CONTEXT-DEPENDENT ACCURACY OF THE BG-COUNTER REMOTE MOSQUITO SURVEILLANCE DEVICE IN NORTH CAROLINA

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ABSTRACT. There is a clear need for improved vector surveillance approaches that are affordable, labor efficient, and safer than traditional methods. The BG-Counter (Biogents USA, Moorefield, WV) is a device for remotely monitoring mosquito activity in combination with the BG-Sentinel (Biogents USA), a widely used trap for the collection of host-seeking mosquitoes. The BG-Counter uses a wireless connection to provide real-time counts of mosquitoes captured by the BG-Sentinel, allowing users to remotely monitor mosquito populations. This study tested the effectiveness of the BG-Counter in 5 North Carolina counties. A total of 96 trap-days resulted in the collection of >45,000 individual mosquitoes representing 35 species. *Aedes albopictus* was the most common species collected in all counties, except for New Hanover County where *Culex nigripalpus* was the most common. The mean daily accuracy ranged from 80.1% (New Hanover County) to 9.4% (Jackson County). There was a significant linear relationship between the actual number of mosquitoes collected and the device counts at all sites except Jackson County, the site with the lowest relative mosquito abundance compared with nontarget organisms. A linear regression of daily BG-Counter accuracy and the daily proportion of mosquitoes to the total number of arthropods collected revealed a significant positive linear relationship, supporting the premise that the BG-Counter is less effective when the relative abundance of mosquitoes is low. Mosquito surveillance programs using the BG-Counter should recognize its context-dependent accuracy and routinely evaluate the accuracy of the device based on local conditions.

KEY WORDS   *Aedes albopictus*, *Culex nigripalpus*, BG-Sentinel

INTRODUCTION

Increasing urbanization, global transportation, and weakened public health infrastructure for vector surveillance and control continue to exacerbate the emergence of vector-borne diseases across the world (Gubler 1998, Gould et al. 2017). Several arboviruses, such as West Nile, dengue, chikungunya, and Zika viruses, have emerged within the USA in recent decades, resulting in tremendous economic costs and substantial morbidity and mortality (Barber et al. 2010, Barrett 2014, Lee et al. 2017, Rosenberg et al. 2018). Likewise, rarer endemic arboviruses such as California serogroup (chiefly La Crosse virus), eastern equine encephalitis, and St. Louis encephalitis viruses collectively cause approximately 100 cases of encephalitis in the USA annually (Rosenberg et al. 2018). Globally, the persistent burdens of dengue, malaria, and other emergent mosquito-borne diseases necessitate improved mosquito surveillance tools that are affordable, accurate, and practical (Krockel et al. 2006, Fournet et al. 2018, Paixao et al. 2018). There are additional ethical concerns for some surveillance methods, such as the commonly used "human landing catches" (HLCs) where humans are used as bait to attract mosquitoes. Methods such as the HLC may provide accurate assessments that are representative of actual human biting rates, but are increasingly recognized as invasive and/or unethical as the techniques potentially pose unnecessary disease risk to those performing the collections (Aultman et al. 2000, Achee et al. 2015).

The BG-Sentinel (Biogents USA, Moorefield, WV) offers an alternative method for capturing primary vectors of mosquito-borne diseases, namely container-inhabiting *Aedes* spp., with results comparable to HLCs (Krockel et al. 2006, Bhalala and Arias 2009, Li et al. 2016). Like many other traps designed to collect host-seeking mosquitoes, there are cost considerations and field labor requirements for deploying, retrieving, and identifying mosquitoes in each collection. Mosquito surveillance sites may range from urban households, rural woodland vernal pools, saltwater marshes, and other locations that may be difficult and time consuming to routinely assess. A new device from Biogents, the BG-Counter, aims to increase the utility of the BG-Sentinel for mosquito surveillance by counting the number of mosquitoes collected by the BG-Sentinel and remotely reporting the data to the user in 15-min intervals. The BG-Counter transmits information

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wirelessly to a Web-based server, providing real-time mosquito counts and allowing users to remotely control the device's activity. It aims to differentiate mosquitoes from other arthropods using an infrared sensor that sorts individual insects by size, providing counts of "small" and "large" nonmosquitoes, with contemporaneous counts of the mosquitoes collected by the BG-Sentinel. This device may improve mosquito surveillance and control efforts by collecting temporal (i.e., diel) trends and abundance data defining the host-seeking activity of mosquitoes that can be assessed by mosquito control personnel immediately. The BG-Counter has the potential to increase the efficiency of surveillance and control programs by reducing the need for daily transportation and collection costs. Furthermore, the device may provide immediate data on the effectiveness of mosquito control efforts (adult suppression), population emergence "spikes," and the impacts of meteorological events (e.g., rainfall, high winds) on mosquito abundance remotely. Although the BG-Counter cannot discriminate among mosquito species, the specificity of the BG-Sentinel to discriminate mosquitoes from nontarget organisms is potentially valuable for vector control and public health programs.

A comparative field evaluation of the BG-Counter is not readily available in the published literature, but a limited number of reports suggest that the BG-Counter is an effective tool in a number of organized mosquito control districts in Florida, Louisiana, Illinois, and California (Biogents 2019, Clifton et al. 2019, Lucas et al. 2019). These states generally have a robust mosquito control infrastructure, highly abundant nuisance mosquito populations, and endemic mosquito-borne diseases. However, such extensive and organized mosquito programs are not widely present in North Carolina, which sees annual transmission of West Nile virus, eastern equine encephalitis virus, and the state's most burdensome mosquito-borne arbovirus, La Crosse virus (Utz et al. 2003, Byrd 2016). The greatest incidence rate of endemic arboviral disease (La Crosse encephalitis [LACE]) in North Carolina occurs in western North Carolina (WNC) (Haddow and Odoi 2009). The WNC region has relatively low mosquito diversity and abundance compared to the Piedmont and Coastal Plains regions. Large-scale organized mosquito control programs are nonexistent in WNC, and surveillance is limited to a few programs within local health departments. Thus, our original interest in the BG-Counter was to evaluate it as a potentially useful tool for mosquito surveillance and risk assessment at households with higher risk for LACE, as recent evidence suggests that the risk of LACE persists at some residences in WNC over multiple years (Byrd et al. 2018). Similarly, throughout the continental USA, West Nile virus disease incidence varies regionally, and some areas that have higher annual incidence rates often do not have organized mosquito control programs with routine surveillance. Thus,

tools to reduce the burden of surveillance efforts are needed. The BG-Counter may improve disease control interventions by providing information about the active times of host-seeking mosquitoes, which would inform public health officials of the most effective times to implement adult control measures, increase public health messaging (i.e., public service announcements), and measure the successes of control and/or prevention efforts.

The BG-Counter manual (version dated July 2019) states that "small Diptera, such as chironomid midges and fungus gnats, and other insects with a similar size cannot be reliably differentiated" (Biogents 2019). Mosquito abundance and diversity varies across North Carolina with predictable diversity for some species (e.g., container-inhabiting *Aedes* spp.) and a paucity of published regional diversity records for other species (Reed et al. 2019). However, it is generally known that mosquito diversity and abundance in the Piedmont and Coastal Plains regions of North Carolina are much higher than in WNC, where the LACE burden is highest. Thus, the usefulness of the BG-Counter may be limited in WNC LACE-endemic counties, where mosquito abundance is low. The purpose of this study was to evaluate the effectiveness of the BG-Counter for vector surveillance in North Carolina, with the specific aim of examining the accuracy of the device in different contexts of mosquito abundance and diversity. To test this hypothesis, we compared the ability of the BG-Counter on a BG-Sentinel to accurately count mosquitoes at 5 sites in North Carolina with different mosquito abundances and diversity.

MATERIALS AND METHODS

We deployed BG-Sentinel traps with BG-Counters in 5 North Carolina counties (Fig. 1): Jackson (WNC), Haywood (WNC), Wake (central North Carolina), Pitt (eastern North Carolina), and New Hanover (eastern/coastal North Carolina). Most sites were at previously established mosquito surveillance locations. We baited each trap with a BG-lure (Biogents USA, Moorefield, WV) and CO_2 gas released from a compressed cylinder at the counter's default CO_2 rate (50 g/h). During the collection period (2017: September–November, 2018: June–August) the traps remained at the same location in each county. All trap-days (intervals between catch bag deployment and retrieval) were 24 h except for one 48-h trap-day in Jackson County. For each trap-day we identified the mosquitoes to species with a taxonomic key (Harrison et al. 2016) and counted the nonmosquitoes. In a subset (approximately 20%) of collections we further enumerated the non-Culicidae arthropods by taxonomic order. We calculated the proportion of mosquitoes relative to the total number of arthropods collected for each trap-day by dividing the total number of mosquitoes collected by the total number of arthropods collected.
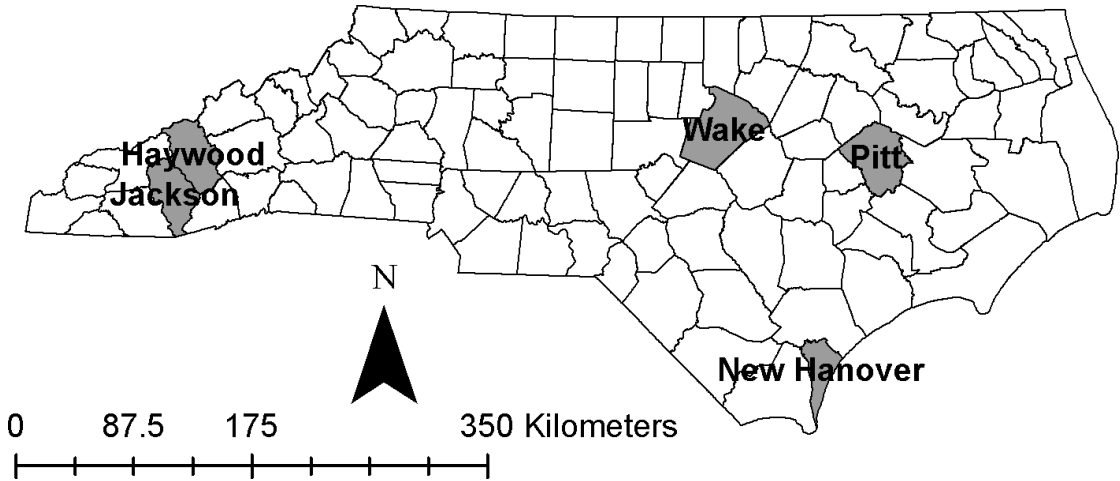
Fig. 1. Map depicting the 5 study sites (counties) located within the Mountain (Haywood and Jackson), Piedmont (Wake), and Coastal Plains (New Hanover and Pitt) regions of North Carolina.

We calculated the accuracy of the BG-Counter for each trap-day by determining the proportion of BG-Counter mosquito counts that could be attributed to actual mosquitoes captured by the BG-Sentinel (i.e., Actual Count ÷ BG-Counter Count × 100). This formula assumes that the BG-Counter overcounts the number of mosquitoes. When the BG-Counter underreported the numbers of actual mosquitoes, we used an absolute (ABS) value in these instances (i.e., 1 − ABS[1 − (Actual Count ÷ BG-Counter Count)] × 100). This revised formula produces the same accuracy value for trap-days when the BG-Counter overreported the number of mosquitoes but provides values below 100% for those days when it underreported. Accuracy of 100% would indicate that the BG-Counter counted exactly the number of mosquitoes captured by the BG-Sentinel. Lower accuracy means that BG-Counter counts were higher or lower than the true counts due to overcounting or undercounting the number of mosquitoes present in each collection bag. We compared the mean daily accuracies of the BG-Counters among the 5 counties with analysis of variance and used linear regression to measure the relationship between actual mosquito counts and BG-Counter counts for each collection site. We also used linear regression to compare the daily accuracy of the BG-Counter across all sites with the proportion of mosquitoes collected to other arthropods with log transformations. Statistical analyses were performed using RStudio (version 1.1.463), and figures were created using ggplot2 version 3.1.0 (RStudio Team 2016, Wickman 2016).

RESULTS

A total of 96 trap-days resulted in the collection of >45,000 individual mosquitoes representing 35 species (Table 1); however, >97% of the total collections were represented by 13 species. *Aedes albopictus* (Skuse) was the most common species collected in all counties, except for New Hanover County where *Culex nigripalpus* Theobald was the most common mosquito collected. Collectively, *Ae. albopictus* and *Cx. nigripalpus* accounted for 91% of the total individual mosquitoes trapped in this study. The Pitt trap collected the highest proportion of mosquitoes to other arthropods on average, followed by New Hanover, Wake, Haywood, and Jackson (Fig. 2A). The mean daily accuracy by collection site ranged from 9.4% to 80.1% and the daily accuracy range across all sites was 0–99%. For the majority (91.7%) of the trap-days, the BG-Counter underestimated the trap counts, but it overestimated the counts on 8 of the 96 trap-days.

There was a significant linear relationship between the number of mosquitoes collected and BG-Counter counts for the Haywood, Wake, Pitt, and New Hanover traps (Table 2). The strongest linear relationship was seen at the New Hanover site followed by the Pitt, Wake, and Haywood sites. There was no linear relationship between the actual number of mosquitoes collected and the number reported by the BG-Counter for the Jackson County trap (regression analysis was nonsignificant, $P = 0.65$).

Mean daily accuracy was significantly different among the sites ($F_{4, 86} = 41.09$, $P < 0.001$) (Fig. 2B). Accuracy was the greatest at the New Hanover site, significantly greater than at Haywood and Jackson, but it varied widely across the 12 trap-days (range 40.5–99%) and was not significantly greater than that of Wake or Pitt (Table 3). At the Jackson site the mean accuracy was significantly lower than that of every other site. The mean daily accuracies among the Haywood, Wake, and Pitt sites were not significantly different (Table 3), but the R^2 of the linear regressions of the mosquitoes captured and BG-Counter counts differed greatly among them

Table 1. Number of adult mosquitoes by species[1] collected by BG-Counters in 5 counties of North Carolina.

Species	County					Total	Proportion
	Jackson	Haywood	Wake	New Hanover	Pitt		
Aedes albopictus	93	188	2,005	5	25,919	28,210	0.62
Ae. japonicus	48	26	1	0	0	75	<0.01
Ae. triseriatus	33	39	72	2	95	241	0.01
Ae. vexans	5	0	119	66	14	204	<0.01
Ae. atlanticus	0	0	57	0	5	62	<0.01
Ae. infirmatus	0	0	0	67	0	67	<0.01
Culex pipiens s.l.	1	5	0	682	715	1,403	0.03
Cx. nigripalpus	0	0	32	13,022	0	13,054	0.29
Cx. salinarius	0	0	19	305	0	324	0.01
Cx. coronator	0	0	0	615	0	615	0.01
Anopheles punctipennis	2	8	85	0	28	109	<0.01
Culiseta melanura	0	0	0	306	73	379	0.01
Psorophora ferox	0	0	368	25	0	393	0.01
Unknown Culicidae	0	0	0	56	0	56	<0.01

[1] Species with fewer than 50 individuals captured overall: *Ae. atropalpus*, *Ae. sticticus*, *Ae. hendersoni*, *Ae. dupreii*, *Ae. canadensis*, *Ae. cinereus*, *Ae. sollicitans*, *Ae. taeniorhynchus*, *Ae. atlanticus*, *Ae. cantator*, unknown *Aedes* spp.; *Cx. territans*, *Cx. restuans*, *Cx. erraticus*; *An. crucians*, *An. quadrimaculatus*; *Orthopodomyia signifera*; *Coquillettidia perturbans*; *Ps. ciliata*, *Ps. columbiae*, *Ps. horrida*, *Ps. howardii*; *Uranotaenia sapphirina*.

(Table 2 and Fig. 3). In general, the R^2 and slope of the linear regressions correlated with the average number of mosquitoes collected and the average proportion of mosquitoes collected, except for that of the New Hanover site, which had the highest R^2 and the steepest slope but collected the second-highest proportion of mosquitoes. Taken together, there was a significant positive linear relationship between the proportion of mosquitoes to total arthropods collected and the accuracy of the BG-Counter across all trap-days ($R^2 = 0.71$, $P < 0.01$; Fig. 2C).

We sorted nontargets (i.e., non-Culicidae) by taxonomic order for 9 of the 27 Jackson County trap-days and for all the Haywood County trap-days for a total of 19 trap-day observations of sorted

nontargets. In Jackson County we collected a total of 1,614 nontarget arthropods (daily mean = 179.3, range: 96–480) during that period, comprised mostly of orders Diptera (86.1%), Hymenoptera (3.4%), and Coleoptera (3.4%). In Haywood County we collected 453 nontarget arthropods (daily mean = 45.3, range: 13–110), with the most common orders including Diptera (69.2%), Thysanoptera (12.7%), Hymenoptera (5.4%), Entomobryomorpha (6.5%), Araneae (4.0%), and Coleoptera (3.4%). Although most of the nontarget dipterans in our collections were not identified to a lower taxonomic order, there were Tipulidae (11.4%) and Psychodidae (6.3%) flies that were similar in size to mosquitoes. Mosquito-sized dipterans representing additional families (e.g.,
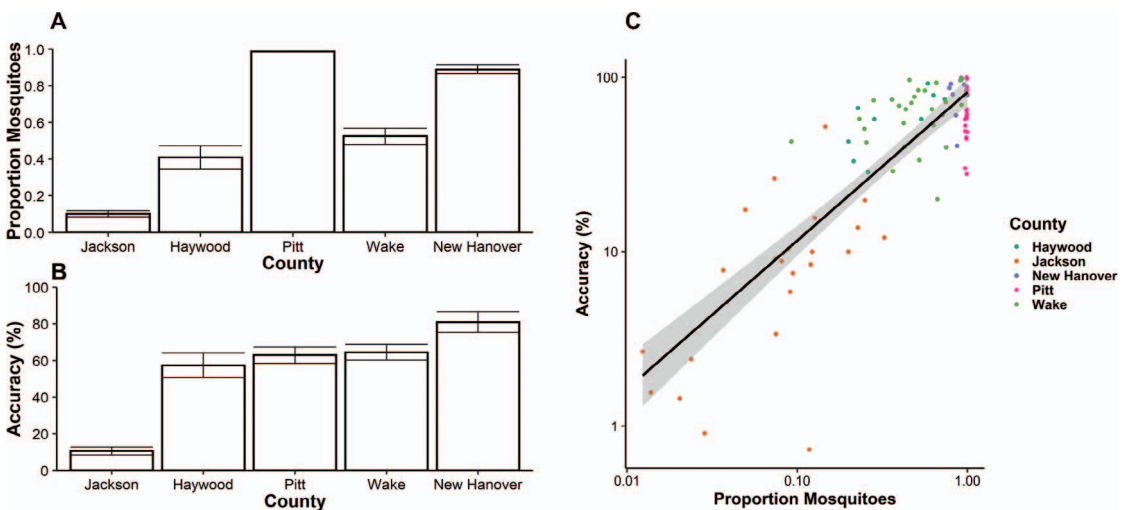

Fig. 2. (A) Mean daily proportion of mosquitoes to other arthropods collected at each site. (B) Mean daily accuracy of the BG-Counter at each site. (C) Linear regression between proportion of mosquitoes and BG-Counter accuracy for all trap-days ($R^2 = 0.71$, $P < 0.01$).

Table 2.   Summary of adult mosquito collections and accuracy data for each county.

County (collection years)	No. collections (n)	Mean daily mosquitoes (minimum–maximum)	Mean daily proportion mosquitoes (minimum–maximum)	Mean daily accuracy, % (minimum–maximum)	Regression line	R^2
Jackson (2017, 2018)	27	7.3 (0–25)	0.04 (0–0.13)	9.4 (0–52)	$y = 0.0083x + 6.38$	0.0085
Haywood (2018)	10	27 (6–60)	0.41 (0.2–0.74)	57 (29–92)	$y = 0.59x + 5.05$	0.56
Wake (2018)	26	96 (12–283)	0.53 (0.09–0.94)	64.45 (20–98.43)	$y = 0.63x + 6.94$	0.64
Pitt (2018)	21	1,278 (230–2,708)	0.99 (0.97–1)	63 (28–99)	$y = 0.70x - 52.62$	0.72
New Hanover (2017)	12	678.8 (44–1,984)	0.89[1] (0.78–1)	80.1 (40.5–99)	$y = 0.80x + 4.97$	0.95

[1] Nontargets were not counted for 2 collection days; $n = 10$.

Drosophilidae, Phoridae, Ceratopogonidae, Dolichopodidae, and Cecidomyiidae) were less commonly observed.

DISCUSSION

To evaluate the effectiveness of the BG-Counter we analyzed both its predictive power and daily accuracy. The difference between those 2 analyses is meaningful and should be taken into consideration when determining whether the BG-Counter will be a useful surveillance tool. A lower accuracy implies that the BG-Counter count is not representative of the actual number of mosquitoes collected during a trap-day, but a high R^2 in the linear relationship between BG-Counter counts and actual counts means that the trap informs users in a predictable manner. For example, if the BG-Counter has a strong linear relationship between actual mosquitoes captured but its accuracy is low, a higher count by the device will correspond with a higher number of mosquitoes even though the actual number reported by the BG-Counter is inaccurate. For users interested in monitoring diel trends in adult activity or determining the effectiveness of adult mosquito control efforts, the accuracy of the BG-Counter is unlikely to be as important as the predictive power, as defined by the slope and goodness of fit, displayed in Fig. 3.

Table 3.   Tukey Honestly Significant Difference contrasts of mean daily accuracy.

Contrast	Difference in means	95% CI	Adjusted P-value
Haywood–Jackson	46.7	27.3–66.1	<0.001*
Pitt–Jackson	52.3	36.9–68.5	<0.001*
Wake–Jackson	53.9	39.3–68.5	<0.001*
New Hanover–Jackson	70.3	50.9–89.7	<0.001*
Pitt–Haywood	5.6	−14.2–25.4	0.93
Wake–Haywood	7.2	−12.0–26.4	0.83
New Hanover–Haywood	23.6	0.58–46.7	0.04*
Wake–Pitt	1.58	−13.5–16.7	0.99
New Hanover–Pitt	18.01	−1.79–37.8	0.09
New Hanover–Wake	16.4	−2.74–35.6	0.13

* $P = 0.05$.

In general, the device was more accurate when deployed at sites where the BG-Counter collected high relative abundances of mosquitoes as compared with other arthropods. The Jackson County trap was placed at the residence of a previous LACE case within a rural forested neighborhood where the primary La Crosse virus vector, *Ae. triseriatus* (Say) and 2 accessory vectors, *Ae. albopictus* and *Ae. japonicus* (Theobald), are commonly collected during routine surveillance (Byrd, unpublished data). The forested area is known to contain multiple tree holes with persistent *Ae. triseriatus* populations from year to year. Forested sites such as the one in Jackson County may be more likely to host a broader diversity and greater relative abundance of non-mosquitoes that are attracted to a BG-Sentinel than seen in other regions where extensive mosquito control activities occur, like New Hanover County, NC. The Haywood County trap was also deployed in WNC but was located at a residence in a suburban area without extensive forested landscape. Despite the regional similarities, the Haywood BG-Sentinel attracted a greater relative abundance of mosquitoes per day than the Jackson trap and its BG-Counter performed more effectively than the one deployed in Jackson County, providing further support for the hypothesis that a low relative mosquito abundance decreases the effectiveness of the BG-Counter.

Although we provide evidence that the proportion of mosquitoes collected by the BG-Sentinel correlates with the accuracy of the BG-Counter, the differences in mean daily accuracy only partially support this hypothesis. The Pitt County trap collected the most mosquitoes in the highest proportions, but only had the second-highest mean daily accuracy. Additionally, while the strengths of the linear relationships between the number of mosquitoes collected and the BG-Counter reported counts were markedly different, the mean daily accuracies did not differ significantly among the Wake, Pitt, and New Hanover BG-Counters (Table 3). The BG-Counter was the most accurate on average at the New Hanover and Pitt sites but with a wide variation in daily accuracy, potentially due to the type and size of nontarget organisms and variation in the mosquito diversity collected by the
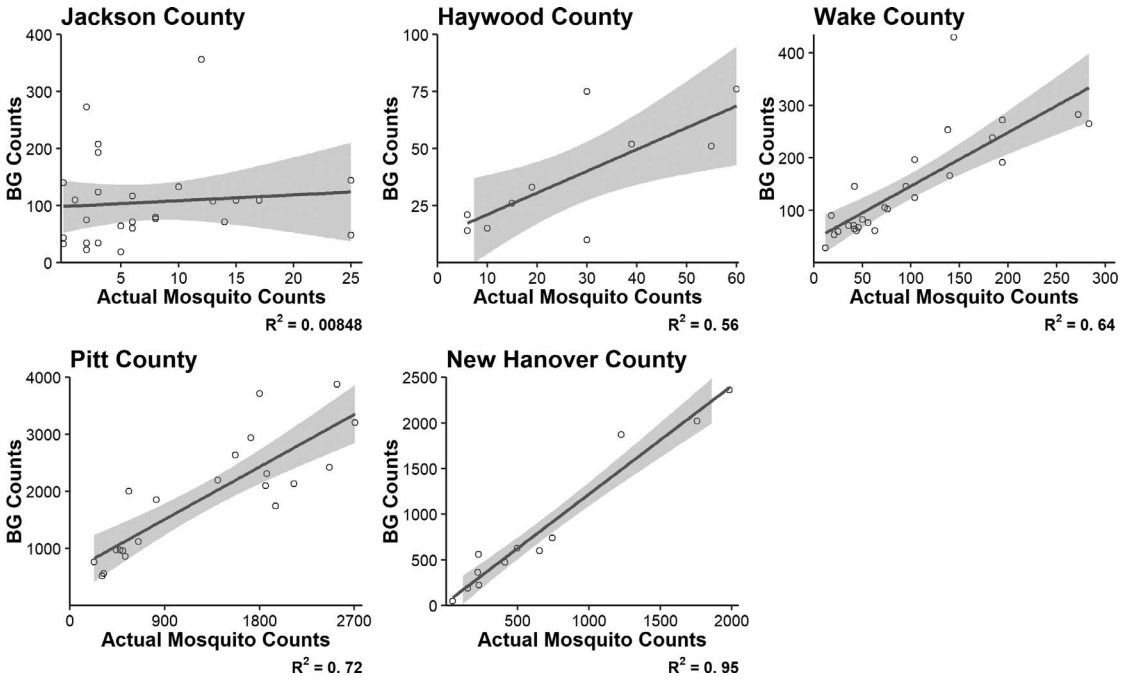
Fig. 3. Linear regressions of actual mosquito counts and BG-Counter mosquito counts for the 5 counties where the traps were deployed.

device. The BG-Counter manufacturer recommends that users evaluate the trap without using a catch bag or use a catch bag for a limited number of hours in order to ensure that the trap contents do not overfill the bag and reduce the trap suction. This may explain the variation in accuracy for some trap collections (i.e., Pitt County) if trap bags became overfilled, allowing some mosquitoes to exit the trap and be recounted.

During this study, several individuals performed trap deployments and collections, arthropod sorting and counting, and mosquito identifications within the represented counties. To ensure that the poor performance of the BG-Counter in Jackson County was not due to user error, the device was deployed for more independent trap-days at the Jackson site than in any other county ($n = 27$) across 2 different field seasons (2017 and 2018). The site of deployment in Jackson County is a location where routine mosquito surveillance is performed with the BG-Sentinel by the Western Carolina University Vector-Borne Infectious Disease Laboratory. The low abundance of mosquitoes collected in this study is typical for the site (data available upon request). The Jackson BG-Counter regularly reported daily mosquito counts in the hundreds, while the most mosquitoes collected in a day was 25. Such significant count inflation cannot simply be accounted for by user error. Sources of error unrelated to the relative abundance of mosquitoes, such as environmental interference with the device's sensors or other malfunctions, were not tested in this study.

The BG-Counter manual (version dated July 2019) suggests that CO_2 be used to improve classification accuracy (Biogents 2019). In this study we used CO_2 at the standard rate provided by the BC-Counter device. However, even with the use of CO_2, in some instances we captured nontarget arthropods at abundances higher, often by an order of magnitude, than the captured mosquitoes. Indeed, at the Jackson County collection site, a LACE case residence, mosquitoes never accounted for >13% of the total arthropod collection. Other dipterans were very commonly collected at this site, accounting for >85% of the observed nontarget abundance. Many of these dipterans were similar in size to mosquitoes and may have played a role in decreasing the accuracy of the device.

In this study we found that the effectiveness of the BG-Counter is context dependent, with the proportion of mosquitoes relative to total captured arthropods as a important factor affecting the counter's accuracy. At times, the counter can provide a highly accurate report (>99%) of the number of mosquitoes collected by a BG-Sentinel. However, even when deployed at a site with favorable entomologic conditions, the accuracy may vary. In areas where the relative mosquito abundance is especially low, the BG-Counter may be ineffective and unable to provide data that are reflective of the actual number of mosquitoes captured by the BG-Sentinel. In areas were mosquito abundance is especially high, the converse is likely true. Public health and mosquito control programs considering the BG-Counter should

be aware of the device's limitations, and question if their surveillance sites are appropriate for effective use of the device. The BG-Counter was otherwise durable, easy to set up for data retrieval, and informative when the abundance and relative proportion of mosquitoes were high.

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