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Predicted reduction in transmission from deployment of ivermectin-treated birdfeeders for local control of West Nile virus

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Abstract

Ivermectin (IVM)-treated birds provide the potential for targeted control of *Culex* mosquitoes to reduce West Nile virus (WNV) transmission. Ingestion of IVM increases mosquito mortality, which could reduce WNV transmission from birds to humans and in enzootic maintenance cycles affecting predominantly bird-feeding mosquitoes and from birds to humans. This strategy might also provide an alternative method for WNV control that is less hampered by insecticide resistance and the logistics of large-scale pesticide applications. Through a combination of field studies and modeling, we assessed the feasibility and impact of deploying IVM-treated birdfeed in residential neighborhoods to reduce WNV transmission. We first tracked 105 birds using radio telemetry and radio frequency identification to monitor their feeder usage and locations of nocturnal roosts in relation to five feeder sites in a neighborhood in Fort Collins, Colorado. Using these results, we then modified a compartmental model of WNV transmission to account for the impact of IVM on mosquito mortality and spatial movement of birds and mosquitoes on the neighborhood level. We found that, while the number of treated lots in a neighborhood strongly influenced the total transmission potential, the arrangement of treated lots in a neighborhood had little effect.

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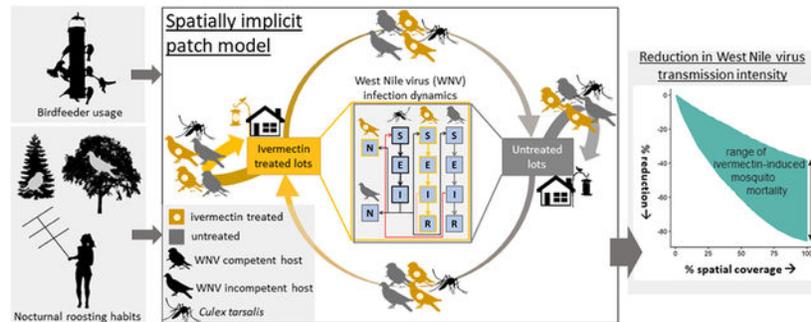
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Declaration of competing interest

BDF, though Colorado State University, has filed a patent application on aspects underpinning this control method.

Increasing the proportion of treated birds, regardless of the WNV competency status, resulted in a larger reduction in infection dynamics than only treating competent birds. Taken together, model results indicate that deployment of IVM-treated feeders could reduce local transmission throughout the WNV season, including reducing the enzootic transmission prior to the onset of human infections, with high spatial coverage and rates of IVM-induced mortality in mosquitoes. To improve predictions, more work is needed to refine estimates of daily mosquito movement in urban areas and rates of IVM-induced mortality. Our results can guide future field trials of this control strategy.

Graphical Abstract



Keywords

Bird dispersal; Endectocide; Spatially implicit patch model; SEIR compartment model; Vector control

1. Introduction

West Nile virus (WNV), the leading cause of mosquito-borne disease in the United States (Rosenberg et al., 2018), is maintained in an enzootic cycle between *Culex* mosquitoes and certain wild birds (McLean et al., 2001; Kilpatrick et al., 2007), but can spill over to infect other hosts including horses and humans when they are bitten by infectious mosquitoes (Kramer et al., 2008). Common bird species involved in maintenance and amplification of WNV in North America include American robins and other passerines including house sparrows, house finches, and Northern cardinals (Kilpatrick et al., 2007; Kramer et al., 2008). The predominant WNV vectors are mosquitoes in the subgenus *Culex* (Turell et al., 2005). For example, in North America, *Cx. tarsalis*, *Cx. restuans*, and species in the *Cx. pipiens* complex are important vectors (Kent et al., 2009; Rochlin et al., 2019). While 80% of infections in humans are asymptomatic, approximately 20% result in a febrile illness and around 1% in a potentially fatal neuroinvasive form of the disease with manifestations including encephalitis, meningitis, and acute flaccid paralysis (Mostashari et al., 2001). Long-term neurological sequelae resulting from infection represent a considerable source of morbidity in patients long after recovery from acute illness (Hughes et al., 2007). In the U.S., average annual disease incidence is highest in the Great Plains region (Centers for Disease Control and Prevention, 2021) due to the extent of suitable larval habitat for *Cx.*

tarsalis in areas of irrigated agriculture in proximity to avian amplification hosts and humans (Kovach and Kilpatrick, 2018, Eisen et al., 2010).

Current WNV prevention and control strategies can reduce the risk of zoonotic WNV transmission but face several limitations. There is no licensed WNV vaccine to protect humans. Therefore, prevention strategies focus on the use of personal protective measures (i.e., applying insect repellent, and wearing long pants and shirts, especially at dusk when mosquitoes are host-seeking) (Gubler et al., 2000) and the control of mosquito populations through removal of larval habitats and application of biological or chemical treatments to kill the larvae or adult stages (Rose, 2001). Practicing 2 personal protective measures can significantly reduce the risk of infection, but widescale adoption by the public is often low due to low perceived risk (Riccò et al., 2021, Loeb et al., 2005). While the use of larvicides can reduce the abundance of *Cx. pipiens* larvae in catch basins (Anderson et al., 2011, Stockwell et al., 2006), a common larval habitat for *Culex* in urban areas, there is currently no evidence to suggest that larviciding alone is sufficient to control mosquito populations to prevent WNV transmission (McMillan et al., 2019). Response to imminent outbreak risk requires rapid elimination of adult mosquitoes. Ground-based adulticide applications can reduce target mosquito populations under ideal conditions, but estimates of the effects on WNV transmission are inconsistent (Lothrop et al., 2008, Lothrop et al., 2007, Mutebi et al., 2011, Reddy et al., 2006). Aerial applications of insecticides have greater costs (Barber et al., 2010), but during periods with high zoonotic transmission risk can rapidly reduce the abundance of WNV vectors (Holcomb et al., 2021a) and infectious mosquitoes (Mount et al., 1996, Macedo et al., 2010, Elnaiem et al., 2008, Palmisano et al., 2005, Carney et al., 2008), and have been linked to a reduction in human WNV cases in a treated area versus an untreated area (Carney et al., 2008). However, efficacy varies widely depending on local environmental conditions (Reisen et al., 1984, Nielsen et al., 2007) and adulticide sprays are broad-spectrum and have limited ability to target the bird-biting mosquitoes actively involved in WNV maintenance and zoonotic transmission. In other words, adulticide sprays do not differentiate between mosquitoes and target the “high-risk” ones that have taken a bloodmeal from a potentially infectious bird. Additionally, applications of insecticides often face resistance by some members of the public (Thier, 2001) and the increasing development of insecticide resistance in mosquito populations can render current vector control strategies ineffective (Roberts and Andre, 1994, Liu, 2015).

A promising alternative control strategy that could overcome some of the limitations of current vector control methods is orally administered ivermectin (IVM). It has a different mode of action than that of currently used insecticides, avoiding selection pressures currently driving insecticide resistance (Duce and Scott, 1985, Buckingham et al., 2005). IVM ingestion during bloodfeeding decreases the survival of mosquitoes. Following mass drug administrations of IVM in Burkina Faso, *Anopheles* mosquitoes that fed on treated humans had reduced survival (Sylla et al., 2010, Tesh and Guzman, 1990), reducing cumulative malaria incidence (Foy et al., 2019, Kobylinski et al., 2011). Preliminary lab and field data also indicated increased mortality in *Culex* mosquitoes that fed on IVM-treated chickens and Eurasian collared doves (Nguyen et al., 2019). Pilot trials of IVM-treated wild birds and backyard chickens provided support for the efficacy and feasibility of IVM-treated birds to act as a WNV control strategy, pointing to a reduction in the level of WNV

transmission near treated feeders and chicken flocks due to IVM (Holcomb et al., 2021b). However, the full impact on mosquito populations and WNV transmission was unclear due to small sample sizes and low WNV infection rates in *Cx. tarsalis* that limited statistical power for comparisons.

Ivermectin treatment of wild birds that are commonly fed upon by WNV vectors has the potential to reduce viral transmission, thereby reducing the need for broad applications of insecticides. House sparrows (*Passer domesticus*), house finches (*Haemorhous mexicanus*), and mourning doves (*Zenaida macroura*) are common bloodmeal hosts for *Cx. tarsalis* and *Cx. pipiens* (Francy et al., 1967; Kent et al., 2009; Thiemann et al., 2012) that often visit backyard birdfeeders (Horn et al., 2014; Galbraith et al., 2015). Additionally, blue jays (*Cyanocitta cristata*), common grackles (*Quiscalus quiscula*) and red-winged blackbirds (*Agelaius phoeniceus*) often visit birdfeeders (Horn et al., 2014) and are occasionally fed upon by *Culex* (Molaei et al., 2006; Molaei et al., 2007; Kent et al., 2009). Of these species, all except mourning doves are highly competent, or able to infect mosquitoes with WNV; doves have low to no competence (Komar et al., 2003; Wheeler et al., 2009). Thus, these species could effectively disseminate IVM to bird-biting mosquitoes involved in enzootic WNV transmission, preventing subsequent bloodmeals at which WNV transmission could occur. However, variation in the WNV competence of these common bloodmeal sources might modulate the achievable reduction in WNV transmission.

An additional factor that could further influence IVM efficacy at controlling WNV is the feeder usage and dispersal dynamics of birds using IVM feeders. Feeder usage patterns will influence the attainment and duration of mosquitoicidal levels of IVM in the blood of birds. Nocturnal roost constancy, i.e., the probability a bird uses the same roost on consecutive nights (Heisterberg et al., 1984), and proximity of roosts to birdfeeders would thus influence the potential spatial extent of this control strategy's effect by modifying the distribution and number of treated bloodmeal hosts present in the local area. Knowledge of the biology of key bird species during the peak WNV transmission season (Jun-Sep) is limited because most ornithological studies have focused on the earlier breeding season and later fall migration periods (Cornell Lab of Ornithology, 2021). Assessment of nocturnal roosts is generally performed in conjunction with seasonal changes in communal roost composition (Heisterberg et al., 1984, Caccamise and Fischl, 1985) or reproductive strategies (Scardamaglia et al., 2018). To our knowledge, no study has assessed nocturnal roost locations of backyard birds in relation to birdfeeders.

In this study, we assessed the feasibility and efficacy of deploying IVM-treated bird feed in neighborhoods to reduce WNV transmission through two interrelated approaches: (1) field characterization of birdfeeder usage and nocturnal roost locations of targeted, common backyard species and (2) model-based estimation of the optimal deployment strategy of IVM-treated birdfeeders. We used a combination of field studies and modeling to synthesize field and laboratory evidence to establish data-based theoretical expectations for the effects of IVM-treated birdfeed on neighborhood-level WNV transmission, accounting for uncertainties about avian and mosquito population dynamics. For the first approach, we monitored birds using a combination of radio telemetry, radio frequency identification, motion-activated cameras, and point counts in a neighborhood with untreated birdfeeders.

For the second approach, we developed a spatially implicit compartmental patch model of WNV transmission on the neighborhood scale in the presence of IVM-treated birdfeeders, using results from fieldwork to parameterize the bird populations and spatial dynamics. Using the model, we assessed the efficacy of implementation in terms of the density and spatial arrangement of treated feeders as well as the treatment of bird populations broadly versus based on their WNV competence.

2. Methods

2.1. Ethics statement

This study was carried out in strict accordance with the UC Davis Institutional Animal Care and Use Committee (IACUC) Protocol #20980 (reviewed and approved on February 6, 2019) and with an inter-institutional agreement with the Division of Vector-Borne Diseases of the Centers for Disease Control and Prevention (reviewed and approved on June 15, 2020). The UC Davis IACUC adheres to the Office of Laboratory Animal Welfare Health Research Extension Act of 1985 (Public Law 99–158) as well as the United State Department of Agriculture’s Animal Welfare Act. UC Davis is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) and has an Animal Welfare Assurance (number A3433–01) on file with the Office of Laboratory Animal Welfare (OLAW). Work in the natural areas was approved by the City of Fort Collins (permit #4919647–43). Capture and auxiliary tagging of birds was authorized by federal bird banding permit (#22866) and Colorado Parks and Wildlife permit (#TRb3531).

2.2. Methods overview

To characterize birdfeeder usage and nocturnal roosting habits, we tagged and monitored six species of common backyard birds [blue jay (*Cyanocitta cristata*), common grackle (*Quiscalus quiscula*), house finch (*Haemorhous mexicanus*), house sparrow (*Passer domesticus*), mourning dove (*Zenaidura macroura*), and red-winged blackbird (*Agelaius phoeniceus*)]. We used radio telemetry to determine the spatial distribution of nocturnal roosts on sequential nights. We used radio frequency identification to assess birdfeeder usage patterns. Comparing bird species detected at feeders with motion-activated cameras with those detected in the area during point counts, we determined the proportion of WNV competent and incompetent species visiting birdfeeders.

We also developed a spatially implicit patch model to capture WNV infection dynamics with self-medication of birds at IVM-treated feeders and dispersal of birds and mosquitoes across household lots in a neighborhood. We used our field-derived nocturnal roost locations to parameterize Gaussian dispersal kernels for avian dispersal across lots. In each patch (IVM-treated and untreated), WNV dynamics between mosquitoes and birds proceeded according to a susceptible-exposed-infected-recovered (SEIR) compartmental model, accounting for variation in avian host competence and increased mosquito mortality following a bloodmeal on an IVM-treated bird.

2.3. Field study on avian nocturnal roost location and birdfeeder visitation

2.3.1. Birdfeeder site selection and monitoring—We selected five locations in eastern Fort Collins, Colorado (Fig 1) comprising neighborhoods adjoining a central natural area (River Bend Ponds Natural Area; 40.57186°N, 105.02690°W) to place bird feeders during the summer of 2020 (June–September). We selected sites which provided spatial coverage of the area and in locations where we observed our six target species. We arranged four of the sites approximately 700 m away from our main site and placed a single tube feeder (classic tube feeder, New Hyde Park, NY, USA) on a shepherd’s hook at approximately 2-m height at each of these sites. Sites were located in the natural area ($n = 2$) and adjacent neighborhoods ($n = 3$) to represent the land use types present. At the main site (site 1), we established two sub-sites at opposite ends of the property (approximately 37 m apart) to capture movement of birds within the property. We placed five total feeders (four tube and one platform) at this site with two tube feeders on a pole in the northwestern section (site 1a; placed 22 Jun and 24 Jun) and two tube and one platform feeder suspended on clotheslines in the southeastern section of the yard (site 1b; tube feeders placed Jul 1 and platform placed Jul 9). We established the other sites on 24 Jun (sites 2, 3, and 5) and 9 Jul (site 4).

At all locations, we hung each tube feeder under a plastic squirrel baffle (40-cm diameter Perky-Pet, Denver, CO, USA) and attached a 5-cm diameter metal spring toy (Slinky, Bedwina, New York, NY, USA) to the pole to minimize squirrel access to feeders. We visited the feeders every 1–2 days to refill seed [manually mixed Royal Wing Classic Mix (white millet, milo, wheat, black oil sunflower seed, and cracked corn) and Royal Wing Nyjer seed (Tractor Supply Company, Brentwood, TN, USA)].

We placed a motion-activated trail camera (Trophy Cam HD Essential E2, Bushnell, Overland Park, KS, USA) at each feeder location ($n = 6$). Cameras were placed on the same day or day after the feeder was placed except at site 4 where there was a lag of 13 days due to a delay in acquiring the final camera. At the end of the season, we inspected images to visually identify avian species visiting the feeder each day. Visits were defined as activities involving direct or indirect contact with the feeder or feed, including landing on a feeder, ingesting seed from a feeder, or foraging for fallen seed below a feeder. We noted any issue with the camera or feeder limiting or preventing us from categorizing the full range of species present at a feeder location each day.

We performed weekly point counts at all feeder sites 1–5 for five consecutive weeks starting 7 Aug to document the bird species present in the area. At each site, we recorded visual or auditory detections of species during a 10-minute interval onto an eBird checklist (Cornell Lab of Ornithology, 2021). We used detections to characterize the species composition of the area around each site. From these data and the identification of species visiting the birdfeeders, we calculated the proportion of WNV competent or incompetent bird species present in the area that interacted with the feeder, as determined by images from the motion-activated cameras at each site. We used results from previous experimental studies (Komar et al., 2003, Wheeler et al., 2009) to classify the competency of birds for WNV. Comparing the species detected with motion-activated camera (numerator) and point count

data (denominator), we also determined the proportion of the target species that were detected in the area that interacted with the feeder at each site.

We removed feeders and cameras from the study sites on a single day at the end of the study (Sep 8) except site 1a where the feeders and camera were removed on Aug 31 following squirrel damage to the antennas on the radio frequency identification readers.

2.3.2. Mist netting and tagging birds—We collected birds of our six target species using 38-mm polyester mist nets (Avinet Inc, Portland, ME, USA) at our main site (site 1) over the course of nine days during the period of 3–17 Aug. Nets were closed and secured when researchers were not present at the site. We identified each captured bird to species, weighed it, and took wing cord and tail measurements. We also determined the age (i.e., hatch year, after hatch year, second year, after second year, or unknown) and sex (female, male, or unknown) of each bird according to Pyle (1997) based on morphological measurements, molt patterns, plumage, and presence of cloacal protrusion or brood patch.

We attached a radio transmitter (PicoPip Arg376; mass = 0.83–0.94g, Lotek, Newmarket, Ontario, Canada) to a subset of the captured birds with a backpack harness (Rappole and Tipton, 1991) using a 1-mm fabric-covered elastic cord. We aimed to tag five individuals from each of the six target species. Prior to release, all birds were placed in a flight cage for observation to confirm that neither the harness nor tag interfered with flight and ambulation.

All birds were marked with numbered USFWS aluminum leg bands (U.S. Department of Interior Bird Banding Laboratory) and the majority also received a passive integrated transponder (PIT) tag affixed to a colored plastic leg band (Darvic leg bands, Avinet Inc, Portland, ME, USA) on the opposite leg if the combined weight of the tag and bands did not exceed 3% of body weight. We affixed the PIT tag to the plastic leg bands initially using super glue (Gorilla Glue, Cincinnati, OH, USA) and then transitioned to using a UV resin (Solarez, Vista, CA, USA) because super glue resulted in a brittle finish and PIT tags were easily dislodged during handling. During the last half of tagging, we encased individual PIT tags in heat-shrink tubing (Electro Insulation Corporation, Arlington Heights, IL, USA) prior to attaching to the leg band with resin to improve adhesion, as previously described (Bridge and Bonter, 2011). Following placement on the bird, the ends of the colored leg band were sealed together using a handheld thread burner (Beadsmith Thread Zap II, Carteret, NJ, USA) to prevent unconnected ends from snagging on objects and impairing the bird or resulting in the loss of the band.

2.3.3. Radio telemetry—We performed radio telemetry using a Yagi antenna and a Biotrack receiver (Lotek Wireless Inc, Newmarket, Ontario, Canada) to determine the nocturnal roosting location of tagged birds from 5 Aug – 8 Sep 2020. Telemetry began after dusk (starting at 8:00 PM) to ensure that birds had settled into their nocturnal roosting location and was performed at unique locations (range 3–16 total locations per night) across the broad area to obtain at least two bearings per detected bird. At each telemetry location, a GPS point (e-trex 30x, Garmin, Schaffhausen, Switzerland) was taken and bearings were obtained for signals using a compass. Relative signal strength and notes on unusual sounds (i.e., strong/loud, weak/quiet, distorted) were also recorded. In some instances, the exact

roost was located and was visited on subsequent nights to record the presence of tagged birds. The exact location could not be ascertained for all detected birds as roosting locations often were located within private property.

Using the same methodology, we also performed telemetry during daylight hours (9:00 AM – 7:00 PM) from 25 Aug – 3 Sep. The goal of diurnal telemetry was to confirm tagged birds remained in the study area during the day and to relocate birds we were unable to detect during nocturnal telemetry.

We used the triangulate function in the *radiotrack* package (version 0.0.0.9000; Dang, 2020) in R statistical software (version 4.0.2; R Core Team, 2021) to estimate the location of each of the detected birds using the maximum likelihood estimator. For any estimated locations that were implausible (i.e., those outside the study site, at distances beyond the detection ability of the receiver) or that resulted in calculations that did not converge, bearings were re-examined and corrected to produce plausible estimates (i.e., based on previous and future locations for that bird and comparison of bearings with field notes to correct errors).

For species that had at least two individuals with at least three nocturnal re-sighting locations, we calculated the probability a bird remained in the same roosting location on sequential nights (i.e., roost constancy; Heisterberg et al., 1984) and the mean distance between nocturnal roosting locations on sequential nights. To estimate the area a bird utilized for nocturnal roosting sites, we calculated the maximum Euclidean distance between any two locations for each individual and averaged by species to estimate a species' average “nocturnal home range”.

2.3.4. Radio frequency identification—To monitor visitation to feeders by PIT-tagged birds and characterize feeder usage patterns, we placed an Arduino-based radio frequency identification (RFID) reader (Bridge et al., 2019) on each feeder. Due to delays in shipping, we placed three RFID readers on 13 Aug and the remaining six on 20 Aug. Readers were removed concurrently with feeder removal (i.e., 31 Aug or 8 Sep). For tube feeders, we secured the 100-mm diameter loop antenna around the tube at the level of the upper openings to provide perching locations for birds visiting the feeder, thereby increasing the probability of successful detection of the tag. For the platform feeder, we placed the antenna in the bottom of the feeder with a small volume of seed on top so as not to cause a visual anomaly to deter visitation. The microprocessor was housed in a plastic container with a snap-on lid (Sistema KLIP IT sandwich containers, Moscow, Russia) and attached to a 12V battery through a voltage regulator to maintain a constant supply of 5V. We set the reader to poll for tags every eight seconds from 5:00 AM to 10:00 PM each day to balance energy consumptions and temporal scale of detection.

For each detected bird, we calculated the duration of individual visits, number of visits, and total duration of visits during each day.

2.4. WNV transmission model with the impact of IVM-treated birdfeed

2.4.1. Spatial parameterization of patch model—We defined our model for a neighborhood 1,500 m in length, subdivided into 75 individual properties (20 m long), each

representing a typical lot associated with a single household. We chose a one-dimensional representation of a neighborhood for model tractability, with the implicit assumption that there is no difference in movements in one or two dimensions. We categorized each property as treated if it had an IVM-treated feeder or untreated otherwise. We represented dispersal of *Cx. tarsalis* mosquitoes and birds (WNV competent or incompetent) across the properties in the neighborhood with Gaussian kernels. The group-specific kernels were parameterized based on mean dispersal distances from literature (*Cx. tarsalis*) (Dow et al., 1965, Bailey et al., 1965, Reisen et al., 1992, Barker et al., 2009) and our nocturnal telemetry results (birds). We used data for house sparrows to parameterize the dispersal of WNV competent birds and data for mourning doves to parameterize the dispersal of WNV incompetent birds. We constrained bird movement spatially by truncating the tails of the dispersal kernels beyond a species' "nocturnal home range" (i.e., observed area encompassing nocturnal roosting locations), similar to Moffitt et al (2009) to account for movement of species within their home range. We parameterized the diameter of the "nocturnal home range" as the mean maximum distance between nocturnal roosting locations for all individuals of that species (Table B in S1 Appendix). Note our use of "home range" differs from the oft-used term in ecology to characterize the area utilized by an individual during normal activities of gathering food, mating, and caring for young (Odum and Kuenzler, 1995, Harris et al., 1990, Burt, 1943). Here, home range was defined as the area a bird utilizes for nocturnal roosting only (i.e., during evening and nighttime biting periods of WNV vector mosquitoes).

We simplified the neighborhood-level model to a two-patch, spatially-implicit model with an IVM-treated and an untreated patch; this results from a weighted representation of the neighborhood such that all IVM-treated lots were represented by the treated patch and all untreated lots by the untreated patch. Group-specific movement parameters among patches were obtained with integrodifference equations (Kot and Schaffer, 1986, Van Kirk and Lewis, 1997). That is, integrating and summing dispersal kernel over the arrangement of treated and/or untreated properties in our neighborhood resulted in the movement rates within and between treated and untreated patches for each species following Baskett et al. (2006) (see Mathematical Details in S1 Appendix). We used the `integral2` functions in the *pracma* package (version 2.2.9; Borchers, 2019) in R (version 4.0.2; R Core Team, 2021) to perform integration.

2.4.2. West Nile virus compartmental model structure in patches—We modified a previous compartmental model by Hartley et al. (2012) to represent WNV transmission dynamics in IVM-treated and untreated patches (Fig 2). We simplified the previous SEIR model to include two classes of avian hosts (WNV competent and incompetent hosts) and added IVM-treated and untreated host classes for both host competencies. The assumptions of the model were as follows:

1. Avian hosts were either WNV competent or incompetent. Following infection, a competent host developed a moderate to high viremia, had a high probability of infecting feeding vectors, and sometimes succumbed to the infection. Incompetent species did not achieve high enough viremias to infect vectors, thus acting as dead-end hosts for the virus and diverting bites away from competent hosts (Komar et al., 2003, Reisen et al., 2005). While we intended these two

classes of species to represent any avian host fed upon by mosquitoes, we chose to use house sparrows and house finches to parameterize competent hosts and mourning doves to parameterize incompetent hosts.

2. All avian hosts were either treated or untreated. Regardless of WNV competency status, birds could feed at IVM-treated feeders in treated patches and self-medicate. The ingested IVM rapidly entered the bloodstream (Canga et al., 2009, Moreno et al., 2015) and biting vectors had a relatively high probability of dying following a bloodmeal on a treated bird (Nguyen et al., 2019). Unless a bird subsequently visited a treated feeder, blood levels of IVM declined over time, transitioning the bird to the untreated status in which biting vectors did not ingest sufficient amounts of IVM to cause mortality (washout period of two days, see Table A in S1 Appendix). Each treated lot in the neighborhood contained a treated feeder, but birds did not always visit it when in that lot (probability of feeding < 100%). Birds in untreated lots did not have access to IVM-treated feed.
3. For the competent avian hosts, we followed the number of susceptible (S_X) to infection, infected, but not yet infectious (E_X , during the intrinsic incubation period), infectious (I_X), or immune for life after recovering (R_X) from infection over time for different categories of treatment ($X=CU$ for competent and untreated, CT for competent and treated) (Fang and Reisen, 2006, Nemeth et al., 2009). The total population was $N_X=S_X+E_X+I_X+R_X$.
4. For incompetent avian hosts, we followed the total population size (N_X) over time for treatment groups ($X=IU$ for incompetent and untreated, IT for incompetent and treated) because the individual susceptible, exposed, infected, and recovered categories were not applicable.
5. For adult mosquitoes (M), we followed the number of susceptible (S_M) to infection, infected, but not yet infectious (E_M , during extrinsic incubation period), or infectious (I_M) over time. Mosquitoes remained infectious for life (Reisen et al., 2006a) and fed on hosts in proportion to abundance in the patch (Chaves et al., 2010). The total adult mosquito population was $N_M=S_M+E_M+I_M$. We used *Cx. tarsalis* as vectors, the predominant WNV vectors in western North America (Eisen et al., 2010).
6. The gonotrophic period (GP), or the number of days between bloodmeals for mosquitoes, was three days, which is shorter than previous estimates but was selected to reproduce seasonal dynamics of WNV infection from field surveillance data for the study area. Longer gonotrophic periods resulted in mosquito biting rates that were insufficient to sustain realistic levels of WNV infection (see Mathematical Details in S1 Appendix for selection).
7. Mosquito eggs were rarely infected from vertical transmission (Goddard et al., 2003, Reisen et al., 2005). Adults emerged from uninfected (P) or infected (Q) eggs as susceptible (S_M) or infectious (I_M), respectively.
8. The growth of mosquito and bird populations was logistic with respective rates of birth and non-disease death. Environmental carrying capacity for mosquitoes

varied seasonally (see details below), and environmental carrying capacity for avian hosts was constant across the season.

As implemented in the previous compartmental model (Hartley et al., 2012), extrinsic incubation period (EIP) and environmental mosquito carrying capacity (K_M) were temperature-dependent parameters. We used loess-smoothed daily mean temperature data (Jun 1-Sep 15, 2007) extracted from the PRISM Climate Group database (PRISM, 2007) for Fort Collins, Colorado to model these parameters according to the following relationships that have been outlined previously (Hartley et al., 2012, Barker et al., 2013). Briefly, the extrinsic incubation period, or number of days for an exposed mosquito to become infectious, was modeled as $EIP = 1/(-0.132 + 0.0092 \times \text{temperature})$ based on a previously published regression of median extrinsic incubation rates for *Cx. tarsalis* (Reisen et al., 2006a). Because the environmental carrying capacity for *Cx. tarsalis* cannot be measured directly, we approximated it following (Barker et al., 2013) based on a generalized time series of trap counts six days later using the following assumptions that allowed us to capture the observed timing of increasing and decreasing mosquito populations. Starting with the observed average number of *Cx. tarsalis* per trap-night from Fort Collins for 2007 on a weekly scale (Fauver et al., 2016), we used linear interpolation to obtain a generalized average nightly trap count abundance. We multiplied this by the gonotrophic period (3 days) to scale the proportion of female mosquitoes that would be host-seeking each night to the total population size and by a scaled number of hosts [i.e., (competent + incompetent birds) / 5]. We scaled the number of hosts based on the assumption that a trap would represent multiple hosts because birds often roost in groups and thus each individual does not have the same “attractiveness” as a single trap (see Mathematical Details in S1 Appendix). We set the scale factor for hosts to reproduce a realistic total number of mosquitoes present in a neighborhood based on expert knowledge. To match the timing of observed peak abundance (Fauver et al., 2016), we used mosquito trap counts offset by six days when calculating carrying capacity. We scaled the nightly trap count by gonotrophic period because, on average, only 1/GP of female mosquitoes would be host-seeking on a given night, but the model required an estimate for total number of females in all stages of the gonotrophic cycle (e.g., laying eggs or resting).

In order to reproduce realistic seasonal WNV infection dynamics observed at the city level (Fauver et al., 2016) on the neighborhood level, we initialized a rate of 0.75 exposed mosquitoes per 1,000 in week 25 (Jun 18–24) in each patch and introduced a total of 2.5 exposed competent birds across the patches at the start of week 28 (Jul 9) (see Mathematical Details in S1 Appendix).

2.4.3. WNV transmission intensity with ivermectin-treated feeders—We numerically integrated the full model (see Mathematical Details in S1 Appendix) of differential equations in each patch and dispersal kernels for the nightly movement of birds and mosquitoes between patches based on the number and spatial arrangement of IVM-treated properties in a neighborhood using R (version 4.0.2; R Core Team, 2021). We initialized the model with 200 total untreated birds (162 (81%) competent, 38 (19%) incompetent) and 3,510 mosquitoes. These totals were divided into the treated and untreated patches based on the proportion of the lots in each treatment status.

We used total infectious mosquito-days in the full neighborhood as the metric of WNV transmission intensity and assessed the change in this metric under two scenarios. In the first scenario, we varied the number and spatial arrangement of treated patches in the neighborhood to determine the optimal spatial design for deploying IVM-feeders. We compared two possible arrangements of treated properties: (1) contiguous, single group of treated properties randomly placed within the neighborhood or (2) randomly placed treated properties within the neighborhood (S1 Fig). Contiguous placement would represent a targeted public health deployment while random placement reflects unorganized election for IVM treatment by individual homeowners. With these placement strategies, we also assessed the change in infectious mosquito-days based on a range of daily probability of mosquito mortality following a bloodmeal on a treated bird (25, 50, and 100%) to explore a range of potential outcomes. We fitted a linear regression model to the change in infectious mosquito-days by number of treated lots to calculate the average percent reduction in infection with increased number of treated lots. We also contrasted the change in infectious mosquito-days in a single treated patch to the whole neighborhood under the above range of daily IVM-induced mortality in mosquitoes (25, 50, and 100%); this assessed the magnitude of direct benefit from placing a treated feeder. In the second scenario, we independently varied the probability that competent and incompetent hosts visited IVM-treated feeders (0–100%) to detect any differential impacts on transmission intensity by bird group treated and assess if feeder design targeting a certain type of bird species reduced infectious mosquito-days over widely treating a variety of species. For all scenarios, we compared the change in transmission intensity to account for the forcing used to recapitulate observed infection dynamics, presenting the mean (95% confidence interval) from 300 iterations of each combination of parameters explored.

2.4.4. Sensitivity Analysis—We performed a global sensitivity analysis of the model as described in Cariboni et al. (2007) with simultaneous random sampling of all parameters from a set of feasible ranges (Table C in S1 Appendix). We generated 300 sets of sampled parameter values for our 26 uncertain variables using Sobol sampling (`sobol.samp` function in the *randtoolbox* package version 1.30.1; Christophe and Petr, 2020) in R (version 4.0.2; R Core Team, 2021), following the suggestion of Matala (2008). We chose Sobol sampling for efficacy and efficiency in sampling from parameter distributions (Kucherenko et al., 2015, Burhenne et al., 2011). We used a random forest model (`randomForest` function in the *randomForest* package version 4.6–14; Liaw and Wiener, 2002) in R (version 4.0.2; R Core Team, 2021) to assess the importance of each parameter with respect to the total infectious mosquito-days. Random forest analysis is an efficient nonparametric method that uses subsets of the data to produce multiple regression trees, which are then used to calculate prediction error and variable importance (in this case, the parameter’s explanatory value for predicting the outcome, total infectious mosquito-days) (Breiman, 2001). Explanatory value was calculated as the difference in prediction error (mean squared error, MSE) using the out-of-bag (OOB) portion of the data with the parameter of interest permuted vs. using the un-permuted OOB portion of the data. We averaged this difference over all trees (Liaw and Wiener, 2002). In the random forest analysis, we randomly sampled eight variables as candidates at each split, the terminal node size was five, and we grew 500 trees.

To compare the relative importance of variables over the range of spatial coverage levels for the neighborhood, we performed a sensitivity analysis as above, constraining the range of number of treated lots to 1–20 (low coverage) or 55–75 (high coverage) when generating sets of parameter values.

3. Results

3.1. Tagged birds

We tagged 105 birds of our six target species with radio transmitters and/or PIT tags over the course of nine days between 3–17 Aug (Table 1). Few blue jays and common grackles were captured, and all house finches had inadequate weight to allow for carrying a radio tag (mean weight: 20.6 g). Therefore, we increased the number of tagged red-winged blackbirds and mourning doves, yielding a total of 28 tagged birds. Due to diminishing returns and to maximize the number of geolocations observed for tagged birds prior to the end of the WNV season, we stopped mist netting before achieving our goal of 30.

A total of 96 birds received a PIT tag mounted on a colored leg band (Table 1). Three house sparrows did not receive a PIT tag because the combined weight of the radio and PIT tags would have exceeded the 3% body weight threshold set for tagging birds. None of the mourning doves received a PIT tag leg band because this species was not expected to visit tube feeders and had not been observed on the platform feeder.

3.2. Radio telemetry

We performed a total of 29 nights of telemetry and obtained 252 unique nocturnal roost locations (Fig 3). Of these, 60.6% ($n = 149$) of roosting locations were within 400 m of the location where birds were tagged, and 5.2% ($n = 13$) were > 2.5 km away from the tagging location.

We performed telemetry during daylight hours for ten consecutive days and obtained a total of 84 unique relocations of 11 birds (S2 Fig). We included the data here for completeness, but we did not include these locations in the analysis because of limited sample size and the data did not inform our parameter of interest, roost-to-roost movements of birds.

Tagged red-winged blackbirds could not be located within the study area after 12 Aug. We engaged in an intensive search in the surrounding neighborhoods and on feeding grounds in the surrounding agricultural areas of Weld County in which red-winged blackbirds had been previously observed but were unable to locate any, presumably because they had moved to more distant foraging and roosting areas. Similarly, we were unable to locate either of the tagged blue jays after Aug 6.

We investigated a tag that had been detected in the same location during both the day and night for several days and reclaimed it from the mud near a roost utilized by a great horned owl (*Bubo virginianus*). It is possible that the tag became detached from the bird during an encounter with the owl, although it is unlikely that tagging had made the bird more vulnerable to predation. We could not be certain about the date this tag was lost because other red-winged blackbirds were roosting in the vicinity over the same period, so

we conservatively excluded all telemetry data related to this bird ($n = 6$), resulting in 246 nocturnal roost locations in the final analysis.

For all birds that were located on consecutive nights [i.e., house sparrows ($n = 5$), mourning doves ($n = 5$), and common grackles ($n = 2$)], we calculated the distances between nocturnal roosting locations on subsequent nights (Table 2). House sparrows had a smaller mean dispersal distance between roost locations than either mourning doves or common grackles and were found in the same roost on consecutive nights more often than these other two species.

3.3. Radio frequency identification

We detected fourteen PIT-tagged house finches at three of the five feeder sites on a total of 27 different days (Fig 4). No detections occurred at sites 3 and 4. Of the 2,082 detections, 1,045 (50.1%) were less than 8 secs (polling interval set on microprocessor) so the exact duration could not be ascertained. Conservatively assuming a duration of 4 secs for these short visits, the average duration per visit was 20.7 secs (max 471 secs) and the daily average total duration of all visits was 398.8 secs (range: 4–3,191 secs). On average, an individual was detected 19.3 times per day (range: 1–78 visits/day).

Due to issues with the backup batteries maintaining time on the RFID readers, we had to estimate the timing of detections for 98 readings (4.7%) based on camera detections and RFID readers on adjacent feeders. Moisture accumulation from thunderstorms (28–31 Aug) on the RFID microprocessor boards and/or batteries caused the readers to overload and were replaced by 1 Sep. Squirrels chewed through the antenna wires on both readers at site 1a on 31 Aug, so these feeders and readers were then removed from the study.

3.4. Avian species composition in local area and at feeder sites

We visually inspected 264,910 images from the motion-activated cameras and recorded the species of birds visiting each feeder per day (Fig 5). Due to sporadic issues with battery life, unintended shifts in camera positioning or limited fields of view, and difficulty maintaining the correct time on cameras, a finer resolution than species per day was not possible.

The comparison of species identified from motion-activated camera pictures (S2 Dataset) and during point counts (S3 Dataset) indicated that approximately 81% of birds observed in the area belonged to species groups (families) known to be WNV competent and 19% were incompetent (S1 Table). Excluding flyover observations (i.e., birds observed in flight across the area, but not utilizing the habitat), approximately 27% of competent species and 7% of incompetent species identified during point counts were detected with motion-activated cameras visiting the feeder in that area. Of the target species detected in the area during point counts, an average of 34% of those species were also detected at the corresponding feeder using motion-activated cameras.

3.5. Baseline WNV infection dynamics in mosquitoes

In the absence of IVM-treated feeders, the simulated WNV infection prevalence in mosquitoes peaked at 11.2 per 1,000 infected or infectious female mosquitoes in week

34 (20–26 Aug; Fig 6). Mosquito abundance peaked in week 27 (2–8 July), decreasing throughout the rest of the season, except for a brief increase in week 32 (6–12 Aug).

3.6. Impact of number and spacing of ivermectin-treated lots on neighborhood-level WNV infection dynamics

The presence of IVM-treated feeders reduced WNV infection prevalence below baseline. Contiguous and random arrangement of IVM-treated lots resulted in nearly identical effects on the simulated WNV transmission risk, as measured by total infectious mosquito-days on the neighborhood scale (Fig 7A). At a realistic but low level of adoption of IVM-treated feeders by homeowners (five treated lots), spatial deployment strategy made no difference. Contiguous placement resulted in a reduction in infectious mosquito-days of 6.72% (95% CI: 6.55–6.90%), and random placement also resulted in a reduction of 6.72% (95% CI: 6.54–6.90%). With twenty treated lots (27% of the neighborhood), a hypothetical level that we considered plausible for a targeted public-health intervention, we estimated reductions of 23.26% (contiguous; 95% CI: 22.96–23.56%) to 23.99% (random; 95% CI: 23.58–24.40%).

If all neighborhood lots were treated, we estimated a best-case reduction of 1,141.5 (85.13%) in infectious mosquito-days if 100% of mosquitoes died following a bloodmeal on a treated bird (Fig 7B); complete elimination of infection was not possible because treated feeders were assumed to represent only a small proportion of birds' daily food sources (aka birds only had a 7–27% probability of feeding at a treated feeder, see Mathematical Details in Appendix 1), leaving untreated birds even if treated feeders were present in all lots. For a daily probability of mosquito death following an IVM meal of 50%, we estimated a maximum reduction of 61.96% (830.9 infectious mosquito-days) and for a daily probability of 25%, we estimated a 37.95% maximum reduction (508.8 infectious mosquito-days) (S5 Fig). Using a linear regression model, we estimated that each addition of one treated lot (continuous placement) results in an average reduction of 0.51% (25% mortality; 95% CI: 0.50–0.52; $R^2 = 0.99$), 0.82% (50% mortality; 95% CI: 0.80–0.84; $R^2 = 0.98$), or 1.07% (100% mortality; 95% CI: 1.01–1.12; $R^2 = 0.95$) in total infectious mosquito-days in the neighborhood, depending on the level of IVM-induced mortality achieved in the mosquito population.

Increasing the number of IVM-treated lots reduced the initial peak number of infectious mosquitoes (Fig 8A), leading to continued suppression of the number of infectious mosquitoes throughout the remainder of the simulated season. The largest magnitude of reduction occurred at the end of season (Fig 8B).

With 50% IVM-induced mortality in mosquitoes, we estimated that IVM-treated birdfeeders would prevent up to 5.6 infections in competent birds (42.52% reduction in infections) with 100% spatial coverage of the neighborhood (S6 Fig).

3.7. Comparison of WNV infection dynamics in treated vs. untreated lots

We estimated reductions in infectious mosquito-days in both IVM-treated and untreated lots with larger magnitudes of reductions estimated in the treated lots (Fig 9). Comparing treatment groups and assuming a daily 50% IVM-induced mortality and at spatial coverages <100%, contiguous placement of lots resulted in, on average, an additional 11.02%

reduction (range: 2.24–16.23% reduction) of infectious mosquito-days in treated lots. Random placement resulted in, on average, a 3.23% additional reduction (range: 1.93–5.33% reduction) of infectious mosquito-days in treated vs. untreated lots.

For a single treated lot, >4x reductions in infectious mosquito-days occurred in the treated lots vs. on the neighborhood scale (Fig 10). With varying levels of assumed daily IVM-induced mortality, reductions in the single treated lot were 3.21% (25% mortality), 6.63% (50% mortality), and 12.1% (100% mortality), indicating some direct benefit from placing a treated feeder.

3.8. Variation in infection intensity with treated feeder usage by WNV competent and incompetent birds

Increasing the daily probability that competent birds visited treated feeders resulted in a larger reduction in the total infectious mosquito-days than for the same increase of daily visitation for incompetent birds (Fig 11A). For example, with five treated patches, 100% daily visitation by competent birds (0% for incompetent birds) resulted in a reduction of 12.9% (95% CI: 12.9–12.8%) while the reverse (i.e., feeder visitation by 0% of competent birds and 100% by incompetent birds) resulted in a reduction of 6.17% (95% CI: 6.12–6.22%).

The magnitude of difference between groups increased as the number of treated patches increased. Since 81% of the birds in the neighborhood were competent, a larger resulting proportion of the population was treated as the daily visitation rate for competent birds increased, resulting in larger reductions in infections in mosquitoes (Fig 11B). For the same resulting proportion of the population treated, we found little difference in the final reduction in WNV transmission if competent or incompetent birds were treated. In other words, host competency had a much smaller impact on WNV transmission levels than the proportion of the avian population treated.

3.9. Sensitivity analysis

The top four most influential parameters were 1) the probability of successful WNV transmission from competent birds to mosquitoes (r_{CM}), 2) birth/death rate of mosquitoes (b_M , d_M), 3) daily IVM-induced mosquito mortality (a_T), and 4) number of treated patches ($n_{treated}$) (Fig 12 and Table C in S1 Appendix). Together, these parameters explained 74.88% of the increase in error when parameters were permuted in the random forest analysis.

At low numbers of treated lots (1–20), the probability of successful WNV transmission from competent birds to mosquitoes and the birth/death rate of mosquitoes were the two most influential parameters, accounting for 85.8% of the increase in prediction error (S8A Fig). In addition to these two parameters, daily IVM-induced mosquito mortality was also important at high numbers of treated lots (55–75; S8B Fig). Under this high coverage, 82.1% of the increase in prediction error occurred when these three parameters were permuted.

All parameters related to the spatial movement of birds and mosquitoes (i.e., bird nocturnal home ranges, daily probability of returning to the roost (i.e., roost constancy for birds) or probability of feeding in the same lot (mosquitoes), and standard deviation of the Gaussian

dispersal kernel) were among the least important parameters, each contributing < 1% of the increase in total prediction error when permuted. This relationship held across all ranges of coverage. Therefore, the earlier conclusion of a greater effect of treatment intensity than spatial pattern on WNV prevalence is robust across parameter values.

The random forest model for the global sensitivity analysis had a mean pseudo R-squared value of 0.905, indicating good model fit and performance.

4. Discussion

Our combined field and model-based investigation of IVM-treated birdfeeders indicated that this strategy could reduce the WNV transmission intensity by as much as 85% in a simulated residential neighborhood, as measured by total infectious mosquito-days. Extensive spatial coverage was required for large reductions. In our model projections, both the probability of IVM-induced mosquito mortality and number of treated lots had strong effects on the magnitude of reduction, while the spatial distribution of treated lots within a neighborhood did not. Treatment of a high fraction of household lots and high IVM-induced mortality rates would be needed to achieve meaningful reductions in infection transmission on the public health-relevant scales of entire neighborhoods. Increasing the total number of treated birds in a neighborhood, irrespective of WNV competency, resulted in a larger expected reduction in WNV transmission intensity than selectively increasing treatment of WNV-competent bird species. Thus, investments in feed formulations specific to a particular species (e.g., treated suet cakes of berries and mealworms to target robins) might not be more efficient than treated seed targeting granivorous birds in general, so long as treated birds are frequently fed upon by WNV-competent mosquitoes.

Our model predicts, larger reductions in infectious mosquito-days in treated lots compared to untreated lots. We also estimated some direct benefit to homeowners placing a treated feeder in their lot, increasing the attractiveness of adopting this IVM control strategy. Given the structure of our model, we could only assess the magnitude of the direct benefit in the minimal example of a single treated lot, and these were marginal (3–12% reduction in the treated lot). However, this points to some direct benefit to homeowners. Additionally, the benefits in treated lots increased as adoption increased, likely due to a combination of direct benefit augmented by additional benefit from treated feed in other lots. Contiguous placement of treated lots resulted in larger reductions in infectious mosquito-days for treated lots compared to random placement, so next-door neighbors might be more willing to participate in coordinated deployments of this strategy (i.e., public health campaign) to protect themselves. As treated birds disperse across the neighborhood from night to night, the presence of treated lots in a neighborhood also affords some protection in untreated lots, but to a smaller degree than in the treated lots themselves. Further work could be done to assess spatial variation in reduced infectious mosquito-days at the level of the lot to identify the magnitude of the direct and indirect benefits from placing treated feeders.

Of the most important parameters identified in the global sensitivity analysis, the number of treated patches and birth/death rates of mosquitoes are the most management-relevant parameters. Vector control or public health agencies deploying IVM-treated birdfeeders

presumably would be able to enlist larger numbers of households in an intervention to increase the number of neighborhood lots with treated feeders. Also, using IVM-treated feeders in concert with other control strategies that affect mosquito birth or death rates (e.g., removal of larval habitats or application of larvicides or adulticides) might enhance the efficacy of IVM.

Nearly half of the reductions in infectious mosquito numbers would occur prior to human infections with WNV. The typical peak of illness onset in Weld County, CO, occurs in week 34 (see S23 Fig in DeFelice et al., 2018). Symptoms usually occur 2–14 days after infection (Petersen et al., 2013) so peak human infections were likely to occur between week 32–33 in our study area. We estimated reductions in numbers of infectious mosquitoes starting in week 25 when exposed mosquitoes were introduced into the neighborhood, thus reducing the risk of human infection starting early in the WNV season. Between 44.1–48.3% of the cumulative total reduction in infectious mosquito-days occurred prior to week 32.

Under maximal assumptions of treatment effect (i.e., 100% IVM-induced mortality, 100% spatial coverage of neighborhood), we estimated that a reduction of 85.13% in infectious mosquito-days could be achieved. This was accompanied by a 63.23% reduction in the number of infections in competent birds (8.4 infections prevented), which could also benefit avian populations, particularly for species such as corvids that suffer high mortality when infected with WNV (Wheeler et al., 2009, Nemeth et al., 2007). Complete elimination of mosquito infections did not occur in our model as only 27% (WNV competent) and 7% (WNV incompetent) of birds visited a treated feeder daily and IVM has a relatively short washout period in plasma (Nguyen et al., 2019, Moreno et al., 2015); only 35.9% of the total bird population was treated under this scenario.

The concentration of IVM in plasma achievable by passive oral administration in feed to wild birds is largely unknown. The relationship of IVM concentration to mosquito mortality is also unclear, and these factors combined have important implications for the effects of the IVM strategy on WNV transmission. Lab studies with colony mosquitoes have estimated an LC₅₀ of 49.9 ng/mL (Nguyen et al., 2019) to 60.6 ng/mL (Holcomb et al., 2021b). However, a strong mosquitocidal effect from the serum of a wild-caught common grackle during a pilot trial vs. control calf serum was observed for *Cx.tarsalis* in a serum-replacement assay (100% mortality within two days), even with a relatively low IVM blood concentration of 5.7 ng/mL (Nguyen et al., 2019). Mortality of 45.6% in three days was observed following a bloodmeal on IVM-treated chickens with serum concentrations of 17–32 ng/mL; concentrations were obtained several hours prior to blood-feeding so the dose to which the mosquitoes were exposed could have been lower (Holcomb et al., 2021b). Further investigation of the impact of IVM on wild mosquitoes is required to refine mosquito mortality estimates for achievable doses.

Feeder usage data from RFID readers provide an initial indication of the dose birds could achieve. While the average house finch spent around six minutes per day at the feeders, the maximum daily duration was >50 minutes. If ingestion rates were constant across visits, a wide variation in dosage between birds could occur. Balancing the IVM:feed ratio with a potentially wide range of ingestion rates might be necessary to reduce the potential for

accidental toxicity, which is known to vary across bird species (Lierz, 2001, Kummerfeldt and Schaefer-Nolte, 1987, Clyde and Patton, 1996). The potential toxicity of treated feed is a limitation of this control strategy and further attention is warranted to minimize this risk. Feeder usage data from this study might not be representative of usage across species or for house finches across the full season.

We predict that increasing daily feeder visitation rates by competent birds can reduce infectious mosquito-days more than the same increase for incompetent birds. While this difference increased as the number of treated lots increased, the effect was not attributable to the competency of the birds, but rather the relative abundance of each group. During fieldwork, we found around 81% of the observed birds around our feeder sites belonged to taxonomic families known to be competent. Because we assumed that mosquitoes bite hosts in proportion to their presence in the environment (Chaves et al., 2010), targeting competent birds increased the total proportion of treated birds more rapidly than targeting the smaller subset of incompetent birds. Comparisons of change in mosquito infections by proportion of the total bird population treated illustrated that targeting WNV competent or incompetent birds did not result in meaningfully different estimated reductions; the increased IVM-induced mortality in WNV vector mosquitoes was a more important driver of reduced infection transmission than the WNV competence of the treated bird. We assumed that mosquito biting was opportunistic (i.e., directly proportional to the fraction of each species present), but there is some evidence that mourning doves account for a large proportion of the bloodmeals by *Cx. tarsalis* in Colorado during the WNV season (range 12–43%) and might be preferentially bitten (Kent et al., 2009, Francy et al., 1967, Hess and Hayes, 1970). Thus, treating preferred bloodmeal hosts, even if incompetent, could have a higher impact than estimated.

The feeder design we used appears to have low attractiveness to our target species or might have been competing with other natural food sources or more established feeder locations in the neighborhood. We estimated that only 27% of competent species and 7% of incompetent species observed in the area visited a feeder, and only 34.6% of our target species present in the vicinity visited a feeder. Quantification and monitoring of other feeders in the area was not feasible so we could not estimate the total number of birds visiting any feeder from our data. Also, various camera issues limited our ability to document the full daily range of species visiting a feeder, meaning our study might have underrepresented the total species diversity that visited feeders. Nonetheless, at the estimated visitation rates of birds, a reduction in infectious mosquito-days of <62% could be expected. Increasing the attractiveness of feeders to a wider proportion of the bird community would be needed to achieve a meaningful reduction in WNV transmission at feasible deployment levels. This could be achieved through the physical design of the feeder, or the composition of the feed mix utilized.

The transmission probability of WNV from birds to mosquitoes, or the host competence of birds, was the single most important parameter from the random forest analyses. Accurately predicting competence is essential in estimating the level of control achievable, especially at operationally plausible levels (< 20 treated lots). WNV competence values for house sparrows and house finches derived from lab studies (i.e., 30–50% probability of infection

per infectious bite (Kilpatrick et al., 2007; Komar et al., 2003)) were insufficient to produce realistic infection dynamics consistent with those observed previously in the study area, so we used a higher competence value typical of corvids to reproduce expected dynamics. This suggests that our modeling framework might not have captured heterogeneity in biting preference, competence, or other factors that maintain WNV transmission in nature. One such factor could be the contribution of hatch-year birds to WNV amplification (Hamer et al., 2008). While we included a birth term in our populations of birds, we did not have differential competencies based on age as this would have increased the complexity of our model. This and other heterogeneities in competence between species and individual birds could be driving infection dynamics in nature while our modeling framework only captured the average effect.

Our study of bird movements took place during August, which is the typical peak of WNV transmission season in northern Colorado. However, bird movement patterns change often and vary widely among species, and patterns earlier in the transmission season might have differed from our observations. Results from the motion-activated cameras indicated that the species composition around some of our feeder sites shifted during our study, with a decrease in daily visitation by red-winged blackbirds and common grackles and an increase in visits by house finches and black-capped chickadees. This shift was corroborated by telemetry; tagged red-winged blackbirds could not be relocated after 11 Aug despite intensive searching and it was presumed that red-winged blackbirds and common grackles were shifting from breeding sites into larger, communal flocks in preparation for fall migration (Dolbeer, 1978, Smith and Bird, 1969). After tagging, the blue jays did not return, potentially due to avoidance of the study site following the tagging event, and they could not be relocated. Pairing the observed shift in feeder visitation with reported shifts in bloodmeal hosts of *Cx. tarsalis* from American robins to house sparrows and doves (Kent et al., 2009, Francy et al., 1967), highlights the potential for variation in the impacts of IVM over the season as the composition of the local avian community changes. However, further work is needed to elucidate the magnitude, if any, of potential changes in impacts of IVM attributable to avian population dynamics throughout the season.

Based on the global sensitivity analyses, all parameters that defined the spatial movement of birds and mosquitoes across the landscape had low relative importance in terms of their effects on total infectious mosquito-days. This could indicate that the spatial aspects of the model were less important than the direct impact of IVM on mosquitoes. Alternatively, the method we used to model the observed spatial aspects of bird movement might have incompletely captured the dynamics that would modify the potential impact of IVM-treated feeders (e.g., direct and indirect benefits of treated feeders). In particular, our use of dispersal kernels resulted in a Markov process for bird movement in which birds' movements were constrained only by their position during the previous time period, ignoring any role memory and habitat quality might play in roost selection and allowed bird populations to disperse across the neighborhood over the season. With this broader dispersal potential, our estimates are likely conservative, representing a spatial averaging of the effect on transmission intensity and not fully capturing focal effects of IVM treatment that we might expect with more localized bird movement. Methods utilized in other systems to model movement in a spatial context have included likelihood procedures with habitat-

dependent dispersal kernels (Vinatier et al., 2011) or mechanistic models with a movement bias to the center of an individual's home range and changes to movement in response to the environment stimuli (e.g., foreign scent marks) (Moorcroft et al., 2006, Moorcroft et al., 1999) to mirror natural processes. These methods or agent-based models might improve spatial accuracy, but would add computational and mathematical complexity, reducing the tractability and generalizability of the model.

A better quantification of *Cx. tarsalis* movements in urban areas is also needed to refine estimates of expected reduction in WNV transmission. Previous mark-release-recapture studies estimate mosquito dispersal in largely agricultural settings that might not reflect those in more urbanized locations. Habitat and land use are known to influence *Culex* dispersal, and mosquitoes tend to disperse longer distances in agricultural settings (Reisen et al., 1992, Reisen et al., 1991, Lothrop and Reisen, 2001), potentially to find bloodmeal hosts, resting locations, and suitable larval habitats. Our choice to use a Gaussian kernel for mosquito dispersal might have allowed unrealistically high probabilities of long-distance dispersal events and artificially elevated movement between patches. This could contribute to the marginal direct benefit we estimated. Estimating mosquito dispersal in such a way as to reproduce mark-recapture studies is difficult. A previous model estimating the dispersal of *Aedes albopictus* in urban areas, simulated the probability of movement between parcels within 200 m (radius of reported daily recapture of released mosquitoes) based on the quality of the parcels with inverse-distance weighting so that movement to nearby parcels was more likely than to more distant ones, modified by the suitability of the new parcel relative to the current one (Montecino et al., 2014). An approach of this nature that accounts for landscape features and explicitly truncates dispersal distances could more accurately capture the factors driving the movement of *Cx. tarsalis* in neighborhoods and refine our estimates.

While not a significant factor in our study, the spatial distribution of treated lots could be important in larger neighborhoods (> 75 lots). In these settings, the spatial extent of the whole area would be much larger than the total movement of individual birds, thus resulting in multiple localized groups. Therefore, random allocation of treated lots might be more efficient than a single contiguous treatment block on these circumstances. At smaller scales (< 75 lots), spatial allocation is unlikely to be important as the total neighborhood size would represent only a small portion of avian nocturnal ranges.

While the previous compartmental model effectively reproduced seasonal infection dynamics on the scale of 20 km-square grids (Hartley et al., 2012), in this study it was necessary to maintain early season WNV infections by forcing the introduction of exposed mosquitoes and birds to match realistic infection dynamics observed at the city level (Fauver et al., 2016) on the neighborhood level, indicating that the modeling assumptions and disease processes do not translate directly across spatial scales. Focusing our model on the neighborhood level enabled us to estimate the effect on the scale on which deployment would occur but did not consider the spatial structure and surroundings of the neighborhood that might influence pathogen transmission dynamics and the efficacy of the intervention. Additionally, our decision to use least-squares optimization to select these forcing values resulted in a fractional number of introduced birds. However, as we needed to introduce

birds into the system to cause the dramatic increase in infection, this could indicate that immigration of infected mosquitoes or birds plays a role in initiating or sustaining local infection dynamics.

Our approach of pairing fieldwork with model development allowed us to collect and incorporate firsthand data into our modeling framework, thus improving the biological validity of our modeling approach. Taken together, our findings demonstrate that deployment of IVM-treated birdfeed in neighborhoods could reduce local WNV transmission intensity, as measured by total infectious-mosquito days, if several key conditions are met. Both the probability of IVM-induced mosquito mortality and number of treated lots strongly affected the magnitude of risk reduction, whereas the spatial distribution of treated lots within a neighborhood did not. Further work is needed to estimate the daily probability of IVM-induced mortality in mosquitoes to refine the achievable reduction in WNV transmission under field conditions. Increasing the total number of treated birds in a neighborhood, irrespective of WNV competency, reduced WNV transmission intensity, indicating that effective IVM deployment would target a wide variety of backyard bird species. Our fieldwork and modeling results are a step towards designing effective randomized controlled trials of IVM-treated birdfeed. They also provide a view of the intersection of feeder usage and nocturnal roosting habits of common backyard birds during the period of typical WNV amplification following the avian breeding season. Our results also open the possibility that IVM treatment of the maintenance hosts for other mosquito-borne disease systems could act as a viable control strategy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

All relevant data are within the manuscript and its Supporting Information files. Raw telemetry data available upon request to protect the privacy of individuals who participated in the study.

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Highlights

- Ivermectin (IVM) is a promising tool for targeted West Nile virus (WNV) control.
- We used fieldwork and modeling to estimate impact of IVM-treated birdfeeders on WNV.
- We monitored birdfeeder usage and nocturnal roosting habits of common backyard birds.
- Model included IVM-induced mosquito mortality and host and vector spatial movement.
- We predicted reductions in WNV with large spatial coverage and high IVM efficacy.



Fig 1. Birdfeeder placement in Fort Collins, Colorado.

The main study site (site 1) contained two sub-sites on opposite ends of a single property with two tube feeders at 1a and two tube and one platform feeder at 1b. The remainder of the sites had a single tube feeder. Green outlines indicate natural areas managed by the City of Fort Collins. Grey lines trace paved roads. Geographic boundaries of city-owned parcels were obtained from the Colorado statewide tax parcels shapefiles prepared by the Governor's Office of Information Technology (2020) and road centerlines were obtained from the 2020 TIGER/Line shapefiles prepared by the United States Census Bureau (2020).

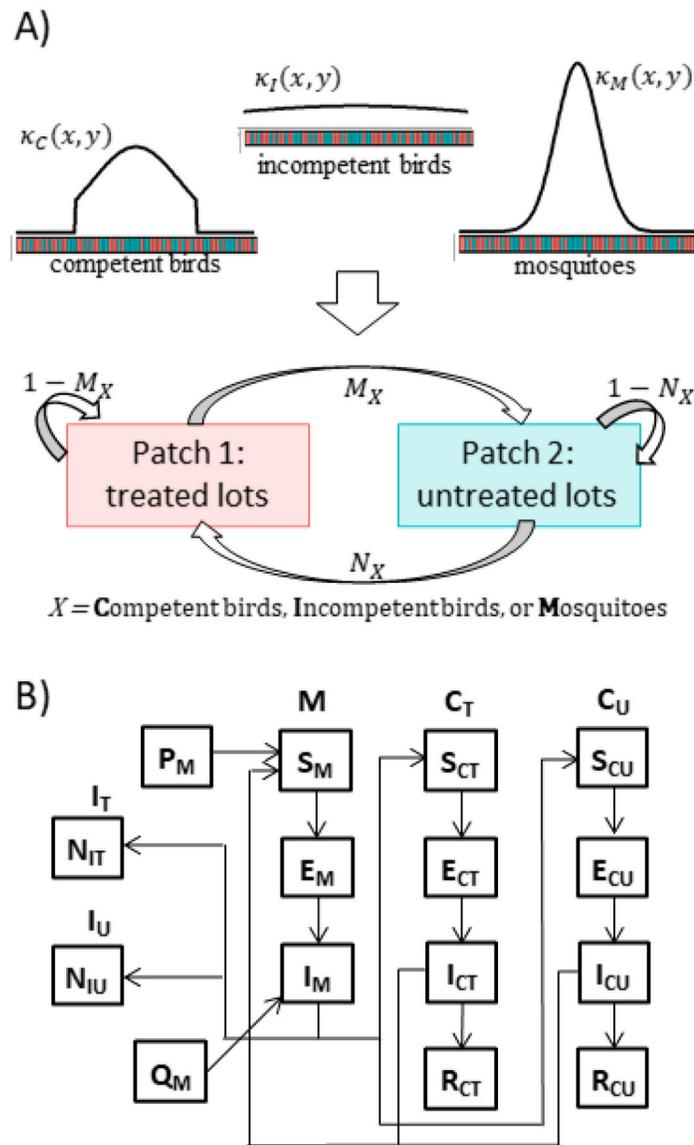


Fig 2. Schematic of SEIR model developed for West Nile virus (WNV) transmission with ivermectin (IVM)-treated birdfeeders.
 A) Integration of group-specific normal dispersal kernels ($\kappa_X(x,y)$) across the arrangement of treated and untreated lots in the neighborhood determines movement parameters between patches in the spatially implicit patch model (X is competent birds (C), incompetent birds (I), or mosquitoes (M)). M_X is the movement rate for group X from IVM-treated to untreated patch and N_X is the movement rate for group X from untreated to treated patch. In each patch, an SEIR model B) represent WNV transmission between avian hosts and mosquitoes. Avian hosts are categorized as WNV competent (C) or incompetent (I) and can be either treated (T) or untreated (U). Adult mosquitoes (M) emerge from either uninfected (P) or vertically infected (Q) eggs. Over time, competent birds were susceptible (S_X) to infection, infected, but not yet infectious (E_X , during the intrinsic incubation period), and infectious (I_X) or immune for life after recovering (R_X) from infection, with a total population of $N_X = S_X + E_X + I_X + R_X$ ($X = CU$ for competent and untreated, CT for

competent and treated). Similarly, adult mosquitoes were susceptible (S_M) to infection, infected, but not yet infectious (E_M , during extrinsic incubation period), or infectious (I_M) with a total population of $N_M = S_M + E_M + I_M$. Because incompetent birds did not contribute to transmission, we tracked the total population (N_X) of incompetent birds over time for treatment category ($X = IU$ for incompetent and untreated, IT for incompetent and treated). See section 2.4.2 of the Methods and Mathematical Details in S1 Appendix for complete explanation.

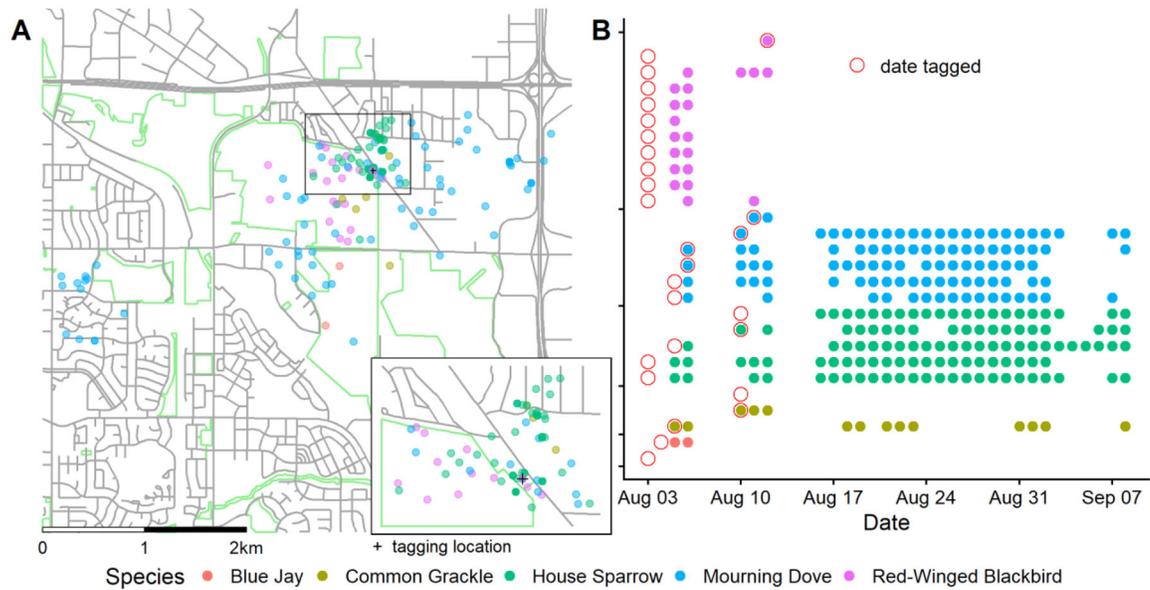


Fig 3. Detection of avian roosting sites.

(A) Locations of avian nocturnal roosting sites determined from triangulation of radio telemetry data or visual location. Inset of area around tagging location. Green outlines indicate natural areas owned by the City of Fort Collins. Grey lines trace paved roads. (B) Date of tagging and nocturnal relocation of species using radio telemetry or visual location. If an individual was relocated at night on the day it was tagged, the two shapes coincide for that day. Ticks along the y-axis delimit groups of individuals of the same species. Geographic boundaries of city-owned parcels were obtained from the Colorado statewide tax parcels shapefiles prepared by the Governor’s Office of Information Technology (2020) and road centerlines were obtained from the 2020 TIGER/Line shapefiles prepared by the United States Census Bureau (2020).

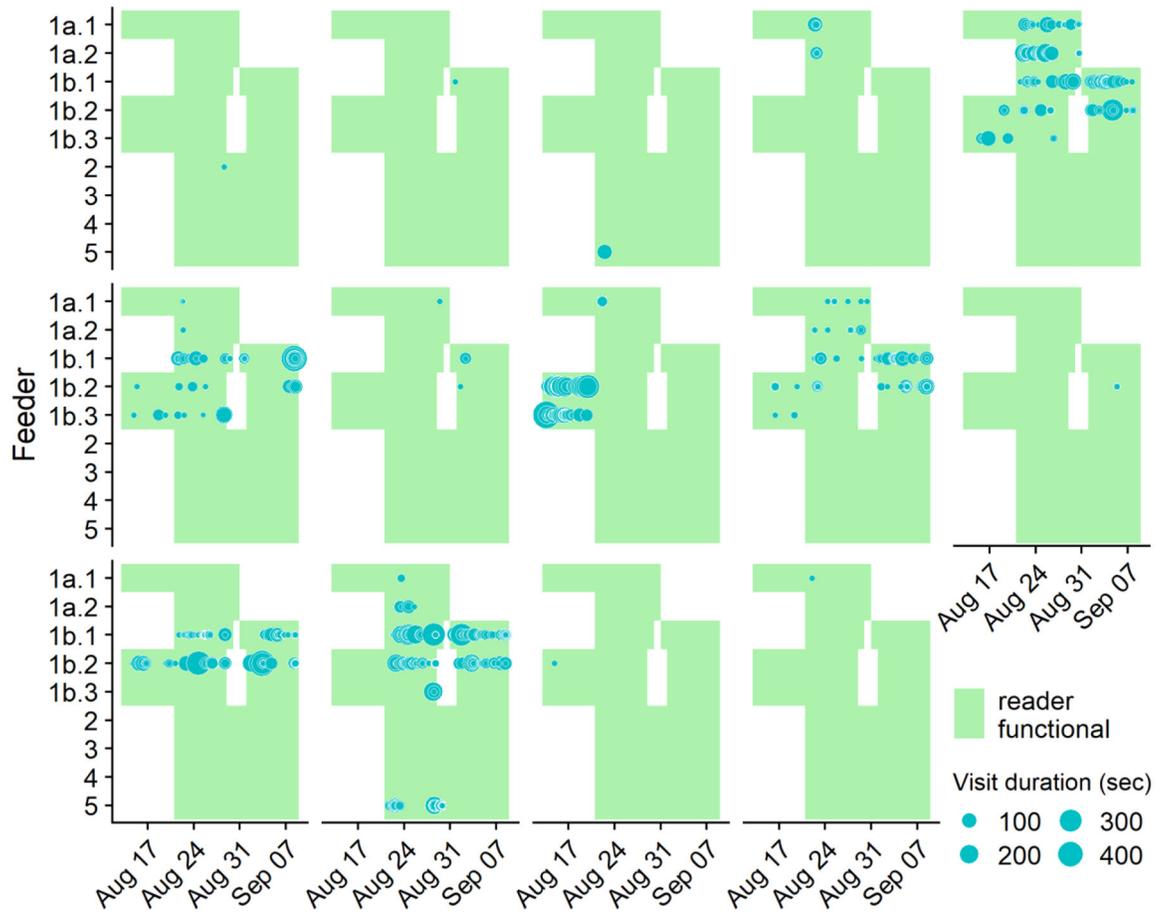


Fig 4. Duration of individual bird visits to feeders with radio frequency identification (RFID) readers.

We detected visits from 14 PIT tagged house finches at our birdfeeders. Individual feeders in the main site, site 1a (1a.1 and 1a.2) and 1b (1b.1, 1b.2, and 1b.3), are indicated. Date ranges with functional RFID readers in place are indicated in green shading.

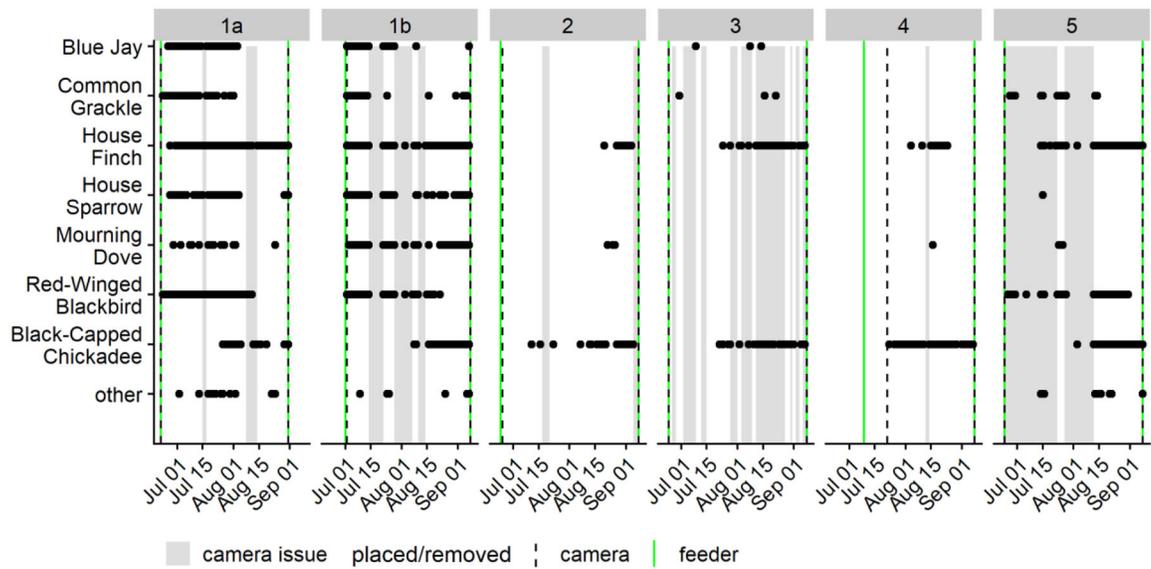


Fig 5. Daily detections of species visiting feeders based on motion-activated cameras. Date feeder and camera placed at site indicated. Camera issues included dead batteries, fallen cameras, and incomplete fields of view that could have prevented fully documenting the full range of species present. Other species observed included American robin (*Turdus migratorius*), orchard oriole (*Icterus spurius*), American goldfinch (*Spinus tristis*), brown-headed cowbird (*Molothrus ater*), and Northern flicker (*Colaptes auratus*).

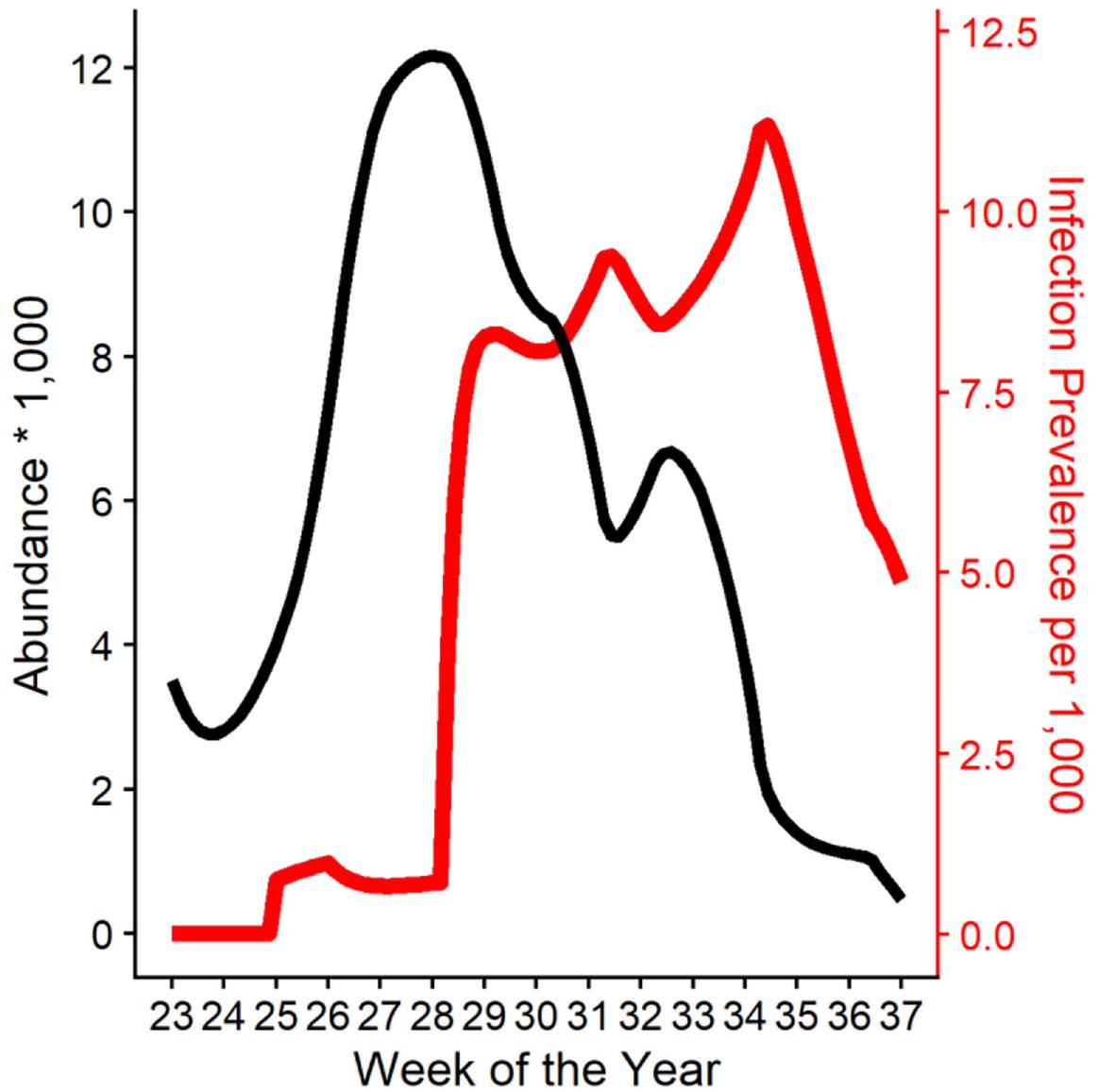


Fig 6. Baseline West Nile virus (WNV) infection dynamics in *Cx. tarsalis* in absence of ivermectin. WNV infection prevalence (number of infected and infectious mosquitoes per 1,000) and abundance of female *Cx. tarsalis* per day.

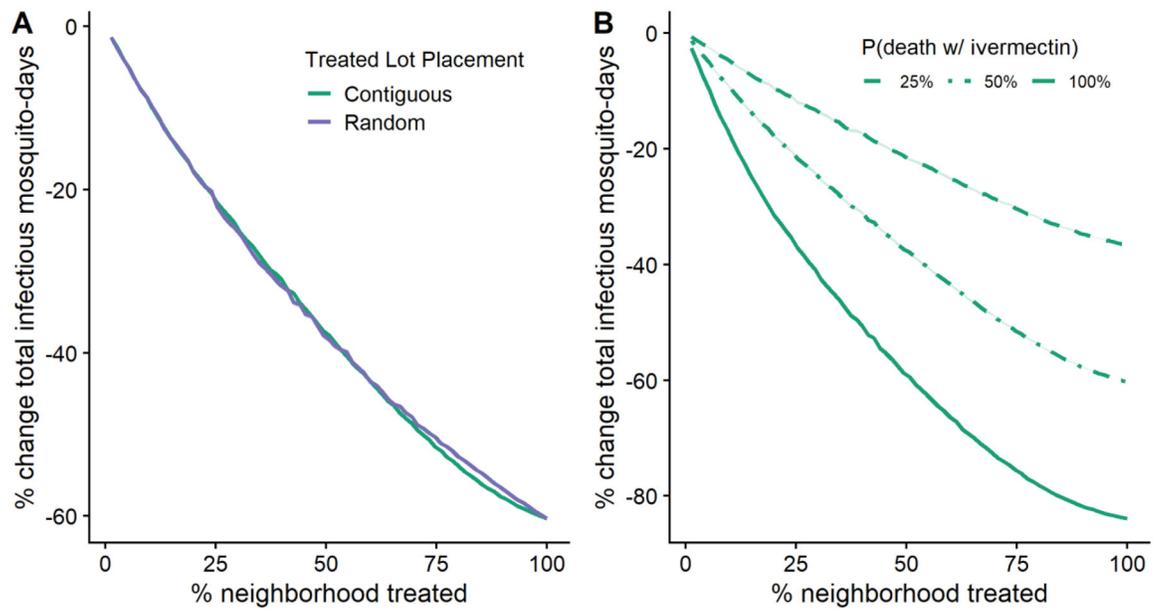


Fig 7. Reduced infectious mosquito-days with increased ivermectin-treated lots and daily probability of mosquito death following a bloodmeal from an ivermectin-treated bird.

Percent change in infectious mosquito-days with A) contiguous versus random placement of ivermectin-treated lots in neighborhood (75 total lots), assuming a 50% daily probability of mosquito death following a bloodmeal on an ivermectin-treated bird, and B) contiguous placement of ivermectin-treated lots in neighborhood with 25, 50, or 100% daily probability of mosquito death following a bloodmeal on an ivermectin-treated bird.

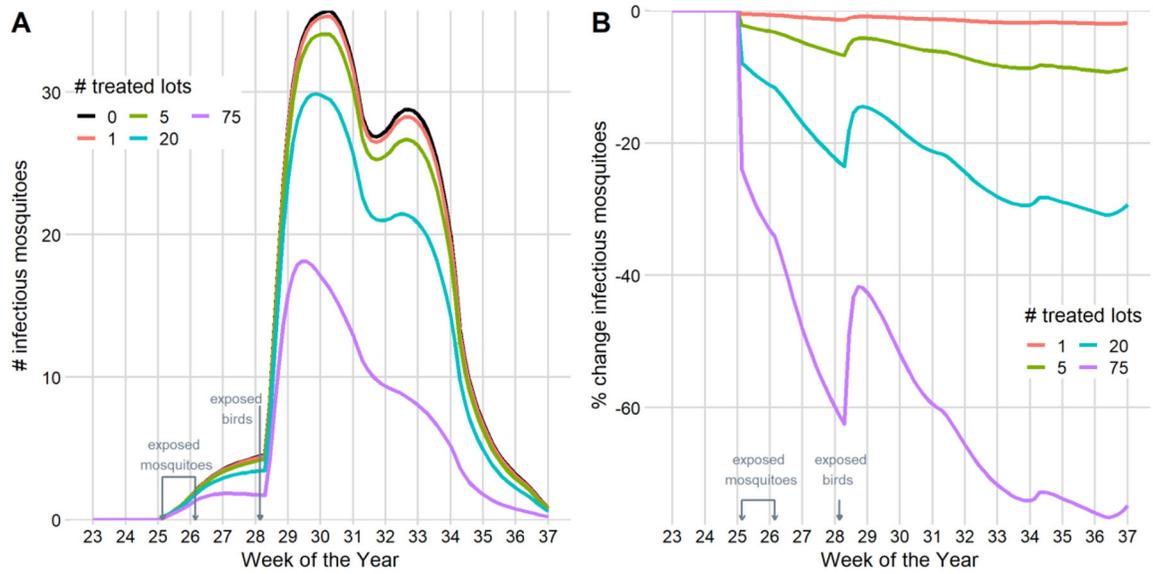


Fig 8. Reduction in daily infectious mosquitoes at the neighborhood scale with IVM-treated lots. A) Daily number and B) percent change in number of infectious mosquitoes in the neighborhood (75 total lots) with contiguous placement of treated lots. Calculations assume a daily 50% IVM-induced mortality in mosquitoes. To reproduce realistic dynamics, infected mosquitoes (E_M) were maintained at a constant daily prevalence of 0.75 mosquitoes per 1,000 females during the indicated date range, and 2.5 exposed birds were added on the indicated day.

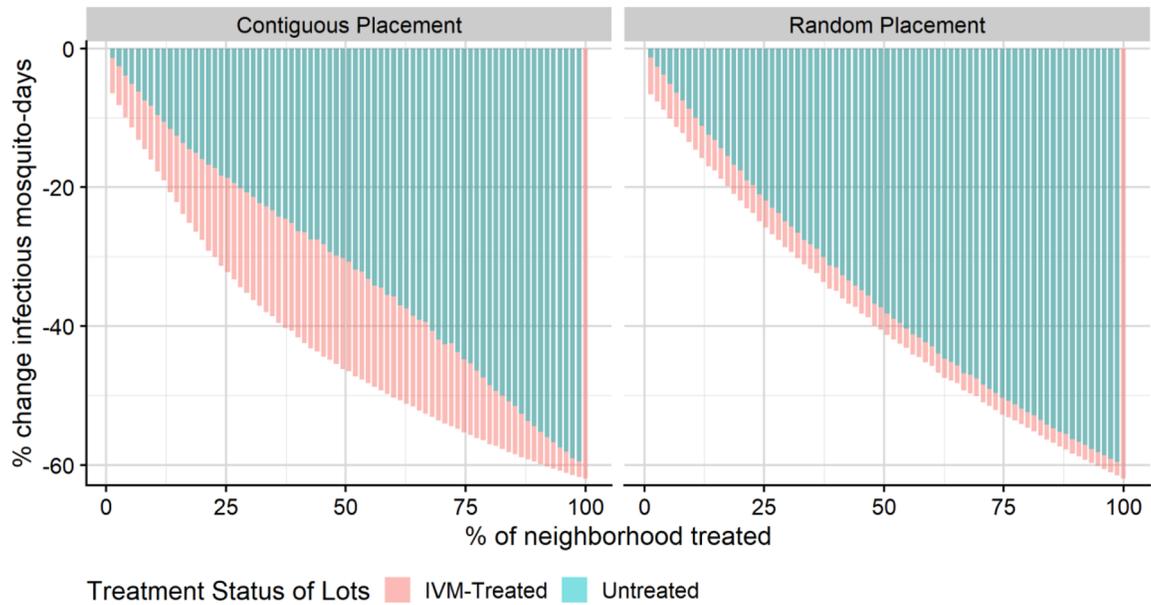


Fig 9. Percent reduction in total infectious mosquito-days in IVM-treated versus untreated lots. Change assumed 50% daily IVM-induced mortality in mosquitoes with contiguous (left) or random (right) placement of treated lots. The entire neighborhood consisted of 75 lots.

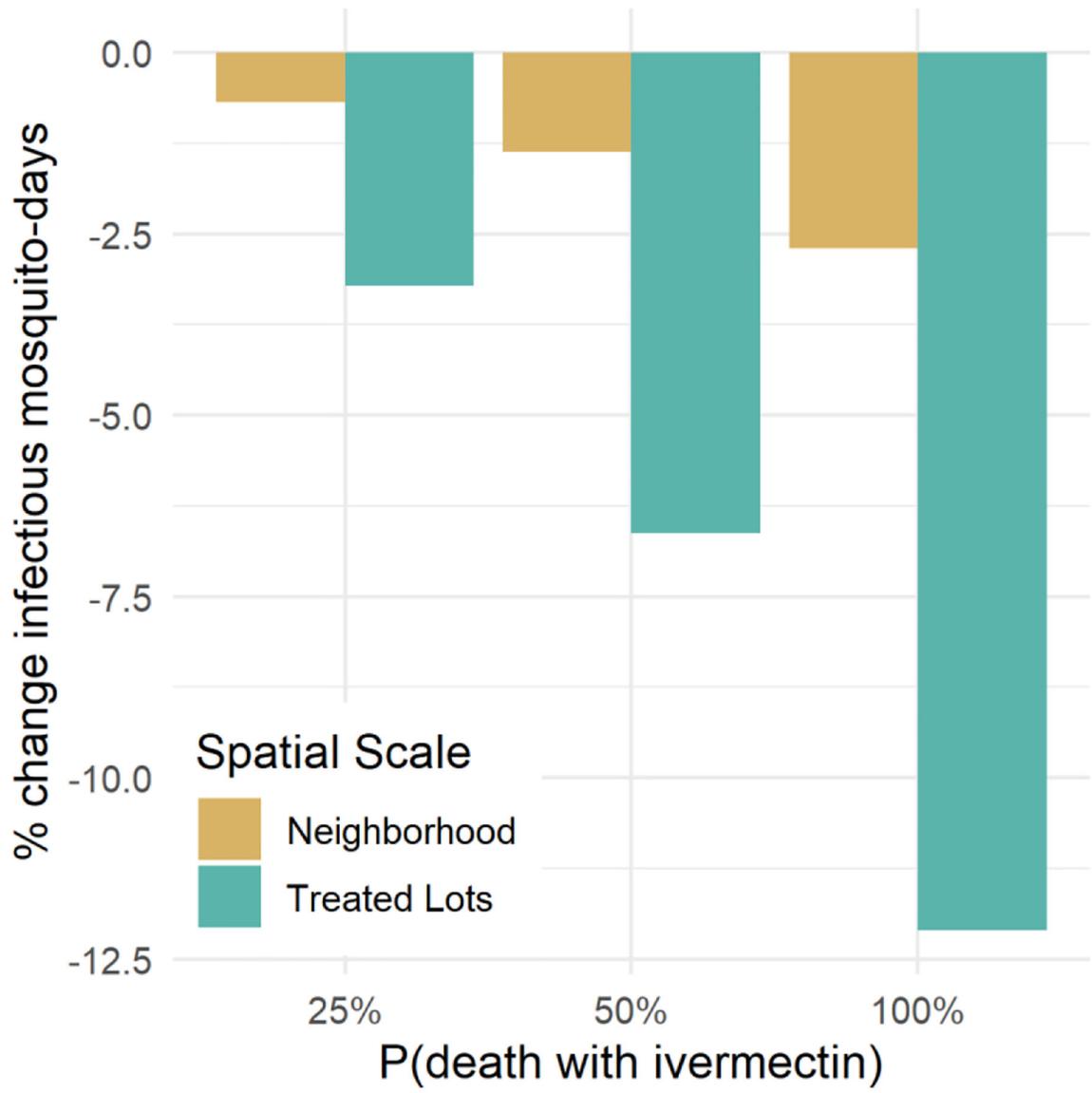


Fig 10. Reduction in infectious mosquito-days with a single treated lot.
 Comparison of overall, neighborhood-wide change versus change in the treated lots only.
 The entire neighborhood consisted of 75 lots.

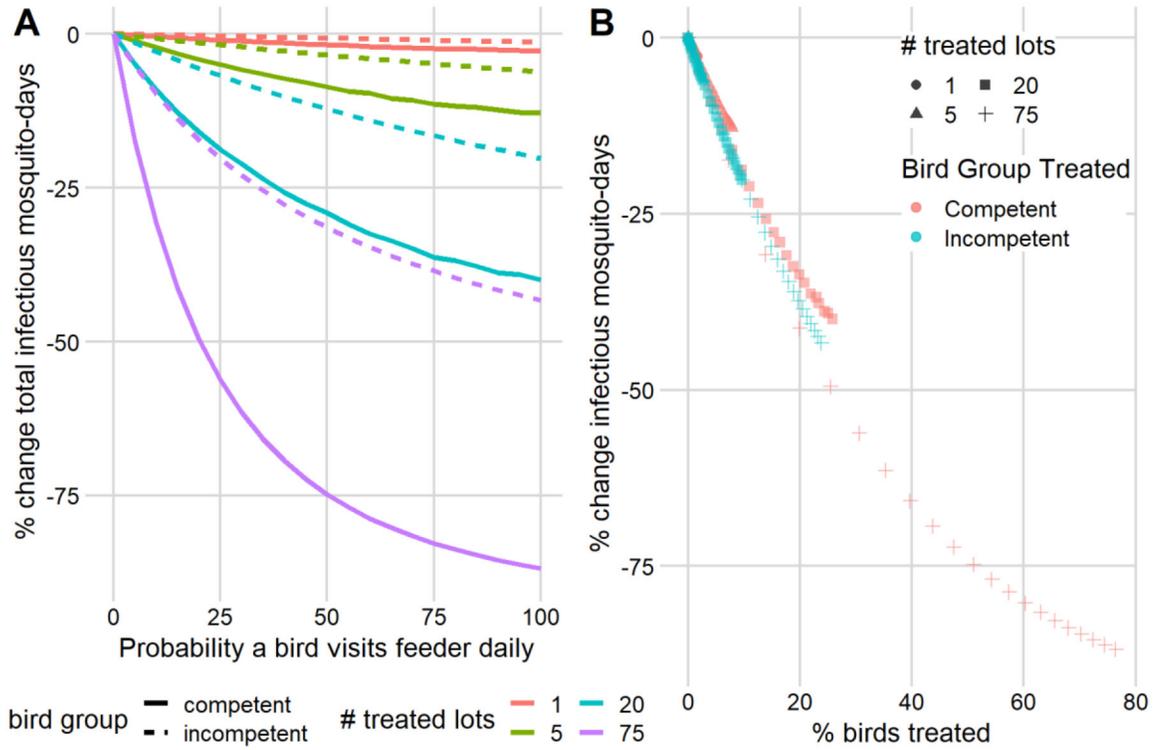


Fig 11. Reduced infectious mosquito-days with increased treated feeder visitation by competent and incompetent birds.

A) Change in infectious mosquito-days with increase in the daily visitation rates by each group of birds in four deployment scenarios of contiguous treated lots (1, 5, 20, and 75 treated lots). B) Accounting for the assemblage of the bird community (81% competent, 19% incompetent), the final proportion of treated birds in the neighborhood under each scenario and corresponding change in infectious mosquito-days. In each calculation, probability of daily feeder visitation was held constant at 0 for the other group.

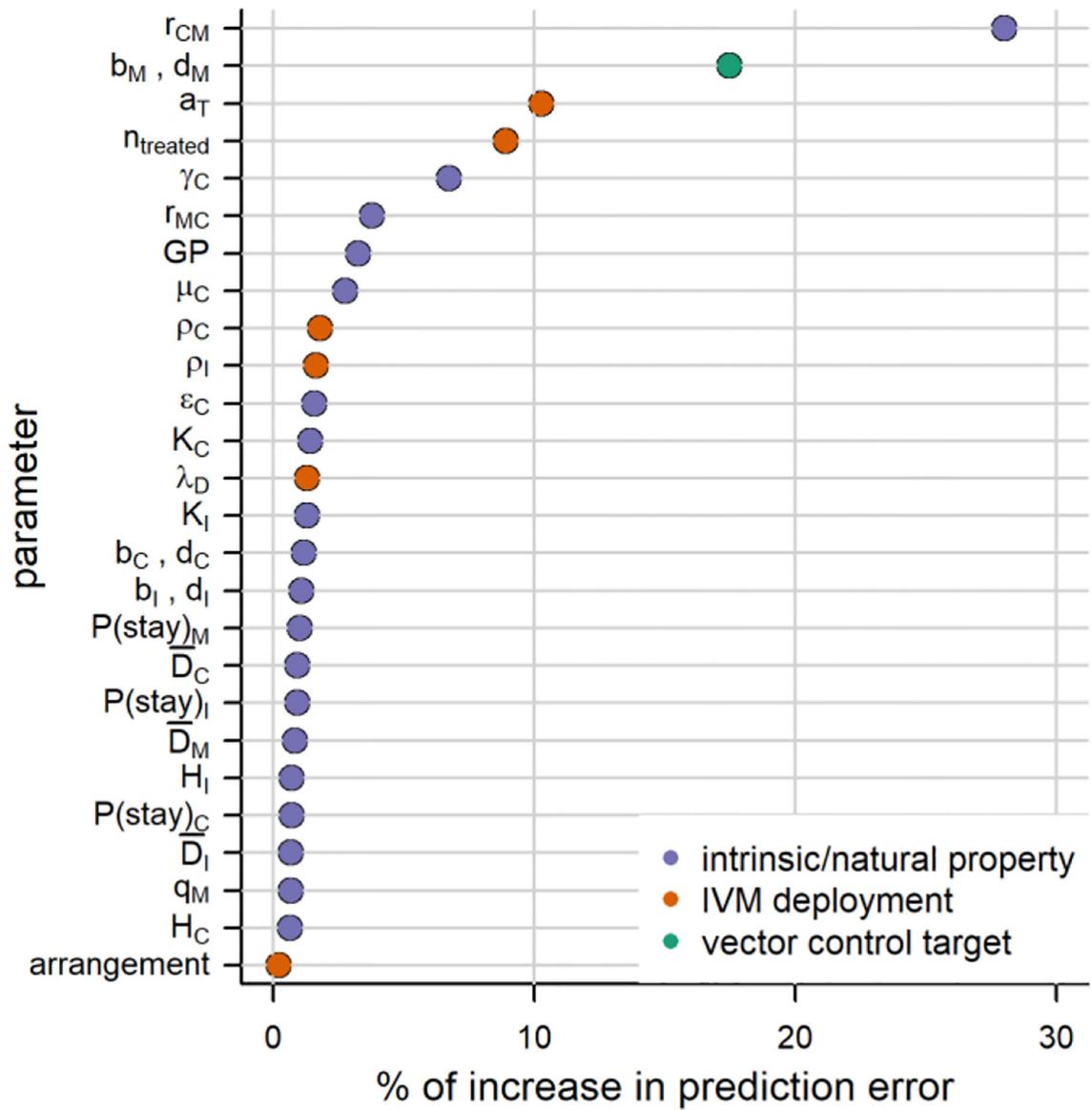


Fig 12. Contribution of parameter to increased prediction error. Increase in mean squared error of prediction when parameter was permuted in random forest analysis with higher error indicating higher relative importance. See Tables A–B in S1 Appendix for symbol definitions. Parameters modifiable with IVM deployment or vector control indicated.

Table 1.

Radio telemetry and radio frequency identification tagging of six common backyard bird species.

Species	Radio tag only	Radio + PIT [^] tag	PIT [^] tag only	Total tagged
Blue jay (<i>Cyanocitta cristata</i>)	0	2	0	2
Common grackle (<i>Quiscalus quiscula</i>)	0	3	0	3
House finch (<i>Haemorhous mexicanus</i>)	0	0	53	53
House sparrow (<i>Passer domesticus</i>)	3	2	0	5
Mourning dove (<i>Zenaida macroura</i>)	6	0	0	6
Red-wing blackbird (<i>Agelaius phoeniceus</i>)	0	12	24	36
Total	9	19	77	105

Tagging occurred in Fort Collins, Colorado during August 2020.

[^] Passive integrated transponder (PIT) tag for radio frequency identification system.

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Table 2.

Characteristics of movement between avian nocturnal roost locations.

Species	Total nights bird detected	Night pairs bird detected	% roost constancy [^]	Mean (m) [‡]	Standard deviation (m) [‡]
House sparrow (<i>Passer domesticus</i>)	25	21	57.1	223.7	153.5
	23	20	45.0	316.4	152.0
	24	23	60.9	165.7	129.3
	19	14	71.4	91.3	55.0
	21	19	68.4	200.6	133.1
	Average		60.6	219.3	149.4
Mourning dove (<i>Zenaida macroura</i>)	16	12	8.3	394.5	511.3
	19	15	6.7	939.5	703.4
	19	15	0.0	1,246.0	773.9
	20	15	40.0	755.5	303.8
	22	19	63.2	205.4	131.5
	2	1	0.0	88.6	NA
	Average		19.7	780.9	685.5
Common grackle (<i>Quiscalus quiscula</i>)	11	6	66.7	141.7	168.2
	3	2	0.0	778.0	202.0
	Average		33.3	459.9	397.5

Mean and standard deviation of distances between nocturnal roosting locations on consecutive nights.

[^] Percent of night pairs in which bird located in same roosting location.

[‡] Mean and standard deviation of distance between consecutive nocturnal roosts when roost location changed.