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Abstract: An overview of ARS research in the field of veterinary pests is presented. Results of research from the past three years on ticks, fire ants, nuisance flies, mosquitoes, sand flies and black flies, among others, are included. Where applicable, significance of research is discussed.
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1 INTRODUCTION

Research on arthropod pests of veterinary importance has always been a high priority with ARS and this still holds true today. Surprisingly, the project which generates the most inquiries from the general public is the screwworm, *Cochliomyia hominivorax* (Coquerel), eradication program. Although this program has been largely invisible within US borders since 1966, the public is still aware that the program existed, and that it was a USDA program. The screwworm eradication program is possibly the most important pest eradication program to be attempted, saving countless millions of dollars and greatly improving the heath of livestock and humans in the USA and Latin America.

Just like the screwworms of years gone by, significant problems of veterinary importance still face ARS research personnel today. Despite the fact that many scientists are working on pests of veterinary importance in many programs, the scope of this paper will permit only an overview of some of the larger projects on the major pest groups. Red imported fire ants, *Solenopsis invicta* Buren, have been a major problem in the southeastern USA for a number of years and their range continues to expand despite the best efforts of ARS scientists and their collaborators at other institutions. However, new biological control agents have been found in South America and some of these show promise in upcoming area-wide control projects. Ticks on livestock and wildlife, such as deer, continue to pose zoonotic health risks in many parts of the USA. Because of tick resistance to currently registered pesticides and the development of few, if any, replacement pesticides, ARS scientists must continue to look for new approaches to tick management in rural and urban settings. With the recent entry of West Nile Fever into the USA, the management of mosquito populations in urban areas becomes an even more important challenge. Protection systems must be satisfactory for humans as well as animals, particularly horses, and personal and area-wide protection must be achieved by environmentally friendly means. Thus the aim is the discovery of new attractants and repellents, and the development of new and more effective traps and trapping systems. These are the projects that are most timely, and these will discussed most thoroughly herein.

2 LABORATORY LOCATIONS AND MAJOR RESEARCH PROJECTS

Research on pests of veterinary importance is performed at a number of ARS locations, but at four laboratories it is the major thrust. These locations are the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, Florida, the Knipling Bushland Livestock Insects Research Laboratory (KBLIRL) in Kerrville, Texas, the Midwest Livestock Insects Research Unit (MLIRU) in Lincoln, Nebraska, and the Arthropod-borne Animal Diseases Research Laboratory (AADRL) in Laramie, Wyoming. An overview of these locations and a brief mention of other locations will present a picture of ARS research in the field.

2.1 CMAVE, Gainesville, Florida

CMAVE was created in May of 1996 by merging the Insect Attractants, Behavior, and Basic Biology

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Research Laboratory (IABBBRL) with the Medical and Veterinary Entomology Research Laboratory (MAVERL—formerly the Insects Affecting Man and Animals Research Laboratory). Scientists in the former MAVERL still work primarily on pests of medical, veterinary and household significance, and sometimes it is difficult to separate pests into individual categories. Current information on the scientists and their work can be found at the CMAVE web page (http://cmave.usda.ufl.edu/).

The Imported Fire Ant and Household Insects Research Unit, (http://cmave.usda.ufl.edu/~ifahi/) works primarily with the red imported fire ant, other pest ant species and termites. Although the various pest ant species and termites warrant the research, the red imported fire ant is the largest part of the effort simply because of the significance of the pest. Fire ants have recently spread into Maryland, Southern California, New Mexico and Arizona. Outside the USA, fire ants invaded Australia in 2000. A major research effort has been the development of baits for environmentally safe management of fire ant populations.[1] Results include the development of commonly used products such as hydramethylnon (Amdro), 1-(8-methoxy-4,8-dimethylnonyl)-4-(1-methylethyl)benzene (ProDrone) and fenoxycarb (Logic).[1] Research has also been performed to better define how ants use pheromones to facilitate interactive communication within and between colonies.[2–4] Recognition pheromones could be used to enhance bait acceptance or altered to cause deleterious effects within colonies.

One of the more interesting and unusual projects still in progress at CMAVE started with the discovery and description of phorid flies in the genus *Pseudacteon*.[5,6] These very small flies, while in flight, parasitize fire ant workers by injecting their eggs into the body of the workers. Four days later the phorid larva has migrated to the head of the ant where its chemical activity eventually causes decapitation of the ant. The phorid larva then consumes the contents of the head, and pupates within, using the head as a pupal case. Adults emerge in 2–6 weeks. Injection of eggs occurs so rapidly that the exact place where eggs are injected is unknown. However the coxal areas seem to be the most likely location.[7] After a phorid fly injects her egg into an ant, the affected ant strikes a characteristic stilted pose and freezes for several seconds to a minute as if stunned by the event.[7] When foraging workers realize that flies are nearby, they seek shelter and remain immobile. Ants under attack sometimes assume a c-shape defensive posture. Thus even the mere presence of the flies can interrupt the foraging of hundreds of fire ants for long periods. This not only adversely affects colony activity, but gives competing ant species an opportunity to forage unopposed.[6] An illustrated key to 18 South American species of *Pseudacteon* decapitating flies that attack the *Solenopsis saevissima* complex has recently been published in English and Portuguese.[8]

Pseudacteon tricuspis Borgmeier has been released in Florida and several other states and is permanently established at eight to ten sites. Populations are beginning to expand rapidly out from several release sites. As of fall of 1999, *P tricuspis* populations occupied more than 50 square miles of area around Gainesville, Florida (http://cmave.usda.ufl.edu/~ifahi/). Because of the successful field trials with *P tricuspis*, this fly is one of two *Pseudacteon* species to be incorporated in the area-wide fire ant control project which began in the May of 2001.[9]

Some very exciting and timely work at CMAVE has become even more important because of the invasion of West Nile Fever into the Western Hemisphere. Although some may consider West Nile Fever to be a medical entomology problem, it is actually a zoonotic problem because it involves a transfer of pathogens from birds to humans and horses via mosquitoes. Scientists from the Mosquito and Fly Research Unit (http://cmave.usda.ufl.edu/researchunits/mosqflyindex.html) have been associated with the problem since late 2001, using state-of-the-art traps to survey mosquito populations in the North-eastern states where West Nile Fever was first confirmed. Initial studies are currently in progress so publications have not yet appeared in the journals, but look for periodic updates on the CMAVE web page. Traps used in the surveys are propane-powered with counter flow design.[10] These traps are highly attractive to mosquitoes because they use propane to produce their own heat and carbon dioxide. Similar traps are also being used in studies at the University of Florida Horse Teaching Unit where several horses have already died from West Nile Fever.

In conjunction with the development of improved mosquito trap design, scientists from the Mosquito and Fly Research Unit have also tried to determine what chemicals emanating from the human skin are attractive to mosquitoes. Skin volatiles were collected by two different methods and analyzed.[11,12] Results have enabled the development of chemical preparations that are more attractive on human skin than the naturally produced volatiles. Results have also enabled the development of chemical preparations that nullify the attractiveness of human skin volatiles when applied to human skin (http://cmave.usda.ufl.edu/researchunits/bernier.html). Essential oils have been tested as repellents for mosquitoes. Several essential oils, such as clove, peppermint and thyme oils, show some potential for further development, but user acceptance of associated oil fragrance may be a limiting factor in applied use.[13] Additional papers are in preparation.

Also associated with biological control of mosquitoes, scientists at CMAVE have found a new baculovirus from the mosquitoes *Culex nigripalpus* Theobald and *C quinquefasciatus* Say.[14] An exciting aspect of this project is that the scientists found the mechanisms for activating and deactivating the virus, and these mechanisms are related to the concentrations of certain cations in the water containing the mosquitoes and the virus. Percentage infection was positively correlated with the concentration of magnesium ions in solution. Other activators included ions of barium, cobalt, nickel and strontium. Increased concentrations of calcium ions in the presence of magnesium ions resulted in a decrease in infection. Other deactivators included ions of copper, iron and zinc.[14] With this information there is a tremendous possibility for development of this virus for applied mosquito control after additional testing. *Culex nigripalpus* and *C quinquefasciatus* are both known to transmit West Nile Fever. The baculovirus has been found to be a very unusual member of the family Baculoviridae[15] and its complete genomic sequence has been described.[16]

In another area of insect attraction, CMAVE scientists are investigating the chemical cues that attract the blood-feeding stable fly, *Stomoxys calcitrans* (L). Although stable fly attraction has been studied in the past, the number of chemicals shown to be attractants or repellents is few, and trapping systems currently used in the USA are based largely on visual components. When stable flies were exposed to chemicals known to be attractive in a triple cage olfactometer, differences in attraction were found between air-flow rates, adult age and time of day,[17] but results were more interesting when stable flies were exposed to their own feces and fecal extracts.[18] Feces were collected by suspending small ($6 \times 6\ cm^2$) squares of kitchen sponge in stable-fly colony cages. Stable flies resting on the sponges made fecal deposits which were exposed to other stable fly adults in the olfactometer. Compared with the other putitatively 'known' chemical attractants, the feces and feces extracts were highly attractive and showed promise for increasing trap efficiency in the field. Additional studies are needed to identify the attractant materials in the feces.

Identification of cuticular hydrocarbons has been done for many years at CMAVE, originally in search of attractive sex pheromones that could be incorporated in trapping systems to increase trap efficacy. Recently, cuticular hydrocarbons have been used as chemical taxonomic systems to help identify differences or similarities in insects that cannot be defined visually or behaviorally. Although some work has been done with tabanids and mosquitoes,[19,20] additional work has been done with parasitic wasps that play a part in the natural control of nuisance flies on livestock facilities.[21,22] Some of the wasp species appear visually, to be closely related but no definite relationship could be established without the use of cuticular hydrocarbons.

Filth flies are becoming increasingly important as problems with two major fly pests have become more acute. Stable flies have become a serious problem on the great plains, but the situation and related projects will be discussed under the section devoted to the Mid-west Livestock Insects Research Unit (MLIRU) in Lincoln, Nebraska. However the second nuisance fly, the house fly, *Musca domestica* L, has become a serious problem in the urban/rural interface. Major discount, supermarket and department store chains claim that house flies are their major pest insect inside and outside of stores. In fact, a recent fly outbreak in a rural community surrounded by poultry farms involved the house fly predator, *Hydrotaea aenescens* (Wiedemann), which had been shown previously to disperse from one farm to another, but not to houses or commercial facilities.[23] A major deficiency in developing management programs for these flies is the lack of information about fly behavior in urban situations. As a result, research is underway to improve attractants and traps and to adapt agricultural systems to fit urban needs. A new trap being evaluated and improved for both rural and urban systems is a one that has sound, visual and heat components. Most sound-related traps on the market are of questionable value, but in this case the sound definitely improves trap efficiency. A paper is in preparation and updates will appear on the CMAVE web page. Because house flies are still considered a serious pest in large livestock and poultry facilities, work has continued at CMAVE by various scientists to solve problems caused by house flies and other pertinent pests, particularly in the areas of trapping,[24] biological control,[25,26] behavior[27] and mechanical control.[28]

2.2 KBLIRL, Kerrville, Texas

KBLIRL was the home of some of the original work on screwworm eradication, but in recent years the focus has been on horn flies, *Haematobia irritans* (L), and cattle fever ticks, *Boophilus microplus* (Canestrini), among others. In the past 5 years, tick research has become predominant with the two main applied projects being the fight to keep *B microplus* south of the Rio Grande, and the development of the 4-poster passive topical treatment device to manage ticks on cattle and wildlife, primarily the white-tailed deer. Information on these and other fascinating tick-related projects can be found on the KBLIRL web page (http://www.csrl.ars.usda.gov/kbuslirl/).

The Tick Research Unit provides technological support to the APHIS Veterinary Services in their continuing efforts to protect the US livestock industry from debilitating tick species found primarily on cattle. Unit scientists are also responsible for development of novel technology for management of ticks on white-tailed deer and thus to protect humans and livestock against ticks and tick-borne diseases. Besides the main laboratory in Kerrville, the Cattle Fever Tick Research Laboratory facility is located in Mission, Texas.

Past research has shown that white-tailed deer are reservoir animals that maintain tick populations in nature. In Texas, these ticks may be *B microplus*, the species that transmits Texas cattle fever, while in other parts of the country, notably the north-eastern part, these ticks may be the blacklegged tick, *Ixodes scapularis* Say, the species that transmits Lyme disease. Because deer populations have become so large, and because of their freedom of movement, it became evident that tick control on cattle and management of zoonotic diseases like Lyme disease would be impossible if ticks could not be managed on white-tailed deer populations.

The project to control ticks on white-tailed deer is based largely on an ingenious new device called the 4-poster passive topical treatment device (Fig 1).[29] This device attracts deer with an untreated feed bait (whole kernel corn). While feeding on the corn, deer self-apply acaricide to their head, ears, and neck as they contact pesticide-treated rollers located at the corners of the feeding areas. The device is acceptable to all deer whether or not they have antlers (Fig 2). The acaricide used in the 4-poster is a $20\,\mathrm{g\,liter^{-1}}$ pour-on formulation of amitraz (Point-Guard, Hoechst Roussel Vet, Warren, NJ), which has been shown to be relatively harmless to beneficial insects and wildlife. In early field studies, the efficacy of the 4-poster was determined by confining nine white-tailed deer in a 22.3-ha tick-infested deer-fenced treatment pasture and allowing them to feed at a treated 4-poster unit. In a similar control pasture, nine white-tailed deer were allowed to feed from an untreated 4-poster unit. After the 5-month treatment period, ticks in the treatment pasture had been reduced by 91.8%.[29] Population development of the ticks had also been retarded as indicated by the lack of engorgement in the attached ticks. Thus nymphs had not fed enough to molt to the adult stage and females had not fed enough to complete oviposition. Control may therefore actually be greater that the 91.8% given above. Additional field studies indicated that the 4-poster gave results similar to those achieved with amitraz-treated corn, and that either or both could be used in area-wide control programs.[30]

The 4-poster unit is the basis of a project in five North-eastern states to control ticks on the white-tailed deer herds and 20–30 4-posters will be used on every 50–55 acres of treatment area. This project is a cooperative effort between scientists at KBLIRL, USDA scientists at the Henry A Wallace

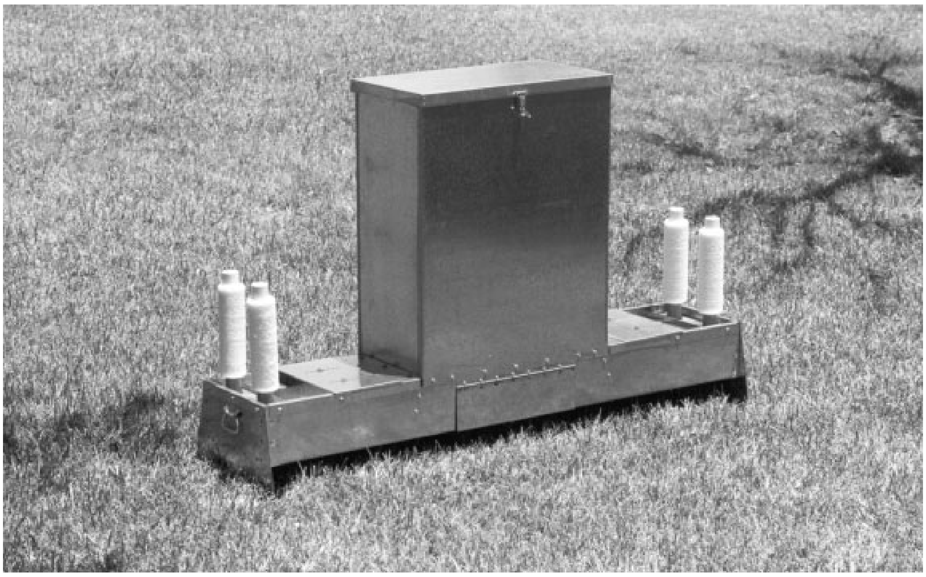

Figure 1. The '4-poster' passive topical treatment device (Photo JM Pound).

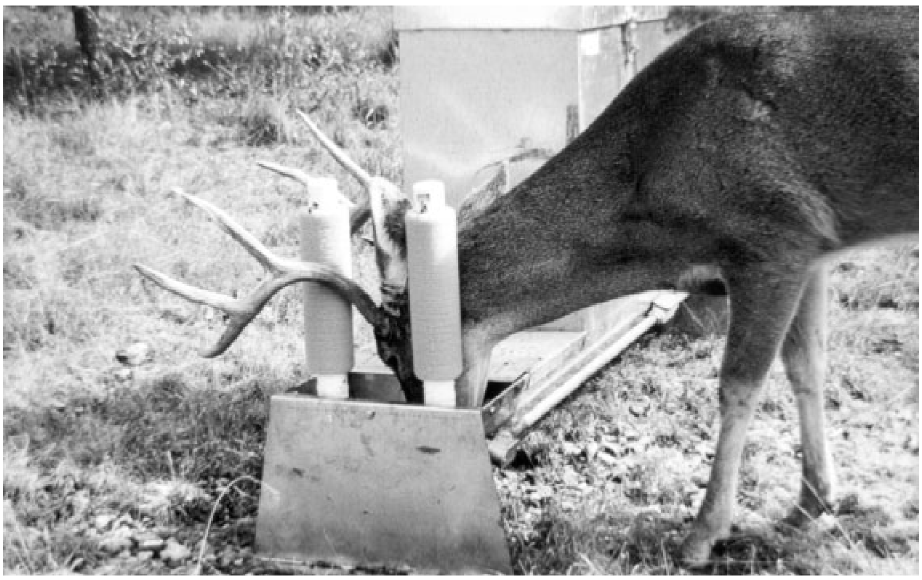

Figure 2. Buck white-tailed deer with antlers feeding at a '4-poster' passive topical treatment device (Photo JM Pound).

Beltsville Agricultural Research Center (HAWBARC), Beltsville, Maryland, and other scientists in Connecticut, New York, Rhode Island and New Jersey. A project goal is a 90% reduction of blacklegged tick nymphs at each of the 1280-acre treatment sites after a 5-year period. Reduction of nymphs is critical because it is this stage that most commonly transmits Lyme disease to humans. In the first 2 years of the project, nymphs of *I scapularis* had been reduced by 59–71% in Maryland alone. Updates on this project can be found on various ARS web pages because the project is still in progress and no final publication is yet available (http://www.nps.ars.usda.gov/programs/programs.htm? NPNUMBER = 104).

At the KBLIRL unit in Mission, Texas, there is a constant effort to prevent *B microplus* ticks from entering the USA from Mexico on livestock, primarily cattle. Cattle are inspected and if necessary treated in Mexico to eliminate tick populations. Personnel at the Mission unit monitor pesticide use, look for alternative pesticides that could be used for treating cattle and check the resistance levels in tick populations of pesticides currently in use. This is a constant on-going project and updates can be found on the KBLIRL web page (http://www.csrl.ars.usda.gov/kbuslirl/). Meanwhile scientists from both KBLIRL facilities have begun to characterize various aspects of permethrin resistance and coumaphos susceptibility found in a strain of *B microplus* ticks from Mexico.[31] Widespread of resistance in Mexican tick strains, particularly to coumaphos, is of great concern because coumaphos is the only acaricide available to treat tick-infested cattle at the Texas–Mexican border. Pyrethroid resistance seems to be a country-wide problem in Mexico,[31] but it is still important to understand the mechanisms of pyrethroid resistance. In *B microplus* ticks it appears that pyrethroid resistance is related to novel mutations in the sodium-channel gene or metabolic detoxification.[32] Undescribed mutations existing in other regions of the sodium channel may also be involved in resistance development, and studies are underway to further investigate these possible sites.

Although emphasis at KBLIRL has been on tick research, research on pesticide resistance has continued with the horn fly. The horn fly is a major cattle pest in many states, and in some regions of the country horn fly resistance to pyrethroids has almost completely removed this class of pesticide as an alternative to organophosphates. To preserve the currently used organophosphate compounds, resistance mechanisms are being further investigated. Because esterase activity has been widely studied in relation to organophosphorus resistance development, esterase profiles were studied in field populations of horn flies in Texas.[33] Diazinon is one of the main pesticides used for horn fly management and a number of specific esterases appeared to contribute to the development of diazinon resistance. Two esterase cDNAs were cloned from a diazinon-resistant field population of horn flies that showed qualitative and quantitative differences in esterases when compared with susceptible flies. One esterase transcript was found at levels five times higher in diazinon-resistant horn flies than in susceptible horn flies.[34] Differences in gene expression in resistant and colony-reared flies were at first thought to be related to diazinon resistance, but this was later disproved.[35]

2.3 MLIRU, Lincoln, Nebraska

MLIRU has traditionally been involved with stable fly and house fly research, particularly as it relates to population management in feedlot situations. In the last decade, MLIRU has also been doing screwworm research, primarily to support the APHIS screwworm eradication program in Latin America. Emphasis seems to be shifting more towards screwworm research, but stable fly research is still an integral part of the program.

There is a major emphasis in the screwworm program on the development of a male-only primary screwworm strain to facilitate sexing procedures at the mass-rearing facility currently located in Tuxtla Gutierrez, Mexico. Other projects include the study of screwworm cytogenetics and the use of molecular techniques to determine genetic variation in the primary screwworm. MLIRU has a number of strains of primary screwworm in their quarantine facility and research is underway to develop procedures for preservation of screwworm germplasm. In preparation for future cryopreservation studies, MLIRU scientists have successfully permeabilized screwworm embryos.[36] Survival in this preliminary study was about 60% for first-instar larvae, but lower in pupae and adults. Additional work is needed to perfect the process, and the development of a special diet and rearing procedures will help increase survival after treatment. There is an effort to enhance screwworm rearing procedures and work has recently been done to evaluate acquisition of fatty acids from phospholipids present in the larval diet.[37] In addition, MLIRU scientists are developing new strains of screwworms and continuing to provide support to the USDA-APHIS / Joint Commission's Screwworm Eradication Program. Most of the other projects are in progress and papers have not yet been published.

As mentioned above, the stable fly has become a problem of new and unexpected proportions in the Midwestern states. Stable flies have been traditionally associated with Midwestern feedlots, but they were not a pest of range cattle simply because suitable habitat for immature development was not available in rangeland situations. However, in the past 20 years there has been an increase in producer complaints and a definite increase in stable fly populations on animals. The common complaint in Nebraska and Kansas is that after a rain in early June there are suddenly large populations of stable flies on the animals. Because there is no synchronized eclosion in higher Diptera and because the appearance of the flies was usually associated with rain, a stable fly dispersal phenomenon similar to that documented in Florida has been suspected. In 1996, a team of scientists from CMAVE and several area universities investigated the problem in western Kansas and results suggested that flies trapped during the study could have dispersed long distances to reach our study site.[38] In 1997–98, a band of traps was placed from the Nebraska border to the Oklahoma border in eastern and central Kansas and stable flies were captured weekly or twice weekly during the months of April and May. An increase in stable flies was noted after the passage of cold fronts and whenever the winds were southerly. Results indicate that spring populations of stable flies in Kansas could be originating from as far south as Louisiana, Texas, or New Mexico (JA, Hogsette, unpublished data). A team of MLIRU and university scientists has evaluated the economic impact of stable flies on range cattle and found a significant reduction in weight gains resulting from fly feeding and fly worry.[39] This is more than enough reason to justify the critical need for stable fly management programs for range cattle.

2.4 AADRL, Laramie, Wyoming

The mission of the AADRL is to conduct basic and applied studies on the arthropod-transmitted viral diseases of domestic animals. Scientists from AADRL and HAWBARC, Beltsville, Maryland, studying various aspects of Lyme disease investigated the patterns of attachment by adult *I scapularis* on white-tailed deer and horses. On deer, the ticks were attached mainly on the outside of the ears, the head, neck and brisket. On horses, ticks were mainly on the chest, the axillae of the front and rear legs, or under the jawbone. These studies show where these animals should be inspected when looking for ticks and also indicate where tick repellents or toxicants should be applied.[40] AADRL scientists also made an evaluation of a 1995 vesicular stomatitis outbreak in the western states to better understand the role of arthropod vectors in relation to risk factors that promote high rates of transmission, such as livestock in western river valleys, insect blood feeding, and transport of animals from one area to another for recreational purposes.[41] An excellent review about the relationship between vesicular stomatitis, horses and fly vectors was also prepared.[42]

Culicoides research has been a major thrust of the AADRL for a number of years. In a major taxonomic breakthrough, electrophoretic analysis showed the *Culicoides variipennis* complex to consist of three separate and distinct species. The three species are sympatric over much of their ranges and can now be distinguished visually through taxonomic keys. Results may benefit US export regionalization programs in relation to movement of livestock and animal germplasm from bluetongue-free regions of the USA to bluetongue-free countries. Animals in the upper mid-west and north-eastern U.S. are relatively bluetongue-free because the non-vector, *C variipennis* (Coquillett), is prevalent in this area. Conversely, livestock in areas populated by another member of the *C variipennis* complex, *C sonorensis* Wirth & Jones, have high to moderate bluetongue seroprevalence rates because *C sonorensis* is a bluetongue vector. Bluetongue virus cannot enter into the *C variipennis* gene pool via *C sonorensis* because the two species do not interbreed.[43] An additional study indicates that habitats inhabited by immature stages of the *C variipennis* complex may be regulated by chemical factors. For example, larvae of *C variipennis* and *C sonorensis* can tolerate similar levels of nitrates and phosphates, which may explain why they are found together in sediments containing livestock wastes. Boron levels seem to separate the three species, with *C variipennis* tolerating low levels, *C sonorensis* tolerating moderate levels, and *C occidentalis* Wirth & Jones tolerating extremely high levels. A better understanding of the chemical factors that separate the members of the *C variipennis* complex will give further insight into the epidemiology of bluetongue disease.[44]

In one final study, AADRL scientists used the repellent action of a permethrin spray, wipe-ons and pour-ons to protect ponies and cattle from the bites of mosquitoes and black flies. Feeding by *Aedes dorsalis* Meigen and *A melanimon* Dyar was reduced 79–88% at 4 days post-treatment on heifers treated with pour-on concentrate and a whole-body spray. Feeding by *Simulium bivittatum* Malloch and *S griseum* Coquillett was reduced by 96% to >99% 4 days post-treatment. On ponies treated with a permethrin fly wipe, feeding by *S bivittatum* was reduced by 98 and 87% at 1 and 7 days post-treatment, respectively.[45] Permethrin repellency might be used for protection of horses against the feeding of West Nile vectors, but more studies are needed to evaluate mosquito feeding behavior in the presence of the selected repellents.

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