick **Ixodes ricinus**. Natural size 1.5 mm. Photo: G. Wife & T.G.T. Jaenson.*

*Nymf av den vanliga fästingen **Ixodes ricinus**. Naturlig storlek 1,5 mm. Foto: G. Wife & T.G.T. Jaenson.*

rensis) and babesiosis (*Babesia divergens, B. microti*) (Jaenson 1999). Therefore, a simple and cheap method of protection of man and domestic animals against ticks is needed.

Preliminary observations suggested that MyggA®, a commercially available mosquito repellent containing N,N-diethyl-3-methyl-benzamide (= N,N-diethyl-*m*-toluamide = deet) and the essential oils of lavender, geranium and roses, repels nymphs of *I. ricinus* (T.G.T. Jaenson, unpubl. observations). Moreover, studies in other countries have shown that some tick species are indeed repelled by deet in laboratory experiments (Mehr et al. 1995, Dautel et al. 1999) and in the field (Evans et al. 1990). Other studies have shown that certain plants contain substances, which repel or kill ticks (Sutherst et al. 1982, Malonza et al. 1992, Mwangi et al. 1995, Ndungu et al. 1995, Panella et al. 1997).

”A repellent is a chemical that, acting in the vapour phase, prevents an arthropod from reaching a target to which it would otherwise be attracted” (Browne 1977). The aim of this study was to investigate if MyggA and its components have repellent effects on the nymphal stage of *I. ricinus*. We tested the repellency of MyggA and its components on *I. ricinus* both in the laboratory and in the field.

Material and methods

MyggA (”roll-on”) contains (i) deet (19% w/w (weight/weight)), (ii) “active perfume”, i.e., terpene fractions of plant oils of e.g. lavender, geranium and roses, and (iii) putatively “inactive ingredients”, which are all well known ingredients of cosmetic products. The exact composition of MyggA is the manufacturer’s classified information and cannot, therefore, be disclosed.

One field study was conducted using the cotton flannel cloth dragging method (Mejlon & Jaenson, 1993). A white cloth, 1x1m, was attached to a 1 m long wooden pole to both ends of which a cord was tied (Fig. 2). The cloth, lying horizontally over the ground vegetation, was dragged over the vegetation by pulling it (from the center) via the cord attached to either end of the pole. Stops were done every 10 m when the cloth was turned over to count and collect all nymphs that had attached.

MyggA (19% deet) was sprayed to cover a large proportion of the downward surface of the cloth. During the experiment the cloth was pulled with the treated side downwards, towards the ground vegetation, to maximize the effect of the treatment. The control cloth was an identical cloth, but untreated with MyggA. The cloths were pulled in a randomised order between the persons. Each cloth was pulled for 2,500 m parallel to the other cloth and was turned over every 10 m to count and collect all nymphs that had attached. These collections were carried out at Torö (1,300 m), south of Stockholm and at Österlen (1,200 m) in Scania, southeastern Sweden.

Another field study was conducted to evaluate the repellency of deet applied to the legs of light coloured trousers. MyggA (19% deet) was sprayed onto the left leg while the right leg was used as a control. Two persons, with such treated trousers, walked parallel to each other 2,000 m at Torö. Nymphs that attached were counted and collected after every 10 m.

*Figure 2. The cloth dragging method, here demonstrated by the senior author, is commonly used to collect the host-seeking stages of the tick **Ixodes ricinus**, which attach to the lower surface of the cloth when it is pulled horizontally, slowly over the ground vegetation. Photo: Jurek Holzer.*

*"Tygsläpningsmetoden", som här demonstreras av försteförfattaren, används ofta för att insamla de värdsökande stadierna av fästingen **Ixodes ricinus**, vilka sätter sig på den undre ytan av tyget när detta dras horisontellt, sakta över markvegetationen.*

The laboratory experiments were conducted using 5 unfed *I. ricinus* nymphs in a Falcon™ vial in each replicate. The Falcon vial is a 50 ml centrifugal tube, 116x29mm, made of transparent plastic. The control (70% ethanol in water) and test substances (Table 1), diluted in 70% ethanol in water, were applied with pipettes to cotton cloths. Five drops was the standard application. Each cloth was attached with a rubber band to the open upper end (660 mm^2) of a Falcon vial. Its wall was perforated with small holes to prevent saturation of the air with scent of the experimenter, test substance or control substance. In each replicate, 5 previously unused nymphs were first tested with the control substance for 5 min and then with the test substance for 5 min. To simulate host stimuli to attract the nymphs the same observer held his palm tight to the outside surface of the cloth for each 5 min period. The number of nymphs arriving at the interior surface of the cloth during this period was recorded. For a tick to be regarded as "attached" to the cloth it had to detach all its legs from the vial's surface. Ticks that clung to the cloth were recorded in the protocol as "attracted" while ticks that did not were recorded as "repelled".

In the field experiment the repellency was calculated using the following formula: % repellency = [(no. of nymphs on control blanket - no. of nymphs on test blanket)/ no. of nymphs on control blanket] x 100. The field data were tested with Mann-Whitney *U*-test. In the laboratory experiment % repellency was calculated as [(no. of ticks recorded as ”attracted” in the control vial - no. of ticks recorded as ”attracted” in the test vial)/ no. of ticks recorded as ”attracted” in the control vial] x 100. The laboratory data were tested with the Kolmogorov-Smirnov two-sample test.

Results

MyggA-treated blanket in field test

In the field experiment a total of 528 nymphs was caught, 477 (90.3%) of which were caught on the untreated control blanket. The difference is significant (p<0.0001, N=250). Mean (and median) densities were 4.0 (3.0) nymphs/10m^2 for the untreated control cloth and 0.5 (0.0) nymphs/10m^2 for the MyggA-treated blanket. The repellency was 89.3%.

MyggA-treated trousers in field test

In the field experiments with MyggA-treated garments there were totals of 70 nymphs on the treated legs and 145 nymphs on the control legs. The difference is significant (p<0.0001, N=400). This corresponds to a repellency of 51.7%.

MyggA in the laboratory repellency test

When 5 drops of MyggA were applied to the cloth only 1 of 130 nymphs (0.67%; n = 26 replicates) was "attracted". In the control group 113 of 130 nymphs (75.3%; n = 26) were attracted (Table 1). In the test with one drop of MyggA on the cloth 17 of 50 nymphs were attracted; in the control 48 of 50 nymphs were attracted (Table 1). Thus, in these laboratory experiments MyggA exerted a strong ($p<0.001$) repellent effect on *I. ricinus* nymphs.

Deet in laboratory repellency test
With 98.2% deet no nymphs were attracted to the human skin while in the control 35 of 50 (70%) nymphs were attracted (Table 1). Also, with 19, 10, and 5 % deet (corresponding to ~1.8, 0.96, and 0.48 mg/cm^2, respectively) significantly fewer nymphs were attracted were attracted compared to the control. However, with 1% deet (~0.096 mg/cm^2) the test result (18/50 nymphs) was not significantly ($p>0.1$) different from that of the control (40/50 nymphs). Thus, under these laboratory conditions, deet in the range from 5% to 98% exhibited strongly significant ($p<0.001$) repellent activity on *I. ricinus* nymphs (Table 1).

Plant oils in laboratory repellency test
With the pure (98.2%) essential oils none of 50 nymphs was attracted. Also with the diluted oils (1% = 0.01 mg/cm^2) only 2 of 50 nymphs were attracted (Table 1). These data show that the plant oils, which occur in MyggA, even when diluted, had a strong repellent action on *I. ricinus* nymphs. It is interesting to note that the 1% plant oil solution was significantly repellent whereas 1% deet was not (Table 1).

Deet and plant oils in laboratory repellency test
With five drops of a mixture of 19% (w/w) deet and 1% (w/w) perfume, i.e., plant oils, 0/50 (0%) of the nymphs were attracted to the human skin while in the control 49/50 (98%) of nymphs were attracted. Three drops of 19% deet and 1% perfume resulted in 3/50 (6%) and 48/50 (96%) of nymphs attracted in the test and control, respectively. With two drops of 19% deet and 1% perfume the corresponding data were 13/50 (26%) in the treatment group and 39/50 (78%) in the control group. Thus, a mixture of deet and plant oils exhibited significant ($p<0.001$-0.025) repellent activity on *I. ricinus* nymphs (Table 1).

"Inactive" substances of MyggA in laboratory repellency test
With the putatively "inactive" ingredients of MyggA the numbers of nymphs attracted were 8/50 (16%) in the treatment group and 30/50 (60%) in the control group ($p>0.05$). This shows that the supposedly inactive ingredients did not exert a significant repellent action on *I. ricinus*

Table 1. Outcome of laboratory experiments testing repellency of different substances on ***Ixodes ricinus*** *against control. "n" = number of replicates (5 nymphs per replicate), "p" = probability that frequencies of the two substances compared are similar based on the Kolmogorov-Smirnov test.*

Resultatet av labexperiment där olika substansers avskräckande effekt på fästingnymfer jämfördes med kontroll. "n"=antalet replikat (5 nymfer per replikat), "p" = sannolikheten för att frekvenserna för de två testade substanserna var lika baserat på Kolmogorov-Smirnovs test.

Test and control substance	n	% nymphs attracted		p	% repellency
		Test	Control		
MyggA vs control, 5 drops	26	0.77	79.2	< 0.001	99.0
MyggA vs control, 1 drop	10	34	96	< 0.001	64.6
deet 98% vs control	10	0	70	< 0.001	100.0
deet 19% vs control	10	4	84	< 0.001	95.2
deet 10% vs control	10	14	92	< 0.001	84.8
deet 5% vs control	10	18	90	< 0.001	80
deet 1% vs control	10	36	80	> 0.1	55
"perfume" (98.2%) vs control	10	0	46	< 0.001	100
"perfume" (1%) vs control	10	4	74	< 0.001	94.6
deet 19%+ perfume 1% vs control, 5 drops	10	0	98	< 0.001	100
deet 19%+ perfume 1% vs control, 3 drops	10	6	96	< 0.001	93.7
deet 19%+ perfume 1% vs control, 2 drops	10	26	78	< 0.025	66.7
deet 19%+"inactive ingredients" vs control	10	0	62	< 0.001	100
"inactive ingredients" vs control	10	16	60	< 0.1	73.3

nymphs. In contrast, with the inactive substances and 19% deet none of 50 nymphs was attracted while in the control 31 of 50 nymphs were attracted.

Discussion

The results show that MyggA and two of the three ”types” of its constituents, i.e., deet and the plant oils exerted significant repellent activity against nymphs of *I. ricinus* in the laboratory repellency test. In this ”artificial” test situation the ”perfume”, i.e., a mixture of four different essential oils of plants exhibited a degree of repellency very similar to those of MyggA and 19% deet. The inactive ingredients did not exhibit a significant degree of repellency compared to that of the control on *I. ricinus* nymphs. It is considered likely that in the laboratory method, in which we observed the behaviour of tick nymphs towards a test substance, any repellent, neutral or attractive activity of the substance was relatively easily detected. On the other hand, in the field where we tested the potentially repellent activity of deet by the cloth-dragging method and on trousers it is likely that numerous factors may have partly obscured the repellency. This might explain the greater repellency of deet and MyggA in the laboratory than in the field. On the other hand, we suggest that the test methods used by us in the field situation provide a better picture of the degree of tick protection that would be encountered when ordinary people use MyggA on their clothes.

Deet was discovered and developed by American scientists and patented by the U.S. Army in 1946. It was registered for use by the general public in 1957 (Fradin 1998). Deet is generally considered the most effective and best studied insect repellent on the market. Deet has a remarkable safety profile although toxic reactions have been recorded, especially when the compound has been used extensively on large skin surfaces of children. More than 200 million people use deet-based arthropod repellents every year (Fradin 1998). People who are concerned about the potential toxicity from deet can use the repellent on their clothes instead of on their skin. Kept in a plastic bag, the repellent effect can last for months (Curtis et al. 1987). As a means of tick protection MyggA and similar deet-containing products are preferably applied onto the legs of the trousers as a barrier against host-seeking ticks coming from the ground vegetation onto the clothes. MyggA contains 19% of deet, which is considered to be of a relatively low toxicity. However, it is absorbed by lipid-rich tissues and can cause toxic reactions. Therefore, it is recommended that MyggA and similar products is only used in small amounts and for short periods if applied directly onto the skin. In the case of children, such products shall not be applied directly onto their skin but only on their clothes.

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Sammanfattning

Ixodes ricinus är den i särklass vanligaste av de drygt tio fästingarter som förekommer permanent i Norden. M a o är *I. ricinus* den art som oftast observeras av och på människor. Den överför flera olika, potentiellt sjukdomsframkallande mikroorganismer bland domesticerade och vilda däggdjur och fåglar. Människor drabbas ibland av sjukdom då sådana mikroorganismer via fästingar överförs från djuren till människan. *I. ricinus*-associerade infektiösa virus och mikroorganismer av humanmedicinskt intresse i Nordeuropa omfattar bl a: TBE-virus inklusive louping ill-virus (fästingöverförd hjärn- och hjärnhinneinflammation), Uukuniemi-virus, *Francisella tularensis* (tularemi eller harpest), *Anaplasma phagocytophilum* [anaplasmos; (tidigare *Ehrlichia phagocytophila*; ehrlichios)], *Rickettsia helvetica* (rickettsios/sarkoidos), *Coxiella burnetii* (Q-feber), flera arter inom *Borrelia burgdorferi*-komplexet (Lyme borrelios), *Babesia divergens* och *B. microti* (babesios) (Jaenson 1999). Uppskattningsvis drabbas enbart i Sverige ca 10 000 människor varje år av sjukdom till följd av *Borrelia*-infektion. Ytterligare ca 100 människor drabbas samtidigt av allvarlig sjukdom till följd av TBE-virusinfektion.

Med tanke på alla de infektionssjukdomar som kan överföras från vilda djur till människan via fästingar, speciellt med nymfstadiet av *I. ricinus*, bör man försöka skydda sig mot fästingbett. Harar och rådjur kan släppa ifrån sig blodfyllda fästinghonor som var och en kan lägga upp till 2000 ägg. Därför bör man se till att harar och rådjur inte har tillgång till trädgården, t ex genom att sätta upp ordentliga staket, som når tätt an mot marken (mot harar) respektive är höga (mot rådjur). I tättbebyggda områden med parker och trädgårdar kan en begränsning av antalet harar och rådjur minska risken för fästingangrepp. Sådana åtgärder är sannolikt effektivast i geografiskt isolerade områden (öar, halvöar, etc.). Fuktighetsbevarande vegetation, som är gynnsam för fästingar, kan eventuellt tas bort från tomten för att missgynna fästingarna. Fästingarna sitter i allmänhet på marken eller på låg vegetation (<1 m) då de söker blod. De hamnar därför oftast på benen och kryper uppåt tills de finner bar hud. Barn är kortare än vuxna. Därför fäster sig fästingarna oftare på överkroppen och huvudet på barn. På vuxna personer sitter fästingarna oftare på lägre kroppspartier. I områden där man vet eller misstänker att det finns mycket fästingar kan heltäckande klädsel användas. Långbyxor med byxbenen nedstoppade innanför stövelskaften eller innanför strumporna, och en tröja nedstoppad innanför byxlinningen, ger ett gott skydd. Ljusa kläder kan rekommenderas eftersom fästingarna upptäcks lättast mot en ljus bakgrund. Man bör inspektera hela kroppen åtminstone dagligen, gärna oftare, när man vistas i fästingmarker. Speciellt att tänka på är att kamma igenom håret och leta runt öronen, i synnerhet på barn. Fästingar som infesterat kläder kan avlivas genom att man stryker kläderna med ett varmt strykjärn eller torkar dem ordentligt i ett påslaget torkskåp. En ytterligare metod för att skydda sig mot fästingangrepp är att bestryka sina kläder eller huden med ett kemiskt ämne - eller en kombination av sådana ämnen - som repellerar fästingar.

I Sverige är ”MyggA” ett av de mest sålda preparaten mot stickmyggor och andra blodsugande tvåvingar. Preparatet innehåller ca 19 % av den välkända insektsrepellerande kemikalien deet, dvs. *N,N*-diethyl-3-methyl-benzamid (*N,N*-diethyl-*m*-toluamid). Det innehåller dessutom mindre mängder essentiella oljor av bl.a. lavendel, geranium och rosor samt förmodat inaktiva substanser, som ofta förekommer som tillsatser i kosmetiska produkter. Preliminära observationer tydde på att MyggA repellerar *I. ricinus.* Vi undersökte därför i standardiserade och samtidigt randomiserade fält- och laboratorieexperiment om MyggA har en repellerande effekt på nymfer - det epidemiologiskt viktigaste stadiet - av den vanliga fästingen *I. ricinus.*

MyggA applicerades på ett bomullsflanelltygstycke (1 m^2) som drogs 2,500 m över fästingrik markvegetation. Parallellt med detta drogs ett identiskt kontrolltygstycke utan MyggA. Totalt insamlades 520 nymfer varav 90,3 % fanns på tyget utan MyggA (repellens = 89,3 %). Dessutom applicerades på två personers byxor MyggA på de vänstra byxbenen. De högra användes som kontroller. Personerna vandrade 2,000 m i ett fästingrikt område. 70 nymfer insamlades från de MyggA-behandlade byxbenen och 145 nymfer från de obehandlade (repellens = 51,7 %). Resultaten av laboratorieförsök visade att MyggA såväl som två av dess komponenter, dvs. deet och de essentiella växtoljorna, båda hade höggradiga och signifikanta repellerande effekter på fästingnymfer. Den högre graden av repellens i laboratorie-försöken än i fältförsöken beror sannolikt på att man i de förra försöken sannolikt lättare kan upptäcka om ett ämne är repellerande, neutralt eller attraherande. På grund av en mängd interfererande faktorer är det troligen svårare att upptäcka om ett ämne är repellerande, neutralt eller attraherande med de testmetoder vi använde i fält. Däremot ger våra fältförsök sannolikt en bättre bild av ämnets verkliga effektivitet om det används som skydd mot fästingangrepp.

Som skydd mot fästingar kan deet, MyggA och liknande myggmedel lämpligast appliceras på byxbenen, som en barriär mot fästingar, som oftast kryper från låg markvegetationen upp på kläderna. Eftersom MyggA innehåller det relativt låggradigt giftiga ämnet deet, som delvis tas upp av fettvävnad, bör det användas i måttliga mängder om det stryks direkt på huden. Myggstift och liknande medel som innehåller deet bör ej användas på barns hud utan endast på barnens kläder.

Gråmyran som värmerelikt - falsifierad hypotes

I en ny artikel i Natur i Norr presenteras en intressant funderingar kring gråmyrans utbredning i Sverige som grundar sig på data i form av gamla och nyupptäckta lokaler och en vetenskapfilosofisk betraktelse av vad dessa betyder (Bergsten & Falck 2003). Gråmyran (*Formica cinerea*, Fig. 1) lever på öppna sandiga områden och har tidigare varit rödlistad (Ehnström m.fl. 1993) eftersom denna typ av lokaler växer igen med skog på många ställen i landet. Rödlistningen byggde delvis på att arten ansågs vara en värmetidsrelikt som endast fanns kvar på några av de största sandområdena norr om huvudutbredningen i Öland, Blekinge, Skåne och Halland. Ett av de sedan längst kända av dessa områden är Bonäsfältet i Mora och Bo Tjeder var den som först föreslog att gråmyran skulle vara en värmetidsrelikt på platsen, analogt med sandödla och såpört som också finns där (Tjeder 1953). Detta anammades av senare författare, även om förekomster på fem andra sandområden hittades (Lindström & Berglind 1995 och ref. däri). Dessa författare föreslog dock att reliktmönstret även kunde bero på lång kontinuerlig tillgång på öppen sand snarare än varmt lokalklimat i sig.

Johannes Bergsten och Johan Falcks intresse för denna fråga väcktes då de gjorde en inventering av myror på sandområden i Umeås närhet