N O T E

Liquid Chromatographic Detection of Permethrin from Filter Paper Wipes of White-tailed Deer[1]

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White-tailed deer, *Odocoileus virginianus* (Zimmermann), are keystone hosts for the adult stage of lone star ticks, *Amblyomma americanum* (L.), (Patrick and Hair 1978, J. Parasitol. 64:1100 - 1106; Bloemer et al. 1986. J. Econ. Entomol. 79: 679 - 683) and blacklegged ticks, *Ixodes scapularis* Say, (Barbour and Fish 1993, Science 260: 1610 - 1616), both of which transmit a variety of agents that cause disease in humans, including Lyme disease, human ehrlichioses, and human babesiosis in the eastern U.S. (Lockhart et al. 1996, J. Med. Entomol. 33: 153 - 158; Varela et al. 2004, J. Clin. Microbiol. 42: 1163 - 1169). White-tailed deer are also marginal hosts of the cattle tick, *Rhipicephalus (Boophilus) annulatus* (Say), and the southern cattle tick, *R. (B.) microplus* (Canestrini), particularly in the absence of cattle (Cooksey et al. 1989, J. Med. Entomol. 26: 155 - 158). Increasing populations of white-tailed deer along the Texas-Mexico border are compromising efforts by federal agencies to prevent the reestablishment of these vectors of bovine babesiosis from Mexico, where these disease vectors are endemic (George 1990, J. Agric. Entomol. 7: 119 - 125, Pound et al. 2010, J. Econ. Entomol. 103: 211 - 218).

In an effort to manage the disease risks posed by the increasing numbers of white-tailed deer and subsequent tick populations in the northeastern U.S. and reinfestations of cattle fever ticks (southern cattle ticks and cattle ticks) in South Texas, the U.S. Department of Agriculture (USDA) - Agricultural Research Service (ARS) developed two passive treatment devices designed to apply permethrin acaricide onto the ears

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and necks of white-tailed deer as they feed on whole kernel corn: the patented '4-poster' Deer Treatment Bait Station and a similar device called a '2-Poster' Deer Treatment Feeder Adapter that attaches to gravity-fed cylindrical feed-chute type deer feeders commonly used by South Texas ranchers (Pound et al. 2000, J. Med. Entomol. 37: 588 - 594). Utilization of these treatment devices by the USDA – Animal and Plant Health Inspection Service (APHIS) – Veterinary Services (VS) – Cattle Fever Tick Eradication Program (CFTEP) has increased in recent years as outbreaks of cattle fever ticks outside the permanent quarantine zone have increased.

Usage of the treatment devices by white-tailed deer is assessed by noting the amounts of corn consumed from the devices on a weekly basis and photographing deer with motion-sensitive cameras where possible, although the expenses of camera equipment and personnel time to review captured images limits the practicality of its use on a widespread basis. To evaluate the efficacy of the treatment devices, white-tailed deer within treatment areas are routinely captured and carefully inspected for the presence of ticks. The necessity of determining the proportion of white-tailed deer in specific areas that are utilizing the treatment devices has led to the development of a simple, efficient, and cost-effective small-scale HPLC procedure for routine monitoring of the presence of permethrin acaricide on deer hair coats.

Sampling for permethrin on deer. After net-gun capture from a helicopter, restrained white-tailed deer were sampled for the presence of permethrin using a 12.5 cm Whatman® No. 1 filter paper disc (Whatman International Ltd., Maidstone, England) as a wipe. Wearing gloves, the person taking the sample vigorously rubbed the filter paper disc on both sides of the animal's head, the front and back of the ears, and both sides of the neck, being sure to rub the pelage both with and against the grain of the hair coat. Once complete, the filter paper wipe was wrapped in aluminum foil and labeled for storage under refrigeration until analysis to prevent cross-contamination between multiple samples. Gloves were changed before sampling each deer.

Analysis of permethrin on filter paper wipes. Wearing protective gloves, each filter paper deer wipe sample was removed from the foil wrap, folded in half, folded a second time along the length, and folded a third time along the length to yield a thick strip of 1/8 the original width. The strip was rolled into a spiral and then placed flat on the bottom of a 20ml glass scintillation vial. Next, 10.0 ml of HPLC grade acetonitrile was added to each sample vial. Vials were then tightly sealed and shaken gently several times. The filter paper was extracted overnight (~24 h), with samples being intermittently shaken 2 - 3 times during this period. Samples (~1 ml) from each vial were removed and filtered through Phenex™ 0.45 µm nylon membrane syringe filters (Phenomenex, Torrance, CA).

The filtered extract was analyzed by HPLC analysis using a Luna 5µm C18(2) 100Å column (250 mm × 4.60 mm; Phenomenex) with a 1100 series isocratic pump, autosampler, and UV-Vis variable wavelength detector (Agilent Technologies Deutschland GmbH, Germany). Degassed acetonitrile/water (80:20) (Burdick & Jackson, Muskegon, MI) was used as the mobile phase with a flow rate of 1 ml/min, column temperature of 37°C and detection at 237 nm. The runtime was 30 min. Permethrin standards of 2, 4, 6, 8, and 10 ppm (96.6%, PESTANAL®, Riedel-de Haën; Milwaukee, WI) were analyzed as a control. Average cost for supplies to determine the presence of permerthrin per filter paper wipe is ~ $10.00.

Increased populations of both white-tailed deer and ticks in the northeastern U.S. and South Texas prompted the need for a way to monitor the use of passive permethrin-based topical application treatment devices by white-tailed deer. Traditional

methods of analysis often require large sample sizes or large volumes of extraction solvent (Williams 1976, Pestic. Sci. 7: 336 - 338; Oehler 1979, J. Assoc. Off. Anal. Chem. 62: 1309 - 1311; Carroll et al. 1981, J. Environ. Qual. 10: 497 - 500; Reichel et al. 1981, J. Assoc. Off. Anal. Chem. 64: 1196 - 1200; Braun and Stanek 1982, J. Assoc. Off. Anal. Chem. 65: 685 - 689; Marie et al. 1982, J. Agric. Food Chem. 30: 558 - 562). Described herein is a simple, small-scale, analytical procedure for assessing permethrin using an approach suitable for routine analysis of large numbers of white-tailed deer samples. Quantification of the proportion of the white-tailed deer population utilizing treatment devices is critical in determining the efficiency of treatment of these types of devices, as well as for making decisions about the numbers of devices to deploy in the field. This small-scale methodology could also be useful in efficacy studies, such as comparing deer use of a newly-designed treatment device with the original or in monitoring the persistence of an insecticide/acaricide on the hair coats of target animals. An added benefit of this type of sampling is that the pelt of the animal is not trimmed, scraped, soaked, or altered in any way by wipe sampling, thus affording no points of entry for infection and no blemishes on the hair coat that might be of concern for producers who are ranching deer for profit. In addition, the growing demand for pesticide residue monitoring in crops, livestock, and wildlife samples makes this type of low cost, efficient sampling method applicable for use by a wide range of regulatory agencies that may have a need to monitor pesticide residues on animals.

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