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Original Communication

# Incorporation of insecticide-impregnated netting in Nzi traps as a labor-saving strategy for controlling tabanids

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## ABSTRACT

Tabanids (Family Tabanidae, Diptera) mechanically transmit important pathogenic organisms such as bovine leukemia virus (BLV), *lentivirus*, pathogen of equine infectious anemia, and *Trypanosoma evansi*, pathogen of Surra. Nzi traps were modified by incorporating permethrin-impregnated netting (Olyset net) instead of plain nylon organdy in the trap canopy as a labor-saving strategy. The Olyset net Nzi trap and the normal Nzi trap captured nearly the same numbers of *Tabanus nipponicus*, the main vector of BLV in northern Japan. In contrast to a few individuals that were knocked down in the normal Nzi trap, most flies that entered the trap were knocked down to the bottom of the Olyset net Nzi trap, eliminating the need for any collecting device, a major labor-saving feature. Altogether, the use of Olyset net Nzi trap is an effective new strategy against tabanids that transmit diseases such as bovine leukemia.

**KEYWORDS:** Tabanidae, *Tabanus nipponicus*, trap, Olyset net, bovine leukemia, Japan

## INTRODUCTION

Tabanids (Family Tabanidae, Diptera) mechanically transmit important pathogenic organisms such as bovine leukemia virus (BLV) [1, 2], *lentivirus*, pathogen of equine infectious anemia [3] and *Trypanosoma evansi*, pathogen of Surra [4]. Among these diseases, bovine leukemia is one of the most serious diseases in Japan [5]. Infection rates of this disease can be up to 100% at many farms in Hokkaido, Japan. The peculiar buzzing of some tabanid species also causes panic in pastured cattle and horses resulting in stress and injuries. For these reasons, tabanids are recognized as one of the most destructive insect pests of livestock during their active seasons.

Although insecticide treatment of livestock can be an effective measure against tabanid attack, efficacy is limited because of fly behavior [6]. Flies land on animals only when they feed, and contact time is short for insecticidal action [7]. Repellents only provide ephemeral relief. Breeding areas are too large for larvicidal treatment for environmental and economical reasons [8].

Under these circumstances, mass trapping is considered to be a promising measure to protect livestock against tabanids, with several efforts being taken to develop practical traps for control purposes [9, 10, 11, 12, 13, 14].

Insecticide-treated nets and fabrics have emerged as important personal protection tools against malaria since the 1980s, and insecticide-impregnated targets are also now routinely used in Africa for tsetse control [15, 16]. Permethrin is often the insecticide of choice for applications where human safety is a prime concern. Peterson *et al.* [17] reported that permethrin is the safest of the various pyrethroid insecticides used in long-lasting insecticide bed nets. Permethrin-impregnated fiber nets are effective against blood sucking mosquitoes [18, 19] and show some potential against tabanids [20]. Permethrin-impregnated insecticidal netting was recommended for malaria control by World Health Organization Pesticide Evaluation Scheme

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(WHOPES) in 2001 [21], with many alternative products also in use now. This type of netting is readily available in Japan and was therefore tested for efficacy in modified Nzi traps, which are effective for many kinds of biting flies [22]. The strategy was to use permethrin-impregnated fiber net (Olyset net) instead of the plain nylon organdy that is typically used in Japan as the netting for the canopy of the trap (Olyset net Nzi trap). This was done to demonstrate the feasibility of controlling tabanids with traps without the need for any collecting device.

## MATERIALS AND METHODS

### 1. Nettings

Olyset net (light blue) is made from more than 150 denier fiber and contains permethrin (2 Wt%, 1000 mg of active ingredient per square meter). It is produced by Sumitomo Chemical Co. in accordance with the World Health Organization specification WHO/IS/NI/331/2002. Nylon organdy or ‘tulle’ (a very fine white mesh, fluorescent) was obtained from the retail market.

Both Olyset net and nylon organdy transmit 86% visible light. Transmittance data of the visible range of both nettings were obtained using a digital light meter (Reed ST-1300) with peak sensitivity at 550 nm (as in human vision). This was done with a simple transmission set-up using a daylight fluorescent bulb (GE F15T8/D). Furthermore, Olyset net transmits 75% ultraviolet light and the nylon organdy transmits 54% ultraviolet light. Transmittance data in the ultraviolet light of both nettings were obtained using a digital ultraviolet light meter (Lutron UV-340, band pass 290-390 nm) with a BlackLight/Blue bulb as the light source.

### 2. Contact effect of the Olyset net

A 500 ml cylindrical plastic container, 12 cm in diameter and 8 cm height, was used for determining the contact effects of Olyset net against *T. nipponicus* Murdoch and Takahasi. Olyset net was used as the top cover of the container and nylon organdy as the bottom.

All flies used in the bioassays were captured just before the tests. For the constant contact to the net, the wings of the flies were cut at 3/4-th length from the apical end and 4 sets of 10 flies were released into separate containers. These containers were placed upside-down for 1, 2, 3 and 5 mins separately for the flies to come into contact with the Olyset net cover. *T. nipponicus* were observed to crawl on the netting for almost the entire period of exposure. At the end of each timed interval, the containers were placed upright and the top covers were replaced with nylon organdy. Flies were fed with 5% honey solution and knock-down numbers were recorded at 0.5, 1, 2 and 24 hrs after contact. A set of 10 flies was also released into a container with only nylon organdy as a control. Observations were repeated 9 times each at the room conditions (23-27 °C; 65-80% Relative humidity). Knock-down rate was adjusted according to the Schneider-Orelli’s formula [23]. Mortality in controls never exceeded 56%.

Data was analyzed by the use of the two-way analysis of variance method after the numbers of knock-down flies were calculated using arcsine conversion method. For studying the effects of contact time, data was reanalyzed using the Tukey’s method.

### 3. Trapping force

An Olyset net Nzi trap and a normal Nzi trap (Fig. 1) were set 20 m apart in a pasture at the experimental farm of Hokkaido University at Shizunai in Hokkaido (42°26’N, 142°28’E) during peak tabanid activity in 2006. Flies were collected by hand or with a hand-held vacuum after an hour, and tallied according to where they were found. In the descriptions below, the cage refers to the collecting cage at the top of the trap, the inside is the inside of the canopy

**Fig. 1.** Nzi trap.

and the bottom refers to the horizontal shelf and ground under the canopy. The flies collected in the cage seemed to be the ones that escaped from the trap and those at the bottom seemed to be influenced by permethrin. The number of flies was counted according to the different parts of the traps from which they were collected. The two traps were rotated after an hour and collections were repeated five times. The numbers of *T. nipponicus* caught by the traps were compared. Data was analyzed using the G-test [24].

### 4. Efficacy

The efficacy of this trapping strategy was tested over several seasons for tabanids of different species and sizes, namely small species such as *T. humilis* (Coquillett) (body length: 10-13 mm) and *T. iyoensis* (Shiraki) (body length: 9-12 mm), medium species such as *T. nipponicus* (body length: 12-17 mm) and large species such as *T. rufidens* (Bigot) (body length: 14-21 mm) and *T. trigonus* Coquillett (body length: 18-24 mm). Olyset net Nzi traps were set for one hour intervals as before and flies were tallied according to where they were caught with one minor change in the procedure as described below. After removing the top cage and collecting the bottom flies, the entire trap was placed into a plastic bag containing some ethyl acetate to get a full count of what remained 'inside'. Surveys of *T. nipponicus* and *T. trigonus* were conducted at the Shizunai experimental farm of Hokkaido University and repeated 45 times, 22 times during 13-15 August in 2007 and 23 times during 14-17 August in 2008. Collecting of *T. rufidens* and *T. humilis* were done at the Omyojin experimental farm of Iwate University at Shizukuishi in Iwate prefecture (39°40' N, 140°56'E) and repeated 32 times during 17-19 August in 2008. *T. iyoensis* was captured in 32 replicates at the International Camping Ground of Toga in Toyama prefecture (36°26'N, 137°02'E) on 31 July and 1 August in 2009.

Data was analyzed using the $\chi^2$-test in order to show the differences among the 5 species according to the different parts of the trap they were captured. After a significant difference was found, data among the two species was analyzed again using the G-test (total 10 combinations) subsequent to the weighted Bonferroni correction.

## RESULTS

### 1. Contact effect

Even control group flies showed knock down after the release and the rate of the knock down increased according to the time after the release. The knock-down number and rate of treated group varied but showed over 97.8% in every group and every observation time (Fig. 2). The adjusted knock-down rate was very high even for the group wherein the contact time was only 1 min (98.1-100.0%). High knock-down effect (97.5-100.0%) was observed even 24 hr after the contact in each treatment group, and significant differences were found only between the control groups ($df = 4$, $MS = 65.7$, $F = 627.4$, $p < 0.01$).

### 2. Trapping force

The Olyset net Nzi trap and the normal Nzi trap captured nearly the same total number of flies, although the numbers of flies captured at different parts of the trap were not same. With the Olyset net Nzi trap, 53.1% of *T. nipponicus* were at the bottom of the trap, versus 0.6% in the normal Nzi trap (Fig. 3) and a significant difference was found between the two materials ($df = 2$, $G = 142.3$, $p < 0.01$).

### 3. Efficacy

The relative numbers of medium and small flies in the collecting cage of the Olyset Nzi trap were higher than those of large flies. A total of 2,882 individuals of *T. iyoensis* (small) were captured and among them, 1,159 (40.2%) were at the bottom. A total of 2,255 individuals of *T. nipponicus* (middle sized) were captured and among them, 850 flies (37.7%) were at the bottom. In contrast to the results for small and medium species, only 9.0% were captured at the bottom of the trap for *T. trigonus*, a large species (Fig. 4). Significant differences were found among the five species ($\chi^2 = 243.4$, $p < 0.01$; $df = 8$, $G = 274.8$, $p < 0.01$) collected at different parts of the trap (Table 1).

The ratio of flies captured at the bottom of the trap showed an inverse proportion with the body size of the flies.

## DISCUSSION

Several authors have studied the effects of treating horses and cattle directly with permethrin to kill

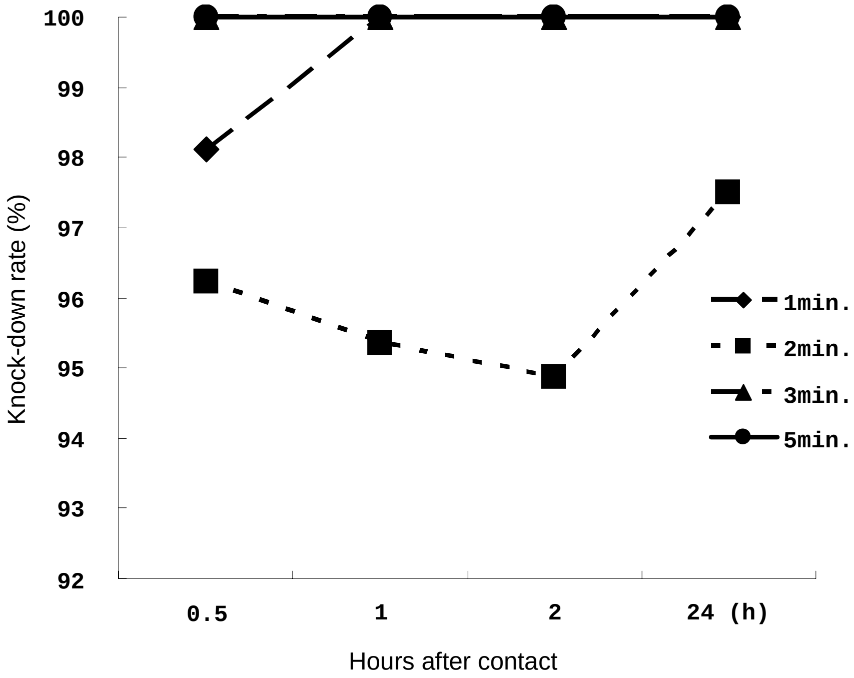


**Fig. 2.** Adjusted knock-down rate (%) after contact.

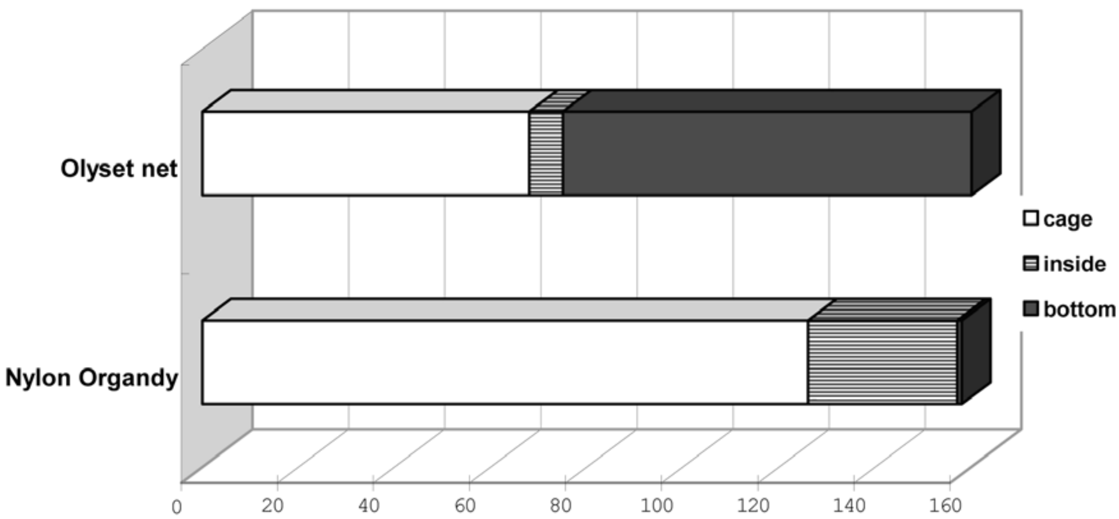


**Fig. 3.** Numbers of *Tabanus nipponicus* captured by the Nzi traps made with nylon organdy and Olyset net.

biting flies, including tabanids [20, 25, 26]. These studies obtained a high fly mortality with this insecticide. There are many studies on the effects of permethrin-impregnated fiber net against mosquitoes [18, 27], but this strategy has hardly been used for other biting flies [20]. They found the efficacies of pyrethroid-impregnated clothing or net against biting flies including tabanids, and also confirmed the potential for controlling them as shown by Schreck *et al.* [25, 26, 28]. The current study showed that knock-down effect of Olyset net against *T. nipponicus* is very high. Therefore, in conclusion, the efficacy of Olyset net examined in this study against tabanid species

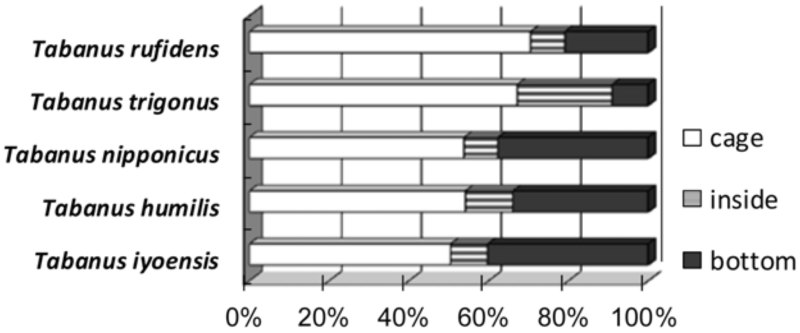


**Fig. 4.** Captured rate of flies at the three parts of the Nzi trap.

**Table 1.** Results of pairwise comparison using G-test weighted by Bonferroni correction.

| | | | | | |
|---|---|---|---|---|---|
| *T. rufidens* | | | | | |
| *T. trigonus* | 57.7* | | | | |
| *T. nipponicus* | 78.9* | 154.2* | | | |
| *T. humilis* | 46.2* | 94.4* | 9.7 | | |
| *T. iyoensis* | 111.9* | 168.2* | 5.9 | 12.7* | |
| | *T. rufidens* | *T. trigonus* | *T. nipponicus* | *T. humilis* | *T. iyoensis* |

Corrected significant G-value with df = 2 was 10.597 ($p < 0.005$).
*shows the significant combination.

is high suggesting many possible strategies for using different kinds of impregnated netting to control biting flies [29].

In the present study, mortality in controls showed over 50%, and one of the reasons for this seemed to be the stress of wing cutting.

Olyset net is designed for indoor use, and hence no information is available on its weathering properties outdoors. No insights were gained in this study as nets were only used for at most a few weeks of cumulative exposure outdoors and no knock-down tests were performed with used netting at various intervals. Results from Hogsette *et al.* [30], showed that some insecticide-treated cloths lost efficacy of the insecticide after outdoor weathering for a period of 90-days. Although the treating method of the net with the insecticide is very effective, the influence of outdoor weathering on the longevity of the insecticide of Olyset net need to be tested for the purpose of the universal use of this trap.

There was no repellency to *T. nipponicus* for the Olyset net as noted by Lang *et al.* [20]. Olyset net and nylon net have similar light transmittance properties, and these two types of nettings captured the same number of *T. nipponicus*. However, the parts of the trap where the flies were tallied were distinctly different. Many flies (79.7%) were in the cage of the normal Nzi trap, but over half (53.1%) of the flies were at the bottom in the Olyset net Nzi trap. It is concluded that this knock-down of the flies is the result of contact with permethrin, the insecticide impregnated into the fiber of the net. There is no need to ‘capture’ or ‘remove’ these flies, which is a very good labor-saving feature when using a trap merely for control. After knock-down, a few flies resuscitated; however, the resuscitation rate was so small to affect the efficacy of the trap.

In general, the tolerance to pesticide is directly proportional to the body size. As expected, the ratio of smaller flies captured at the bottom of the trap was higher than the large sized ones.

Olyset net was determined to have higher knock-down effect and higher trapping force against *T. nipponicus*, the main vector of bovine leukemia in Hokkaido, Japan. These are important characteristics of the material of this labor-saving tabanid trap.

## CONCLUSION

Finally, from the results and characteristics obtained and determined in this study, it is concluded that the introduction of Olyset net Nzi trap is an effective new strategy against diseases such as bovine leukemia transmitted by tabanid.

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## CONFLICT OF INTEREST STATEMENT

The author has no conflict of interest.

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