Subtropical Agriculture and Environments 74:13-20.2023

Mechanical devices for census and detection of off-host larval ticks (Acari: Ixodidae) with emphasis on the Cattle Fever Tick.

Donald B. Thomas[1], Holly D. Gaff[2], Brenda Leal-Galvan[3]

1United States Department of Agriculture, Agricultural Research Service, Cattle Fever Tick Research Unit. 22675 North Moore Field Road, Edinburg TX 78541
2Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529
3Department of Entomology, Texas A&M University, College Station TX 77840

*Corresponding author email: donald.thomas@usda.gov

ABSTRACT

Standard off-host tick census techniques such as drags and CO_2 traps are generally inefficient for larval stages. Also, such techniques expose the investigator to environmental hazards and tick bites. Mechanical devices including vacuums, drones and robotics are alternatives to the traditional techniques. The mechanical devices were tested under field conditions for proof of concept and in side-by-side comparison to the standard techniques in south Texas habitats. The tick-vac collected more ticks than the legging technique, but with lower frequency of positive detections. Thus, the tick-vac has some utility for smaller sampling areas, such as weedy corrals suspected of harboring ticks. Different configurations of Tick-Bots, robotic rovers with flannel flags attached, depended on intensive operator control to avoid obstacles, limiting operation to the line of sight. The use of the Tick-Bot to disrupt clusters of questing larvae was not successful under the conditions of the trial. Surveillance for ticks in the free-living stages remains a challenge.

Additional index words: Tick-Bot, Tick-Vac, *Boophilus*, Drones, south Texas

Hard ticks, (Acari: Ixodidae), are adapted to one of two host seeking strategies. The vast majority, over 98%, are three-host ticks, infesting three different successive hosts during their development. The remaining small minority are one-host ticks that complete their development on a single host. The ecological significance is that the adult, nymph and larva of three-host ticks quest in each life stage for hosts, whereas the one-host tick quests only during the larval stadium. Although in the minority the one-host ticks, such as the cattle fever ticks, genus *Boophilus*, and the winter tick, *Dermacentor albipictus* (Packard) are among the most destructive ticks. The winter tick causes high morbidity to North American wild ungulates (Jones et al. 2019), while the cattle fever ticks are vectors of diseases causing major economic loss to livestock (Suarez & Noh 2011). Because of their economic impact, tick infestations often result in lengthy quarantines of premises. An effective method for detection of the off-host larval stages is desperately needed. Unfortunately, the standard methods, drags and CO_2 traps used for surveying off-host tick populations, are mostly effective against adults rather than the larval stages.

The most widely applied method for tick surveys is the drag technique (Philip 1937). The typical configuration consists of a one square meter cloth supported by a dowel at one end, often weighted at the other, which is pulled by a rope behind the investigator for a predetermined distance through pastures or grasslands. Questing (actively host-seeking) ticks will latch on to the cloth as they would to the fur, feathers or hide of a potential host. For sites where grass is high, or the brush is too dense, a common variation is a "flag" wherein the cloth is attached like a flag to a hand-held pole (Carroll & Schmidtmann 1992).

An alternative method for brushy or thorny vegetation is the use of "leggings" (Shulze et al. 1997) wherein the investigator dons cloth over-pants and walks through the vegetation suspected of harboring ticks. Blakeslee and Bruce (1948) and Wilkinson (1961) used white flannel leggings to sample larval cattle fever ticks and found this method superior to the drag technique in non-improved pastures. There was a *proviso* however in that 60-80% of the larvae dropped off the leggings almost immediately after attaching. Thus, while counts were higher on leggings than on dragged cloths, one would have to shorten transects between samples because of the retention issue. Phillips et al. (2014) used leggings to sample successfully for *Boophilus* larvae in habitats with high density deer populations. Zimmerman & Garris (1985) tested a semi-cylindrical shield (like a riot shield) to collect cattle fever tick larvae in Puerto Rico. The shield was held just above the ground and pushed through the brush ahead of the operator. It collected significantly fewer ticks than the drags or flags in pastures but had the advantage of being sturdier in habitats with heavy brush.

An effective passive method for detection of ticks is based on the attraction to CO_2 gas. Baited devices can be as simple as a cloth or sticky trap placed on the ground with dry ice or pressurized canisters releasing CO_2 gas. Kinzer et al. (1990) and Solberg et al. (1992) studying deer ticks and lone-star ticks reported collecting far more ticks with CO_2 than with drags, flags, or leggings. As a means of sampling larval ticks the CO_2 techniques are generally unproductive (Schulze et al. 1997) because larval ticks do not stray much beyond the hatch site, remaining localized in a cluster rather than scattered in the environment. Consequently, the passive CO_2 method only catches larval ticks if the trap happens to be placed on or very near a cluster. In a comparative study of the active *vs* passive techniques Nascimento-Osava et al (2014) in Brazilian pastures caught larval *Amblyomma* only on drags, none with dry ice. Gherman et al. (2012) tried a combination of drags with and without CO_2. The drags without CO_2 actually captured more ticks, but overall, only 1% of the captured ticks were larval stages. Kinzer et al. (1990) found the CO_2 traps superior for collecting nymphs and adults of lone star ticks, while results with larvae were greatly reduced. Their flags collected greater numbers of larvae by an order of magnitude.

Among the negative aspects in using leggings or drags is that traversing brushy habitat exposes the investigator to venomous reptiles among other hazards, not to mention potentially disease transmitting tick bites. To minimize exposure risk, while increasing larval tick collections, we experimented with alternatives to the “man-powered” methodologies. Wilkinson (1961) reported that an elevated drag, attached to an axle supported by wheels, worked better than the leggings or a standard drag in high grass for cattle fever tick larvae in Australia. Another wheeled sampler, nick-named the “Tick-Bot” (Gaff et al. 2015) was a battery-powered vehicle designed to maneuver through tick infested habitat and treat tick infestations by dragging an acaricide impregnated cloth over the ground behind it. The device was originally designed and tested for utility against lone star ticks and black-legged ticks in eastern U.S. parkland and residential habitat. The utility of this vehicle in south Texas habitat and as a tick collecting device was open to investigation. The following report documents our experience with mechanical devices, the Tick-Vac and the Tick-Bot.

MATERIALS AND METHODS

Study Sites. The primary site of investigation was the Agricultural Research Service, Cattle Fever Tick Laboratory located in Hidalgo County near Edinburg, Texas, U.S. on Moore Air Base, GPS 26°23.9’N; 98° 20.6 W. The pastures are typical south Texas ranchland, classified ecologically as Tamaulipan thornscrub (Correll & Johnston 1970). The terrain here, as over most of the lower Rio Grande valley floodplain is flat, characteristically with shallow podzols and with the hardpan (caliche) close to the surface. The dominant trees are the legume genera *Prosopis* and *Vachiella*. Understory shrubs are *Celtis, Leucophyllum,* and *Condalia*. Common forbs are *Solanum*, *Verbecina* and *Helianthus*. The dominant cover plant however is buffelgrass, *Pennisetum ciliare* (L).

The secondary site was Unit 5 of the Laguna Atascosa National Wildlife Refuge, Cameron County, TX, GPS 26° 28.8’N; 97° 32.2’W. The habitat is coastal prairie dominated by cordgrass *Spartina*, saltgrass *Distichlis,* saltwort *Batis maritima*, and sea oxeye daisy, *Borrichia*. The terrain is level with highly saline loam soils subject to flooding from high tides and storm surges.

Mechanical Devices. The Tick-Vac is a leaf blower (Ryobi Ltd., Hiroshima, Japan) with a two-cycle gasoline engine purchased locally (Fig. 1). The blower tube was simply reversed to the intake port. A knee-length nylon stocking (Leggs) was fit over the nozzle (diameter 11.0 cm) and held in place with a large rubber band reinforced by a hose clamp (Fig. 2). The tube was 122 cm long and the combined weight was 5 kg. At the end of a sample the stocking was placed into a large one-gallon zip-lock bag and labeled. The contents of the bag would be chilled for a short period in a refrigerator then dumped into a white plastic tray for examination with the aid of a dissecting microscope.

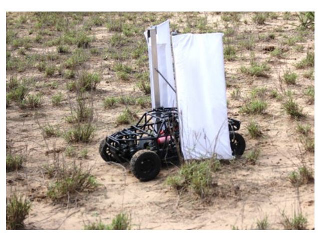
Figs. 1-2. The tick-vac in operation. The intake of the tick-vac with bag secured by hose clamp and rubber band.

Tick-Bot version 1.0 was a battery powered vehicle developed for control of lone-star ticks in Virginia (Gaff et al. 2015). This version is approximately 70 x 30 cm dimension. It is a four-wheel drive vehicle with articulated suspension, locked differentials, driven by a water-resistant electric motor. It has a magnetic field sensor on its front undercarriage that detects and follows a navigation wire at ground level. In this iteration the path of the vehicle is determined by placement of the guide wire. Operational speed was set at 0.3 m/sec with a rechargeable battery pack that allowed 2-3 hours of operation between charges. Mounted on its anterior end is a dozer type brush guard that is designed to push aside vegetation in its path. On its posterior end is a trailer hitch to which a drag-cloth is mounted (Fig. 3).

Tick-Bot version 2.0 was a rover built for Texas using the frame from a manufactured remote controlled off-road model race car of the type sold in Hobby stores. In its commercial configuration it had a gasoline engine with a tubular roll-cage chassis that was capable of speeds up to 90 kmph. The 20 Amp electric motor version was capable of approximately 60 kmph providing sufficient power to overcome obstacles or snags. It was powered by a 200 W-hr rechargeable battery. This four-wheel drive vehicle with slip differentials in each axle was steered and accelerated by means of a hand-held remote control sending a radio signal to the receiver on the rover. The Texas version was 70 cm long and 20 kg in weight. It had 15cm diam Kraken All Terrain tires. The body was outfitted with a pair of hinged frames to which we fitted denim cloth flags (Fig. 4).

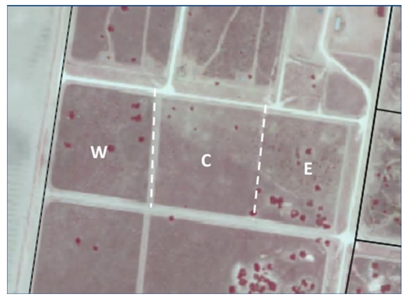
Version 3.0 was custom built for the study by engineers at the Virginia Military Institute. Modifications to improve directional control included go-pro camera, ultrasonic sonar, infra-red and wire whisker sensors. A microprocessor reported data back to the operator with a laptop computer. Instead of a roll cage this version had a higher profile with fat "Baja" buggy tires and a level platform to house the sensors (Fig. 5). It had zero -slip differentials and a higher torque electric motor.

Version 4.0 was a modified version of the 2.0 rover. This version was designed to be a destroyer, or "cluster-buster" rather than a sampler. The concept was to disrupt larval clusters given that clustering is an adaptation to reduce moisture loss. The cloth wings were replaced with wire mesh and the front was outfitted with a stiff dozer type brush guard (Fig. 6).

Figs. 3-6: 3. Tick-bot version 1.0, the Virgina creeper. 4. Tick-bot version 2.0, the Texas rover.
5. Tick-bot version 3.0, high-tech rover provided by VMI. 6. Tick-bot version 4.0, cluster-buster.

Experimental Design. The tick-vac trial was conducted in an 8-ha pasture at the Cattle Fever Tick Research Laboratory. Four naïve calves were artificially infested in early July by gluing an open vial containing ~ 14 d old *Boophilus microplus* (Canestrini) larvae hatched from 500 mg of eggs on the back between the scapulae of each calf. Twenty days after release each calf was manually "scratch" inspected and found to have an estimated 300 to 400 ticks. By allowing the ticks to develop and detach as replete females the pasture became infested. Beginning in October and over the next consecutive 50 weeks each calf was censused manually for the presence of adult ticks. Each week the pasture was sampled by legging transects: one in the eastern, one in the western and one in the middle third (Fig. 7). The leggings were made of flannel cloth covering from the waist to the shoe-tops and were loose fitting to accommodate knee-length snake-boots (Fig. 8). The transect was made by walking the width of the pasture (~150m) and back again, making an effort to brush the leggings against the vegetation. At the end of the transect the leggings were taken off, folded, and placed into a large clear plastic zip-lock bag, prelabeled as east, west, or center. In the laboratory each legging, and the plastic bag) was examined with the aid of an illuminated magnifier. Any ticks were captured on clear adhesive tape which was then transferred to a page of the field note-book for the count and permanent record. On that same day three vacuum samples were taken, also at the east, west and center of the pasture. An effort was made to avoid sampling in a recently sampled location, however the vacuum sampling was focused on loci dominated by grass and forbs, as opposed to the thorny shrubs. Similarly, the legging transect followed a more or less straight line but around rather than through a thorny shrub. To standardize the sample the effort was stop-watch timed to last 3 min and cover a contiguous circular area with a diameter of ~ 5m (approx. 20 m^2). At the end of each vacuum sample the mesh bag was placed in a labeled zip-lock bag and sealed. In the laboratory the contents of the bag were emptied into a white plastic tray and examined with the aid of a dissecting microscope. Larval ticks were handled as previously described.



Figs. 7-8. Map of experimental pasture divided into East, West and Center sampling areas and investigator using leggings in field to census questing ticks.

A first set of trials with the Tick-Bots was conducted at the pastures of Cattle Fever Tick research facility mentioned above. The primary objective was to test the feasibility of operating through south Texas brush habitats. Trials for tick capture efficacy were undertaken mainly at the second site at Laguna Atascosa. On six dates a month apart in 2019-20 simultaneous samples for ticks were taken to compare the Tick-Bot to the tick-vac, leggings, tick drags and a dry ice trap.

An experiment to use the Tick-Bot to destroy clusters of questing larval ticks was conducted at the Cattle Fever Tick Lab. For this trial six canopied sites within a pasture were chosen based on having spaced bunches of buffelgrass. At each site three bunches of grass were artificially infested by releasing two engorged female *Boophilus microplus*. Each bunch of grass was censused using a hand dragged 25x25 cm cloth for 30 sec as described in Galvan et al. (2018) in four weeks successively. Each of the grass bunches was then impacted (run-over) at high speed by the Tick-Bot. Each grass bunch was then censused as before over four successive weeks to measure the effect, if any, on the larval population.

Statistical Analysis. Sample means were compared by pair-wise t-tests assuming unequal variance. The statistical significance of differences of means (p-value) was calculated using the online program QuickCalcs (GraphPad Software), La Jolla, CA.

RESULTS AND DISCUSSION

Vacuum. A method which has not been previously tested for ticks, but which is a standard type of arthropod pest sampler is the use of a vacuum device. Vacuum samplers for pest surveillance came into wide use with the invention of the “D-Vac” (Dietrick et al. 1960). Although more effective than the beating and sweeping methods (Hand 1986) the D-Vac was noisy, bulky and costly. Second generation versions, typically based on a reversed leaf blower design (Holtkamp & Thompson 1985, Harper & Guynn 1998, Buffington & Redak 1998) reduced these objectionable aspects, in particular affording greater mobility. The newer vacuum devices are sufficiently effective against some pests that it has been proposed as control methods (Boiteau et al. 1992). We selected a design with which we had some success in sampling pests in citrus groves (Thomas 2012).

The efficacy of the tick-vac in this experiment compared well to the standard legging technique for censusing larval ticks in the pasture. Although many more ticks were collected by the tick-vac than on the leggings, the legging samples detected ticks somewhat more frequently than the tick-vac (Table 1). Although the pasture was infested, confirmed by the fact that adult ticks were found on the herd animals in 41 of the 50 weeks duration of the experiment, larval ticks were detected in the pasture by either leggings or the vacuum on only 15 dates (Table 1). However, this was not due to a failure by the sampling technique, but rather because the infestation was episodic as shown in Fig. 9. Because the herd was infested at the same time the

Table 1. Summary of results from pasture study with total numbers of fever ticks from weekly samples on cattle (n=4), and off host using leggings (n=3) or tick-vac (n=3).

Number of sample dates =	50
Number of dates ticks detected =	46
Number of dates ticks on cattle =	41
Number of dates off-host ticks detected =	15
Number of dates vac positive =	10
Number of dates legging positive =	11
Number of vac samples =	150
Number of vac positives =	13
Number of legging samples =	150
Number of legging positives =	18
Number of larvae on vacs =	805
Number of larvae on leggings =	280
Number of adults on cattle =	3102

life stages were in synchronized development. Comparing the peaks in larval abundance in the pasture to the peaks in adult abundance on the hosts over the course of the year, it can be seen that the larval peak presaged the adults by about three weeks, which is the mean duration of the on-host feeding phase. Hence there were long periods of many weeks without detections, followed by short periods where both methods detected larvae in the majority of the sample dates (but only in about one-third of the samples). The episodic nature of the infestation as the generations cycled would be more representative of an invasive pest as opposed to a population in an endemic area. Over the course of the year the larval and adult peaks became less episodic and more dispersed.

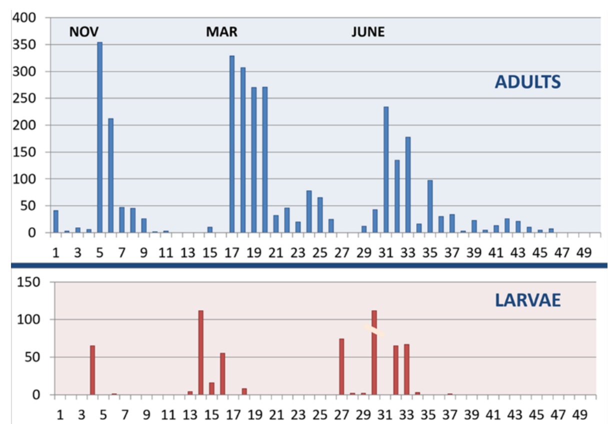


Fig. 9. Ticks collected as adults on-host (above) and off-host larvae (below) by week showing the episodic population cycle and the offset in time between larval and subsequent adult stages.

The mean number (± *sd*) of larval ticks captured per vacuum sample (on dates when larvae were present) was 17.9 ± 91.5 versus the mean number (± *sd*) of larval ticks captured on the leggings at 6.22 ± 18.3

(n = 45 for both). Because the variance was so wide the means were not significantly different ($t = 0.84$, $df = 88$, $p = 0.40$). The clustering of the larval ticks in time and space produced a non-normal distribution and thus the comparison using parametric statistics is of little meaning. Realistically the techniques are so different they should be considered as complementary rather than competing alternatives. The legging walk is a linear transect which would seem to have a greater chance of intercepting a cluster, whereas the tick-vac gives a more intensive search of a smaller locus. For comparison, the largest number of ticks captured in a single legging transect was 109, whereas one vacuum sample had 610 larvae. In that same regard, however, a positive vacuum sample provides information on the particular habitat occupied by the larvae whereas, the habitat occupied by larvae taken after a transect is less certain. Inasmuch as habitat selection by detaching females determines the location of the larval clusters it is logical to conclude that areas most frequented by the host will have greater larval density. For this experiment it is worth noting that the number of detections was only slightly less than equivalent among the three sectors of the pasture with west (13), center (11) and east (7) having positive samples.

An important consideration is the large difference in sampling effort. Although collection time in the field was approximately the same, examination of the vacuum sample in the lab took much greater effort compared to the leggings. The vacuum bag contained typically around 100 gms of plant debris and a considerable amount of mostly live arthropodan by-catch (grass mites were found to be particularly censusable with this technique). Even a negative sample required 15-20 min to sort thoroughly. And while the larvae clinging to the leggings were easily removed with sticky tape, separating larvae from the debris was a multiplier. If larger sample sizes were contemplated a system of Berlese or Tullgren funnels should be given consideration. Currently we have continued the use of the tick-vac for sampling the weeds around corrals and holding pens where infested or potentially infested cattle have been gathered.

Robotics. Version 1.0 of the Tick-Bot had an electric field sensor on its front undercarriage that detected and followed a guidewire at ground level. Thus, the path of the vehicle was determined by placement of the guide wire. On the first day of testing the objective was to challenge the progression through buffelgrass. Buffelgrass is a “bunchgrass” that grows in clumps of long tough stems with bare ground interstices. This creates an uneven surface for the vehicle to traverse. Because the Tick-Bot relies on acquiring a signal from the wire placed on the ground we laid down a course with the wire approximately 50m in length through a dense area of clumps. The first attempt failed because the wire was in some instances elevated above ground level. This caused the signal source to be too close to the bottom sensor of the carriage essentially blinding it and causing it to stop. Thus, the wire was rearranged so that it would lie close to the ground through the interstices among rather than over the bunches of grass. Our second attempt on the improved wire course was better but failed on sharp turns. The Tick-Bot could negotiate broad curves in the course but not sharp angular turns. It would overrun and lose the signal on hard corners. This issue might be mitigated with a change in the programming. To continue we rearranged the course to eliminate the sharp turns. On our third attempt the Tick-Bot was able to complete the course. At one point the battery that fit loosely in a slot on the bottom of the vehicle was knocked out of the slot by the grass. A simple modification using twist-ties secured the battery in its slot. The result of this first set of trials was that the Tick-Bot is mechanically capable of crawling over and through bunches of buffelgrass as long as the signal isn’t lost.

The second trial was to challenge the Tick-Bot 1.0 against thorny shrubs, which might snag and impede the vehicle. We set up a new course, about 50m, along a trail where the wire could be placed flat on the ground but followed the edge of the brush. For this trial the wire trailed through or close to thorny shrubs, *Prosopis* and *Vachiella*. The Tick-Bot was able to push through the shrubs with little impediment. The material in the drag, made of white denim, did not snag on the thorns. So we upped the challenge by connecting flannel side flags. Because the fever tick larvae are expected to quest on the tips of the grasses and forbs, the collecting flag had to match the height of the vegetation. Even in this configuration the Tick-Bot negotiated the thorns with little problem. In one instance the vehicle was stopped by a stump. Our thinking is that if the processor was programmed to back up and attempt again or to veer slightly from the blockage it might enhance the Tick-Bot’s ability to negotiate obstacles.

For a third trail we set a course with the wire, about 50m, in an infested pasture along a trail used by cattle to move between the grazing area and a water trough. For comparison two technicians were sent to walk through the grass nearby the trail wearing flannel leggings. The leggings of one technician had 14 ticks, the other leggings had zero ticks. In two circuits by the Tick-Bot, one tick was captured on the first run and three ticks on the second.

Given the limitations on the speed and necessity of the guidewire we wished to develop and test the feasibility of a roving version of the Tick-Bot. Again, the primary issue was the capability of the vehicle to maneuver through pasture habitats dominated by bunchgrass and thorny shrubs. Preliminary operation confirmed that even at distances in excess of 100 meters there was still strong enough radio signal to maneuver the Version 2.0 rover with the remote control, although that distance is near the end of the line of sight, and the operator was unable to clearly discern obstacles. The utility of a robot to serve as a surrogate for a walking collector is dependent on its capacity to negotiate through habitat avoiding major obstacles and overcoming minor obstacles. Thorny branches are common in the Tamaulipan scrub habitat however the rover powered through these, even with cloth flags mounted on side wings. An important modification was the wings being mounted on hinges such that the wing would fold back when encountering a rigid obstruction or snag. Ultimately it was determined that maneuverability of the vehicle to avoid major obstacles such as tree trunks, fallen branches or clumps of brush, depended on the operator being in visual control.

Hence, we experimented with an aerial drone camera to follow the rover on a video display in real time (Fig. 10). The idea was to see if the investigator could operate the rover and still negotiate obstacles from a drones-eye view. In dense cover this proved difficult because from above the controller could not discern the height of branches, and was unable to judge if the rover had clearance to pass under a branch or not. Ultimately the lack of visibility when operating in brushy habitat proved to be a serious limiting factor. We then directed our efforts with trials on the relatively treeless coastal prairies. But even with the four-wheel drive power delivered through front and rear full differentials which vector torque to wheels that slip, the rover

Figs. 10-11. Drones eye view of the tick-bot and the operator. Tick-Bot 2.0 high centered on *Borrichia* at Laguna Atascosa.

would constantly high-center on the woody sea oxeyed daisy and on the tough cord-grass clumps (Fig. 11).

Resorting to situations where the rover could function without obstruction, we restricted the operation to established trails or roadsides where it could maintain traction, brushing the pathside or roadside vegetation with the wings. In that manner we conducted a trial comparing the Tick-Bot 2.0 against the four other aforementioned methods: the Tick-Vac, the tick-drag, the leggings and the dry ice. The results are shown in Table 2. Clearly, the numbers of ticks collected were so low that no meaningful conclusion can be drawn. Our experience in the coastal areas is that ticks tend to be concentrated in canopied as opposed to the open, exposed habitat where we conducted these trials (Olafson et al. 2018).

In an effort to enhance operator sensibility, a high-tech version, Tick-Bot 3.0 was assembled for us by engineers at the Virgina Military Institute. They mounted a go-pro camera on the front and a variety of sensors that functioned as "feelers," which coupled with a reverse motion could aid in maneuvering around objects. This version was higher profile and heavier than other versions at 25 Kg. It had three limited slip differentials. With the help of these sensors we were able to successfully maneuver the Tick-Bot from one side of the pasture and back.

Table 2. Results of tick collections by method and collection date at Laguna Atascosa. Ticks were adults or nymphs of *Amblyomma* and *Dermacentor*. Monthly sample effort was 100 meters distance for drags, leggings and tick bot (n = 2). 5m diam circle for tick vac (n=2), and 2 hr for dry ice sheet (n=1).

	JUL	AUG	SEP	OCT	DEC	FEB
Drags	1	1	0	0	1	0
Leggings	0	0	2	0	0	0
Tick Bot 2.0	0	0	0	0	0	0
Tick Vac	0	0	1	0	0	0
Dry Ice	0	0	0	0	2	0

Table 3.- Cluster-buster test. Buffle grass clumps at six sites within a pasture, 3 clumps at each site, 1-2 m apart. To census larval ticks each clump was dragged with a 25x25 cm flannel cloth for 30 sec. Numbers of larval ticks per cluster over 8 sampling dates, four before treatment (Sep-Oct) and four post-treatment (Nov-Dec). Treatment date on 1 November 2018. On 30 Oct some clusters were not resampled pre-treatment (na) to avoid disturbing the established population.

Cluster	3-Sep	15-Oct	26-Oct	30-Oct	6-Nov	13-Nov	27-Nov	6-Dec
1A	0	0	0	0	0	0	0	0
1B	0	0	0	0	0	0	0	0
1C	0	0	0	0	0	0	0	0
2A	0	1	69	na	86	38	102	51
2B	0	0	0	0	0	0	0	3
2C	0	3	0	0	9	0	0	0
3A	0	75	120	na	282	14	85	9
3B	0	0	0	0	0	0	0	0
3C	0	0	0	0	0	0	0	0
4A	0	0	0	0	0	0	0	0
4B	0	0	0	0	0	0	0	0
4C	0	0	1	0	0	2	0	0
5A	0	0	0	0	0	0	0	0
5B	0	0	0	0	0	0	0	2
5C	0	0	11	na	19	6	168	48
6A	0	1	1	61	15	38	113	26
6B	0	43	251	na	16	0	20	15
6C	0	1	286	na	316	34	285	16

However, by purposely avoiding areas of dense brush, snags and branches, we were avoiding the very areas most likely to harbor ticks. This presents a dilemma that has been encountered by other researchers. When deploying a method that yields capture data, is that data unbiased, and thus representative of the actual situation? Ginsberg & Ewing (1989) found only intermittent numbers of black-legged tick larvae with their CO_2 traps or flags while they were ample on the host. In another case, Falco & Fish (1992) collected more larvae of *Ixodes* at CO_2 traps than on drags, but the numbers on drags correlated better with the numbers on mice.

Apart from a sampling method, a potential utility of the Tick-Bot could be as a destructive sampler. There is evidence that larval ticks cluster as a survival mechanism by reducing moisture loss (Yoder & Knapp 1999). Clustering also increases the efficiency of attaching to a passing host. By driving through vegetation harboring questing ticks, disrupting the clusters, scattering the larvae, the vehicle might cause substantial reductions in the population. We designed an experiment using Tick-Bot version 4.0 to test this concept with the results shown in Table 3. Comparison of the numbers of larvae in the grass bunches prior to the bust-up to the numbers of larvae afterwards, failed to show any detectable effect. Efforts to control pest tick populations are sometimes efficacious while some efforts only demonstrate the resilience of ticks when challenged by adversity.

ACKNOWLEDGEMENTS

For development of the tick-bot version 3.0 we are grateful to James Squire, Jay Sullivan and Ed Olbrych of the Virginia Military Institute. We thank Roberta Duhaime, USDA-APHIS for suggesting the cluster-buster experiment. In field trials we were assisted by Michael Moses, Brian Rich, Taylor Donaldson and Reyes Garcia. Mention of trade names or commercial products in this article is for information purposes only and does not constitute endorsement by the USDA. The USDA is an equal opportunity provider and employer. This project was funded in part by the USDA-Agricultural Research Service, National Program 104, and Project # 3094-32000-039-00D and in part by a cooperative agreement grant 58-3094-6-0010 to Old Dominion University. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Laboratory Animal Care.

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