

THESIS

EVIDENCE FOR REGIONAL VARIATION IN THE THERMAL BIOLOGY OF
DENDROCTONUS RUFIPENNIS (KIRBY) (COLEOPTERA: CURCULIONIDAE,
SCOLYTINAE) IN COLORADO

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2018

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ABSTRACT

EVIDENCE FOR REGIONAL VARIATION IN THE THERMAL BIOLOGY OF *DENDROCTONUS RUFIPENNIS* (KIRBY) (COLEOPTERA: CURCULIONIDAE, SCOLYTINAE) IN COLORADO

Dendroctonus rufipennis (Kirby) (Coleoptera: Curculionidae, Scolytinae), the spruce beetle, is native to the forests of western North America where it is the primary cause of Engelmann spruce *Picea engelmannii* Parry ex Engelm. mortality. This study is an effort to further the knowledge of ecological conditions that affect *D. rufipennis* flight phenology, abundance, and fitness traits. Spruce beetle populations were monitored for two years at high elevation spruce forest sites; during this period of time 32,881 spruce beetles were captured, as well as significant quantities of off-target species. Trapping sites were classified by variation in thermal trends using hierarchical cluster analysis which revealed statistical support for two groups of sites ('cold' and 'warm'). A logistic function was used to model cumulative proportion of trap capture by day-of-year for all sites; and modeled phenologies indicated that beetles from cold sites terminate their flight period significantly earlier than beetles from warm sites. Additionally, the flight season is prolonged at warm sites, suggesting that hosts at warmer sites are exposed to beetle pressure for longer periods of time. At higher latitudes, the flight period was significantly longer than at lower latitudes, but there was no relationship between spruce beetle flight phenology and elevation. There was a significant female bias in the sex ratio at cold sites, but sex ratios were even at warm sites. Fitness traits (body size) varied along both latitudinal and elevational gradients; a negative correlation between elevation and pronotal width

was observed indicating that beetles were smaller at high elevations, but beetles tended to be larger at high latitudes. Correlations between spruce beetle abundances in traps and the abundances of predators and competitors were evaluated, but there was no evidence that either feeding guild was negatively impacting spruce beetle abundances. The results of this study suggest that fine scale environmental variation must be considered when managing or monitoring spruce beetle populations, as both site-level thermal variability and latitude are associated with variation in the flight period and corresponding biotic pressure on host trees.

ACKNOWLEDGEMENTS

My committee Drs. Seth Davis, Boris Kondratieff, Miranda Redmond, and Sky Stephens, for their contributions. Dr. Donald Bright for his assistance in identification of the beetles mentioned in this study. Dr. James Kruse for the use of a vehicle, without which this study would not have been possible. Dr. Jose Negron for lending me traps and temperature loggers. Dr. Thomas Eager for his sound advice. Matt Hansen for informative conversations. Andrew Mann for help in the field. Abi Cohen for counting thousands of *Polygraphus convexifrons* Wood (Coleoptera: Curculionidae, Scolytinae) from the 2017 catch. Kira Deming for proofreading. My friends and family for their support.

DEDICATION

To my parents.

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CHAPTER 1

THE THERMAL ECOLOGY OF SPRUCE BEETLE

Introduction

The spruce beetle, *Dendroctonus rufipennis* ((Kirby)) (Coleoptera: Curculionidae: Scolytinae) is a conifer-infesting bark beetle that feeds on the phloem of host trees (Wood 1982a) in the genus *Picea*. The range of spruce beetle encompasses most forested areas of western North America due to the broad distribution of its host trees. Spruce beetle populations are comprised of three genetically distinct groups which are differentiated by their host associations. White spruce (*Picea glauca* [Moench] Voss) is the primary host for two northern clades that range from Alaska eastward to Newfoundland, and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) is the primary host for a third genetic group that occurs in the Rocky Mountain region, and extends from Washington state southwards to Utah, Colorado, and Arizona (Maroja et al. 2007). Although southern populations of spruce beetle are capable of colonizing blue spruce (*Picea pungens* Engelm.), blue spruce is a poor host and is generally not affected by outbreaks (Maroja et al. 2007). Throughout much of its range, spruce beetle is considered a pest species, as adult tunneling and larval feeding damage tree vascular tissues and can result in large-scale tree mortality events (Dymerski et al. 2001).

Spruce beetles are associated with moderately pathogenic symbiotic fungi (*Leptographium abietinum* [Peck] M.J. Wingf. 1985) that modify host physiology by reducing concentrations of defensive chemicals (Davis et al., 20018) and greatly accelerate the rate at which host resistance mechanisms can be overcome (Allen et al. 2010). Spruce beetles typically overcome host trees ‘en masse’ via coordinated attack, and the combined challenge from beetle

oviposition, larval feeding, and fungal infection is a primary cause of host mortality. Mass attack behaviors are triggered by the production of a variety of volatile semiochemicals including aggregation pheromones such as frontalin and 1-methyl-2-cyclohexen-1-ol (MCOL) (Isitt et al. 2018). Host produced kairomones, including the monoterpenes α -pinene, myrcene, and terpinolene, synergize with pheromone signals to enhance the aggregation response (Wood 1982b). When a host tree is at full capacity following colonization, accumulation of anti-aggregate pheromones (3-methyl-2-cyclohexen-1-one; MCH) stops new beetles from attacking, preventing overcrowding, and helping to insure the survival of the next generation (Barkawi et al. 2003; Blomquist et al. 2010; Gray et al. 2015).

A warmer climate may increase *D. rufipennis* induced mortality of *P. engelmannii*. Foster et al. (2018) modeled spruce beetle infestations with current and warmer, dryer climate scenarios on the range of *P. engelmannii* in the southern Rocky Mountains. Their model predicts that a warmer, dryer climate will have an amplifying effect on *D. rufipennis* infestations of Engelmann spruce, and these infestations will aid lower-elevation tree species in colonizing higher-elevation forests, displacing *P. engelmannii*. Spruce beetle disturbance coupled with climate change produced a greater reduction in Engelmann spruce biomass than either factor did individually. Due to the reduced range of *P. engelmannii*, after one hundred years climate change had a negative effect on *D. rufipennis* infestations (Foster et al. 2018).

Effects of thermal variation on spruce beetle biology

As poikilotherms, temperature plays a major role in all stages of the life cycle of *Dendroctonus* beetles (Bentz et al. 1991; Gaylord et al. 2008; Hansen et al. 2011; Werner & Holsten 1985). One of the main factors directly affecting the timing and duration of beetle host seeking behavior and flight phenology is their thermal environment (Bleiker & Meyers 2017).

Spruce beetles are biologically inactive until temperatures rise above a lower threshold (approximately 5° C), and activity also typically declines if temperatures exceed an upper threshold (approximately 27°C) (Hansen et al. 2011). In many insect species, developmental rates and periods of biological activity are modeled by the accumulation of thermal units, typically measured in degree-days (°D). One ‘degree-day’ has accumulated if the temperature is exactly 1° above the lower threshold for one day (24 hours)(Wilson & Barnett 1983). A higher number of degree-days is typically associated with warmer locations and reduced developmental times, though this is not always the case.

During the winter, when temperatures are below lower activity thresholds, spruce beetle development is arrested by a cold diapause, which enhances survival in harsh environmental conditions (Masaki 1967; Masaki 1972; Masaki 1978; Roff 1980). Spruce beetles are vulnerable to freezing in the pupal life stage; as a result, diapause is typically initiated as IV instar larvae and teneral adults, which readily survive subfreezing temperatures (Hansen et al. 2011; Logan & Bentz 1999; Logan & Powell 2001; Dyer & Hall 1977). Spruce beetle is the only *Dendroctonus* species known to exhibit obligate diapause prior to reaching sexual maturity (Massey & Wygant 1954; Safranyik et al. 1990; Raffa et al. 2015). Consequently, the accumulation of degree-days and the duration of cold diapause may interact to influence the rate at which new generations of spruce beetle are produced, and spruce beetle generational timing can range from univoltine (one generation per year) at warm sites to semivoltine (one generation every two or more years) at cooler sites, with a three-year lifecycle reported at the coldest sites (Hansen et al. 2011; Werner & Holsten 1985; Massey & Wygant 1954; Dyer 1969; Hansen et al. 2001a; Knight 1961). In semivoltine populations facultative prepupal diapause occurs in fourth instar larvae (Hansen et

al. 2011; Dyer & Hall 1977); however, photoperiod does not appear to influence whether spruce beetle undergo diapause (Hansen et al. 2011).

Timing of generations (i.e., voltinism) of spruce beetle has consequences for forest ecosystems. Univoltine broods increase the potential for new outbreaks to occur and may increase the rate of tree mortality in established outbreaks, due to greater combined biotic (beetle challenge) and abiotic pressure (warmer temperatures) on host trees (Hansen et al. 2011; Reynolds & Holsten 1994; Hansen & Bentz 2003; Berg et al. 2006; Bentz et al. 2001). If temperatures are warm enough, some proportion of the spruce beetle population can be expected to achieve a 1-year life cycle (Hansen et al. 2001b). However, it is not presently understood whether spruce beetle flight phenology or voltinism are selected by processes that occur at a landscape scale or is the product of highly localized variation in thermal conditions.

Univoltine beetles are significantly smaller than semivoltine beetles, accordingly, body mass is a good indicator of voltinism in spruce beetle (Safranyik 2011). Many studies have shown that development time is associated with adult insect mass, and body size is often used as a surrogate for lipid content, flight distance, and overall fitness (Masaki 1967; Masaki 1972; Massey & Wygant 1954; Hansen & Bentz 2003; Safranyik 2011; Robertson 1960; Kamm 1972). Other factors affecting adult size include feeding rate as well as nutritional quality and availability of food (Hilbert 1995). Spruce beetles from warmer sites have shorter development times and may be represented by smaller, univoltine adults (Bentz et al. 2001), though phenological windows can also vary along latitudinal and elevational gradients. For instance, Logan and Bentz (1999) modeled populations of mountain pine beetle (*Dendroctonus ponderosae* Hopkins) at different latitudes and determined that differences in growth rate and size were driven primarily by environmental conditions and not the nutritional content of host

trees. Thus, studying thermal variation as principle abiotic factors affecting beetle body size may provide insight into environmental patterns that drive outbreak behaviors.

While there has been considerable research into the mechanistic effects of temperature on developmental time and adult size of *Dendroctonus* bark beetles (Logan and Bentz 1999; Bentz et al. 2001; Safranyik 2011; Tr n 2007), few studies examine the relationship between thermal trends and variation in spruce beetle flight phenology at regional scales, which could impact local monitoring and pest management efforts. Recent research suggests that the number of days exceeding 13°C (‘threshold days’) is the most accurate thermal metric for predicting the cumulative proportion of spruce beetle trap capture (as a measure of flight phenology) in northern Colorado (Davis and Hansen 2017). Spruce beetle emergence and flight periods are synchronous and emergence behaviors are responsive to the thermal environment. For example, in a laboratory study of Canadian spruce beetle populations, ambient temperatures of at least 4°C lasting 70 days was required for mass emergence (Bleiker & Meyers 2017). In Alaska, field studies of the flight and host colonization by spruce beetle showed that 12.8 °C was the lower threshold for flight initiation, and that peak flight occurred at and above 16 °C (Holsten & Hard 2001). Localized knowledge of thermal trends and how they relate to flight phenology and abundance can enhance our interpretation and prediction of population dynamics and patterns of tree mortality from the level of individual sites to the entire range of spruce beetle in Colorado.

As well as impacting basic insect physiology, the symbiotic fungi of *Dendroctonus* beetles are also highly responsive to thermal conditions, but this varies across beetle-fungus associations and geographic locations. For example, optimal growth of symbiotic fungi associated with *D. frontalis* Zimmermann in the southeastern United States occurs between 15-28°C, with significant declines in growth beyond these thresholds (Hofstetter et al. 2007). In

Arizona, symbiotic fungi of *D. brevicomis* were constrained by a narrower range of temperature thresholds (20-30°C, with evidence for higher thermal tolerances) (Davis et al. 2010). In contrast, fungal associates of the spruce beetle grow across a wide range of temperatures (5-28°C), with extreme reduction in growth beyond 28°C (Davis et al. 2018), potentially indicating that a large range of thermal conditions are suitable for spruce beetle and their fungal symbionts.

In addition to beetle behaviors and impacts on fungal symbionts, beetle pheromonal communications can also be impacted by temperature, with elution rates generally increasing under warm conditions (Holsten et al. 2002). In a laboratory experiment, air temperature was controlled to within ± 1.0 °C and relative humidity $\pm 2.0\%$, and pheromone mass was measured within 0.1 mg at ambient temperature of 20, 25, 30, 35 and 40 °C and relative humidity of 30, 50 and 80%. While not affected by relative humidity, when the ambient temperature increased from 20 to 40 °C, the release rate of the semiochemical MCH (used to repel bark beetles) increased by 6.5-fold (Zhu et al. 2015). A similar experiment was conducted on elution rates for one of the primary aggregation pheromone components (frontalin) of commercial spruce beetle lures. Over a period of two months, a 600 mg pouch of Frontalin at a mean temperature of 17.5 °C eluted at a rate of 6.76 mg/day in the sun, but elution rate was 6.23 mg/day in the shade, an approximately 8% difference (USDA Forest Service, Forest Health Protection 2007). Also, elution rates of secondary compounds produced by coniferous trees, including monoterpenes, are known to increase with increasing temperature (Juuti et al. 1990). This is significant because monoterpenes are used by pioneer beetles during host-finding. Thus, an increase in ambient temperature could affect beetle attack densities by altering emission rates of pheromones and kairomones into the atmosphere (Zhu et al. 2015; Juuti et al. 1990).

Other physiographic factors, including elevation or latitude, also impact bark beetle size and population activity. In a laboratory study of *D. ponderosae* beetles reared from higher latitudes had significantly shorter development time and smaller adult body size than beetles reared from lower latitudes. Numerous other studies of insect size and developmental duration concur with their results (Masaki 1967; Masaki 1972; Masaki 1978; Roff 1980). In a field study, bark beetles at lower elevational bands had longer flight periods: flight initiation occurred earlier and ended later (Williams et al. 2008). Negron et al. (2009) conducted a field study of bark beetle induced mortality of ponderosa pine in Arizona and found that tree mortality increased significantly as elevation decreased (implying increased beetle population activity, abundance, and body size). So, for the bark beetles in the above-mentioned studies, both latitude and elevation have been shown to effect bark beetle size and population activity, likely due to temperature differences, which makes it reasonable to expect that spruce beetle populations from southern latitudes or low elevations will be larger, more fit, and better able to kill trees than smaller ones. Therefore, it is reasonable to examine these physiographic factors on spruce beetle populations as well.

Fundamental ecological interactions such as predation and competition can also regulate bark beetle populations. In field experiments populations of the predatory clerid *Thanasimus undatulus* Say abundances were greater than that of spruce beetles in funnel traps (baited with a standard spruce beetle attractant blend), indicating the checkered beetle is co-attracted to the aggregating pheromones of its spruce beetle prey to a degree that may reduce spruce beetle abundance (Poland & Borden 1997). Field studies have shown that secondary (competing) scolytid beetles pheromones significantly reduced spruce beetle abundance of broods, attacking adults and gallery development (Werner & Holsten 2002). Lab based biological control studies

have demonstrated that increased densities of predator *T. dubius* Fabricius, in the larval form, had a significant reduction in the number of southern pine beetles (*D. frontalis* Zimmermann) emerging from infested logs (Costa & Reeve 2012). Due to their demonstrated potential to regulate bark beetle abundance, the biotic effects of predation and competition also need to be examined.

Spruce beetle continues to be the most dramatic and extensive insect disturbance agent in Colorado's high-elevation forests, with current active infestations in an estimated 83,365 hectares of Engelmann spruce forests, of which over 27,000 were new hectares infested in 2017 (Colorado State Forest Service's Report on the Health of Colorado's Forests 2017). This study is an effort to further our understanding of the basic autecology of spruce beetle as related to beetle flight behaviors, with the goal of modeling flight phenology and abundance based on environmental conditions and biotic factors measured at sites spanning the latitude of Colorado.

CHAPTER 2

ECOLOGICAL FACTORS ASSOCIATED WITH VARIABLE FLIGHT PHENOLOGY AND ABUNDANCE OF SPRUCE BEETLE *DENDROCTONUS RUFIPENNIS* (KIRBY) (COLEOPTERA: CURCULIONIDAE, SCOLYTINAE) IN COLORADO

Introduction

The spruce beetle, *Dendroctonus rufipennis* (Kirby) (Coleoptera: Curculionidae: Scolytinae), has caused considerable economic loss of timber resources and ecological change in the forest systems of western North America. The economic damage is estimated at a loss of 333 to 500 million board feet (mbf) of spruce saw timber annually (Holsten et al. 1999), and the beetle is perhaps the most important natural mortality agent of mature spruce species and their hybrids in North America. In Colorado, an estimated ~720,000 cumulative hectares have been affected by spruce beetle outbreaks since 1996 (Colorado State Forest Service's Report on the Health of Colorado's Forests 2017). Outbreaks can have deleterious effects on forest management efforts by creating hazard trees, unbalancing carbon budgets, or alteration of nutrient cycling processes, hydrological patterns, and fire regimes (Klutsch et al. 2011; Pye et al. 2011; Kurz et al. 2008; Edburg et al. 2012; Jenkins et al. 2008; Jenkins et al. 2012; Mikkelsen et al. 2013). Due to these effects, it is critical to understand the factors that may regulate beetle populations and their periods of activity.

The studies reported here are an effort to further our understanding of the basic biology of spruce beetles, with the goal of optimizing population monitoring efforts. Knowledge of the ecological factors that relate to beetle flight phenology and abundance regionally can enhance

our interpretation and prediction of population trends. To better manage populations, there is a need for studies which elucidate the understanding of the biotic and abiotic variables affecting trap captures and flight phenology (such as thermal trends, latitude, elevation, predators, and competitors/associates). This knowledge is useful to practitioners concerned with reducing the likelihood of epidemic spruce beetle outbreaks. Although there are studies describing interactions between environmental conditions, including drought and temperature, on observed mortality from spruce beetle across forest landscapes and large spatial scales (Berg et al. 2006), it is not yet clear how environmental conditions may relate to variation in actual beetle abundances.

To address this deficiency, I ask the question “What are the factors driving spruce beetle flight phenology, abundance, and fitness traits in the Southern Rocky Mountains?”. I hypothesized that (1) abiotic factors including (a) latitude, (b) elevation, and (c) thermal variability are associated with flight phenology and beetle abundances; and that (2) biotic factors such as (a) predator abundance and (b) competitor abundance affect spruce beetle trap capture abundance. To test these hypotheses, forested sites were classified by variation in thermal trends, and spruce beetle abundances and associated insects were characterized for two years at a weekly interval during the flight period. In addition, I evaluated how beetle fitness traits such as body size and sex ratio vary with the above factors. These studies provide new insights into an array of ecological conditions that affect the basic biology of a destructive forest pest and provide new information that impacts population monitoring efforts and appropriate temporal windows for implementing potential management actions.

Methods and Materials

Site Selection

To identify areas with active populations in order to collect sufficient numbers of beetles for statistical analysis, a total of n=15 sites were selected based on 2015 and 2016 aerial survey polygons of spruce beetle caused tree mortality (USDA Forest Service, Forest Health Protection 2017). Site selection was constrained to occur within 1.6 kilometers of a major highway, using a geographic information system (ArcMap 10.5; ESRI Inc., Redlands, California) (Figure 1).

Forest cover at sites was predominantly Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), with subalpine fir (*Abies lasiocarpa* (Hooker) Nuttall), lodgepole pine (*Pinus contorta* Douglas), and quaking aspen (*Populus tremuloides* Michx.) often present as well. Site locations spanned the latitude of Colorado as well as a range of elevations, and spruce beetle populations were monitored across the growing season (April-September) for two years (2017 and 2018) (Table 1).

Spruce beetle trapping

Beetles populations were monitored at field sites by trapping using standard methods (Lindgren 1983). Traps were serviced at weekly intervals beginning in April and collected until no further captures occurred, typically in August or September in each project year but varied by trapping site, for a total of ten months. The minimum distance between trapping locations was >140 m, but most traps were >1.0 km in distance. Traps consisted of black 12-unit Lindgren funnel traps (Lindgren 1983) hung on available trees at a height of 1 m from the base of the collection cup to the ground surface and were baited with standard *D. rufipennis* pheromone lures containing frontalinal, 1-methylcyclohex-2-en-1-ol (MCOL), and a proprietary blend of host tree kairomones (a proprietary blend of monoterpenes; Synergy Semiochemicals, Victoria,

British Columbia). Although elution rates decline over time from these lures, they typically remain effective throughout a growing season (Davis & Hansen 2017), and accordingly a single lure per site was used each year. Collection cups were supplied with pesticide strips (Vaportape™, Hercon Environmental, Emigsville, Pennsylvania) to prevent sample loss due to predation or intraspecific competition. Voucher specimens of beetles mentioned in the study are deposited in the C. P. Gillette Museum of Arthropod Diversity, Colorado State University.

Temperature measurements

At each site the ambient air temperature was measured at hourly intervals throughout the trapping period using a temperature data logger (HOBO Pendant® Model: UA-002-08 Onset Computer Corporation, Bourne, Massachusetts). Data loggers were covered in white card paper to shade them from direct sunlight and secured with wire at 1.3 to 2.3 m of height on the bole of a *P. engelmannii* at a random aspect. Temperature data from loggers was downloaded periodically during the trapping study to confirm continued function, and data were summarized to yield site-level daily thermal metrics such as daily mean, minimum, and maximum temperature. Traps and temperature loggers were deployed in mid-March of 2017. This ensured that temperature loggers were recording before the temperature threshold of 13° C was passed and that traps were in place before beetles were flying. At selected study sites in 2017, phloem temperatures were measured at 1.3 m of height on the bole of a *P. engelmannii* by drilling a hole, in the direction of the ground, the same diameter as the temperature probe on the north and south aspects. A preliminary analysis revealed that phloem and air temperatures were highly correlated (Table 2). Accordingly, only air temperatures were recorded in 2018, and ambient air temperatures were used in all subsequent analyses. It was also determined that ambient

temperatures were correlated between years at sites (Table 3); accordingly, temperature data was averaged together from 2017 and 2018.

Beetle body size and sex ratio

To evaluate whether beetle fitness or life history traits varied across collection sites or thermal conditions, a subset of captured beetles were dissected to determine sex and measured to determine body size. A total of $n=30$ beetles from fourteen sites were selected randomly from the collection date nearest estimated 50% cumulative trap capture for each site; beetles from the Rabbit Ears Pass site were excluded from this analysis on the basis of $n<30$ beetles captured in 2017. I measured the pronotal width of a total of $n=840$ beetles ($n=420$ beetles in 2017 and $n=420$ beetles in 2018) to the nearest 0.025 mm using an ocular micrometer (VWR® 2305.2 micrometer eyepiece: Graduated Linear Line, Point = 0.2mm/14mm [Widefield 10X/F.N. 23] at 5X magnification using a VWR® Stereo Zoom Binocular Microscope featuring the Greenough Zoom Optical System. The unit offers a 6.7:1 zoom ratio, WD = 113mm, and a magnification range of 0.75X–5X.). Following body size measurement, beetles were dissected using sharp forceps and the presence or absence of male genitalia was used as the determinate of sex, after the characteristics described in Wood (1982a).

Statistical analyses

Effects of abiotic factors on spruce beetle flight phenology and abundance

Sites were classified by thermal variation using a hierarchical cluster analysis (Ward's method using Euclidian distance) (Ward 1963) incorporating a multiscale bootstrap resampling procedure ($n=10,000$ resamples) to generate an estimate of statistical significance, approximately unbiased probability values and bootstrap probability values (approximately unbiased p -values of $p<0.05$ were considered to be statistically supported clusters of sites). These groupings of sites

were based on mean site-level thermal variables: maximum, minimum, and average temperature, total degree days base 13°C, total number of days exceeding 13°C (threshold days), the coefficient of variation in mean temperature, and the temperature range (temperatures were recorded over two growing seasons, from April to September). Clustering analysis was implemented in the R statistical programming language (R Core Team 2017) using the add-on package ‘pvcust’ (Suzuki & Shimodaira 2006).

To test if flight phenology varies across sites classified by thermal variability, I compared trap captures across cluster identities. Beetle abundances at each site were converted to cumulative trap captures (%) for each collection date. As described in Davis and Hansen, cumulative trap captures were fitted to ordinal day (i.e., day of year). I ran multiple candidate sigmoid models of cumulative trap capture using two-, three-, and four-parameter logistic and three- and four-parameter Gompertz functions using the statistical software JMP 13.0 (SAS Institute, Cary, North Carolina), with trapping site and year as random effects. The model with the lowest Akaike’s information criteria (AIC) score was selected. Statistical significance was assessed using a Type I error rate of $\alpha=0.05$. The model selected, a two-parameter logistic function: $\frac{1}{(1+\text{Exp}(-a(\text{Ordinal day}-b)))}$ (where a = the growth rate and b = the inflection point), accounts for 84% of the variance in cumulative *D. rufipennis* trap captures across sites (RMSE = 0.15). Cumulative trap capture data from each site were then fitted to ordinal day with the two-parameter logistic function, using the R add-on package ‘nplr’ (Commo & Bot 2006). Logistic functions were solved to determine the day of year at which specific trap capture thresholds occurred (10, 25, 50, 75, and 90% trap capture). Using one-way ANOVA, I compared the mean day-of-year at which 10, 25, 50, 75, and 90% trap captures occurred between ‘warm’ and ‘cool’ sites (here after “trap capture thresholds”). Statistical significance was assessed using a Type I

error rate of $\alpha=0.05$. Pearson's r was used to analyze whether ordinal day at the above trap capture thresholds were correlated with the physiographic effects of elevation and latitude.

One-way ANOVA was used to determine if there was a significant difference in body size between beetles from warmer cluster sites and colder cluster sites, and least-squares linear regression was used to test the hypothesis that beetle body size and sex ratio varied due to the physiographic effects of elevation and latitude. A chi-square (χ^2) test was used to test the hypothesis that beetle sex ratios were equal between clusters and years. A Type I error rate of $\alpha=0.05$ was used to assign statistical significance to all hypothesis tests.

Effects of biotic factors on spruce beetle flight phenology and abundance

Typically, the presence of predators and competitors are associated with a decline in population abundances of bark beetles (Poland & Borden 1997; Werner & Holsten 2002; Costa & Reeve 2012). Significant numbers of off-target insects (i.e., insects other than spruce beetle) were captured during the trapping period, especially predatory and secondary opportunistic or saprophytic beetles. In particularly high abundances were *Thanasimus undatulus* Say (Coleoptera: Cleridae), a predatory beetle, and *Polygraphus convexifrons* Wood (Coleoptera: Curculionidae, Scolytinae), a secondary colonizer that is co-attracted to spruce beetle pheromones. Accordingly, I used least-squares linear regression to test the hypothesis that spruce beetle abundances in traps would decrease as predator (*T. undatulus*) and competitor (*P. convexifrons*) abundance in traps increased.

Results

Hierarchical cluster analysis revealed statistical support for two groups of sites, one group of sites was characterized by overall warmer temperatures, the other group of sites was characterized by colder temperatures and a higher degree of variability in mean temperatures as measured by the coefficient of variation (refer to Figure 2 for bootstrap probability values). Hereafter, these sites groupings are referred to as ‘cold’ and ‘warm’ sites, and statistically supported clusters were subsequently used as a basis for grouping sites during analysis of flight phenology and beetle traits.

Altogether, 32,881 spruce beetles were trapped over two growing seasons; 22,077 in 2017 and 10,804 in 2018. Although there was considerable variation between the sites in terms of total trap captures (Figure 3), there was not a significant difference in spruce beetle abundances between ‘warm’ and ‘cold’ sites ($F_{1, 10678}=1.268, p=0.260$).

The two-parameter logistic function: $\frac{1}{(1+\text{Exp}(-a(\text{Ordinal day}-b)))}$ where a = the growth rate and b = the inflection point, was used to model cumulative proportion of trap capture by day-of-year for all sites (Figure 4). Averaging captures together across clusters revealed evidence for differential phenology in ‘warm’ and ‘cold’ sites (Figure 5a), with flight periods generally occurring later at ‘warm’ sites. This was supported by a nested ANOVA model, which indicated that there was significant variation in the mean day-of-year at which trap capture thresholds occurred between ‘warm’ and ‘cold’ sites (Table 4). Specifically, subsequent contrast tests revealed that the day-of-year at which 50, 75, and 90% trap capture occurred differed between ‘warm’ and ‘cold’ sites (Table 5, Figure 5b).

Physiographic factors had variable effects on spruce beetle flight phenology. Increasing latitude was associated with later spruce beetle accumulations, with marginally significant

negative correlations between latitude and the mean day-of-year at which 50, 75, and 90% trap capture occurred (Table 6a). However, there were no correlations between elevation and trap capture phenology (Table 6b).

Spruce beetle sex ratios were also associated with site thermal conditions. Female:male sex ratio was 54:46% and 64:36% at cold and warm sites respectively, and this difference was significant ($\chi^2=9.142$, $df=1$, $n=840$, $p=0.002$). However, there was no difference in mean beetle size as measured by pronotal width between cold and warm sites ($F_{1, 838}=1.005$, $p=0.316$); mean pronotal width was 2.320 ± 0.007 mm.

Physiographic factors had little impact on sex ratios but were associated with variation in spruce beetle sizes. Latitude did not have a significant effect on female:male sex ratio ($F_{1, 12}=0.088$, $p=0.771$); however, as latitude increased there was a significant increase in beetle size ($F_{1, 12}=10.164$, $p<0.0078$; Figure 6a). Similarly, elevation had no effect on female:male sex ratio ($F_{1, 12}=0.960$, $p=0.346$), but as elevation increased there was a significant decrease in beetle size ($F_{1, 12}=9.072$, $p<0.011$; Figure 6b).

Effects of biotic factors on spruce beetle abundance

Contrary to expectations of a negative relationship, linear regression showed that as predator (*T. undatulus*) abundance increased there was a corresponding significant increase in *D. rufipennis* abundance ($F_{1, 427}=230.654$, $p<0.001$; Figure 7a). Similarly, as competitor (*P. convexifrons*) abundance increased there was a corresponding and significant increase in *D. rufipennis* abundance ($F_{1, 293}=28.067$, $p<0.001$; Figure 7b).

Discussion

The entire life cycle of *Dendroctonus* beetles is heavily influenced by temperature. One of the main factors affecting voltinism, body size, and flight phenology is the thermal

environment (Hansen et al. 2011; Werner & Holsten 1985; Massey & Wygant 1954; Safranyik 2011; Dyer 1969; Hansen et al. 2001a; Knight 1961). Sites from across the latitude of Colorado were grouped into two distinct groups based on thermal characteristics, and analysis of spruce beetle abundances for two growing seasons (approximately 2,700 trap-days) revealed that flight phenology and sex ratios varied due to thermal conditions, but overall beetle abundance and body size did not. At colder sites spruce beetles flew earlier than beetles at warmer sites, and sex ratios were close to 50:50. In contrast, beetles at warm sites continued to fly and respond to sex pheromones for at least a week or longer on average, and sex ratios were female-biased. This indicates that at warmer locations, spruce beetles have a larger window of opportunity to attack host trees and may be capable of more rapid population growth, which is potentially associated with higher rates of host tree mortality on the landscape. Accordingly, it cannot be assumed that spruce beetle behaviors and life history are consistent across large or small geographic regions, as thermal conditions likely affect spruce beetle phenology on a site-by-site basis, and the timing of management actions may need to be modified.

Although warmer sites experienced prolonged spruce beetle activity, this may be due in part to the re-emergence of adults, as described in Hansen and Bentz (2003). It is known that parent beetles can re-emerge to build a second egg gallery closer to the root collar (Massey & Wygant 1954; Schmid & Frye 1977). To ‘mark’ re-emergent parents, they applied florescent powder on the basal 0.5 m of trees attacked the previous year. They determined that around 2.5% of spruce beetle trap capture was of re-emergent parents and that the peak flight capture for re-emergent parents was one week prior to the peak flight capture of all beetles. However, they found no difference in fecundity between the three brood types (univoltine, semivoltine, and reemergent parents). In a similar re-emergent bark beetle study Bentz (2006) concluded, “When

knowledge of the timing of [first generation] adult emergence is not important, pheromone traps remain a valid tool for mountain pine beetle population management.” The same is true of spruce beetle population monitoring and management: it is not important whether or not re-emergent parents are being captured. It is knowledge of the timing of the host seeking flight of the reproductive population that is important.

While other studies have examined effects of thermal conditions on behaviors and life history of other *Dendroctonus* species (e.g., mountain pine beetle) (Bentz & Powell 2014), this study is the first to examine them for spruce beetle across a large latitudinal and elevational gradient in the southern Rockies. Elevation had no significant relationship with spruce beetle phenology, but beetles flew for longer at higher latitudes. The lack of significant correlations between elevation and the day of year at which trap capture thresholds were reached differs from the results of Williams et al (2008), who found that at lower elevational bands multiple species of bark beetles began their flight period earlier and ended it later than beetles at higher elevational bands. However, Williams et al. (2008) evaluated bark beetle phenology over an approximately 1000 m elevational gradient, whereas the present study only evaluated phenology over an elevational gradient of ~500 m that also varied in latitude and aspect and for a single species of bark beetle.

Many studies have shown that development time is associated with adult insect body size, and body size is often used as a surrogate for lipid content, flight distance, and overall fitness in insects (Masaki 1967; Masaki 1972; Masaki 1978; Roff 1980; Hansen & Bentz 2003; Safranyik 2011; Robertson 1960; Kamm 1972). Physiographic factors including latitude and elevation were associated with variation in spruce beetle body size: as latitude increased, so did beetle body size, but beetles were also smaller at higher elevations. This could indicate that beetles from high

latitudes are generally more fit, but beetles from high elevations are generally less fit (Bentz et al. 2013). Bentz et al. (2001) found that mountain pine beetle reared from high-latitude populations had a significantly smaller adult body size than beetles reared from low-latitude populations; modeled populations of mountain pine beetle from different latitudes yielded very similar results (Logan & Bentz 1999). This suggests that physiographic patterns impacting beetle size are not generally conserved across *Dendroctonus* species, and can vary even among congeners that colonize trees in similar montane environments such as spruce beetle and mountain pine beetle. It is unclear if the the variation in body size observed here is due to different life history strategies of spruce beetle across sites, as we cannot directly determine whether beetles were univoltine, multivoltine, or a mix of both strategies. Safranyik (2011) found that univoltine populations of spruce beetles from warmer sites had no sex bias (50% males: 50% female), but semivoltine populations from colder sites had a 60% to 40% female bias. I report a contrasting result: populations from warmer sites had a 60% female bias, but sex ratio was equivalent at colder sites. Previous studies also indicate that univoltine spruce beetle populations from warmer sites have smaller adults due to shorter development times (Bentz et al. 2001; Safranyik 2011), but my study did not suggest any evidence for body size variation due to thermal conditions.

In addition to abiotic drivers of spruce beetle abundance and fitness, biotic factors including predation and competition also impact populations, and interactions between these trophic levels may exert complex dynamics on spruce beetles (Negron et al. 2009; Poland & Borden 1997; Werner & Holsten 2002; Costa & Reeve 2012). For instance, secondary bark beetles that exploit resources created by tree-killing beetles may impact primary species by increasing competition and predator loads (Boone et al. 2008). I predicted that increasing

abundances of predators and competitors would be associated with a decline in spruce beetle abundance, however, abundances of both predatory beetles (*T. undatulus*) and secondary colonizers that inhabit the same phloem resources (*P. convexifrons*) were associated with an increased abundance of spruce beetle. This indicates that local abundances of spruce beetle are so high that a corresponding increase in the predator and competitor population size produced no observable reduction in *D. rufipennis* populations, this phenomenon was commented on by Furniss and Carolin (1977): “During outbreaks the beetle outruns its natural controls.” In other studies, intraspecific competition for resources has been reported as more limiting than interspecific interactions (Anderbrant et al. 1985; Davis & Hofstetter 2009), which is consistent with the present findings of no negative interspecific correlations. One possibility that explains the observed positive correlations between abundances of *D. rufipennis*, *T. undatulus*, and *P. convexifrons* is that *D. rufipennis* populations in Colorado are large enough to ‘sate’ the effects of predation and competition by providing sufficient prey and habitat resources, respectively.

With active infestations reported on ~83,000 hectares of Engelmann spruce forests in 2017 alone in Colorado (Colorado State Forest Service’s Report on the Health of Colorado’s Forests 2017) there is a need for accurate forest management and population monitoring efforts. For example, spruce beetle populations in non-defended windthrown trees are a concern, as it is thought they can build up sufficient numbers to begin attacking healthy trees (Reynolds and Holsten, 1994; Schmid and Frye 1977). In 1981 a population of *Ips typographus* L. (Coleoptera: Scolytidae) was monitored after a windthrow event of Norway spruce *Picea abies* Karst, in Denmark. The attack densities of standing trees around areas where windthrown trees were removed later, in the summer, were significantly higher than for areas that had the windthrown

trees removed earlier in the season. Wichmann and Ravn (2000) concluded that, "...dispersal may best be countered by removing old breeding sites/windthrown trees in the period between spring-flight and the emergence of the new generation, i.e. by using the windthrown trees as bait trees." Accordingly, management of windthrown tree material is important for reducing mortality risk from spruce beetle, but to be successful management must occur prior to the initiation of synchronous flight. Localized knowledge about the influence of thermal trends and physiography on spruce beetle flight phenology impact such management efforts and could allow practitioners to match their efforts to appropriate temporal windows. Here, I showed that spruce beetle phenology and accordingly appropriate management actions, are likely to vary with both temperature and latitude. Based on the results of my trapping studies, I recommend that windthrow management in colder sites should occur earlier than windthrow management efforts in warmer sites.

This study has demonstrated that using thermal trends to group sites can increase the power of bark beetle phenological models; here, trap capture thresholds occurred significantly sooner at colder sites than for warmer sites. In addition, as latitude increased trap captures tended to occur earlier. Ideally, several years of temperature data would be used to characterize interannual variability in thermal trends, in addition, having several years of trap capture data would increase the accuracy of the ordinal day model in predicting when trap capture thresholds are reached (Weslien 1992). The use of passive barrier traps, which produce trap capture data that is more representative of emergence cage phenologies than pheromone baited traps are, may also be warranted to better gauge flight phenologies (Schmitz 1984).

The results of this study provide new insights into an array of ecological conditions that affect the basic biology of a destructive forest pest and provide new information that impacts

population monitoring efforts and appropriate temporal windows for implementing mitigation actions. Forest managers should take into consideration the effects of thermal variance and a latitude gradient when engaging in spruce beetle monitoring, mitigation actions and future research. Using the methods described above, I was able to accurately model host seeking flight phenologies of spruce beetle populations from sites with differing thermal ecologies, using two years of data. I conclude that a complex suite of factors including thermal conditions, physiography, and associated insect communities can impact observations of spruce beetle flight phenology and fitness-related traits. Future studies could improve the robustness of phenological models by operating larger trapping arrays coupled with temperature sensors (Hansen et al. 2001b) over a period of several years (Davis & Hansen 2017).

Table 1. Colorado study site coordinates and elevation.

Site	Latitude (°)	Longitude (°)	Elevation (m)
Cameron Pass Northeast	40.556399	-105.877274	3025
Cameron Pass Southeast	40.555819	-105.875715	2980
Cameron Pass Southwest	40.539726	-105.880655	3072
Rabbit Ears Pass	40.396725	-106.606305	2815
Willow Creek Pass East	40.345827	-106.095980	2872
Willow Creek Pass West	40.344503	-106.097609	2866
Guanella Pass Bottom	39.649793	-105.708736	3092
Guanella Pass Middle	39.636728	-105.708745	3227
Guanella Pass Top	39.609991	-105.717101	3331
Monarch Pass East	38.504775	-106.328765	3378
Monarch Pass West	38.485979	-106.336259	3334
Spring Creek Pass North	37.986612	-107.241572	3336
Spring Creek Pass South	37.971219	-107.173641	3250
Wolf Creek Pass East	37.480767	-106.802267	3330
Wolf Creek Pass West	37.474650	-106.859370	2931

Table 2. Correlation analysis examining relationships between phloem temperatures at two aspects and ambient air temperature across the growing season at trapping locations in 2017. Correlation of maximum daily temperature between north aspect and south aspect phloem probes and ambient probes were examined at the following Colorado sites: Guanella Bottom, Monarch East, Monarch West, Rabbit Ears, Spring Creek North, Spring Creek South, Willow Creek East, Wolf Creek East, and Wolf Creek West. Phloem S: phloem temperature, south aspect; Phloem, N: phloem temperature, north aspect.

Variables	Pearson's r	n	p
Phloem, S vs. Ambient	0.929	1275	<0.001
Phloem, N vs. Ambient	0.955	1275	<0.001
Phloem, N vs. Phloem, S	0.940	1275	<0.001

Table 3. Interannual correlations for maximum (T_{\max}), minimum (T_{\min}), and mean temperatures (T_{mean}) from 2017-2018.

Variables	Pearson's r	n	p
T_{\max} , 2017 vs. 2018	0.759	125	<0.001
T_{\min} , 2017 vs. 2018	0.794	125	<0.001
T_{mean} , 2017 vs. 2018	0.826	125	<0.001

Table 4. Summary of a nested one-way ANOVA analyzing effects of cluster identity on mean day-of-year at which 10, 25, 50, 75, and 90% trap capture occurred.

Source	df	SS	F	p
Whole model	9	16122	46.154	<0.001
Cumulative proportion [Cluster]	8	15559	50.109	<0.001
Cluster	1	563	14.511	<0.001
Error	65	2522	-	-
Total	74	18645	-	-

Table 5. Summary of contrast tests comparing mean day-of-year at which cumulative trap captures (CTC) occurred between colder and warmer site groups. Degrees of freedom for *F*-tests was 1, 65 for all tests. Statistically significant contrasts are highlighted in bold text.

Contrast test	SS	F	p
'cold' vs 'warm' at 10% CTC	53.63	1.381	0.244
'cold' vs 'warm' at 25% CTC	80.43	2.072	0.154
'cold' vs 'warm' at 50% CTC	112.6	2.902	0.093
'cold' vs 'warm' at 75% CTC	150.3	3.871	0.053
'cold' vs 'warm' at 90% CTC	193.3	4.980	0.029

Table 6. Summarized correlation analysis examining relationship between day-of-year and physiographic factors across five trap capture thresholds. Significant correlations are highlighted in bold text.

Variable	Trap capture threshold	Pearson's r	n	p
(a) Latitude	10%	-0.297	15	0.280
	25%	-0.391	15	0.149
	50%	-0.461	15	0.083
	75%	-0.482	15	0.068
	90%	-0.467	15	0.079
(b) Elevation	10%	-0.063	15	0.821
	25%	-0.112	15	0.688
	50%	-0.158	15	0.572
	75%	-0.186	15	0.506
	90%	-0.196	15	0.483

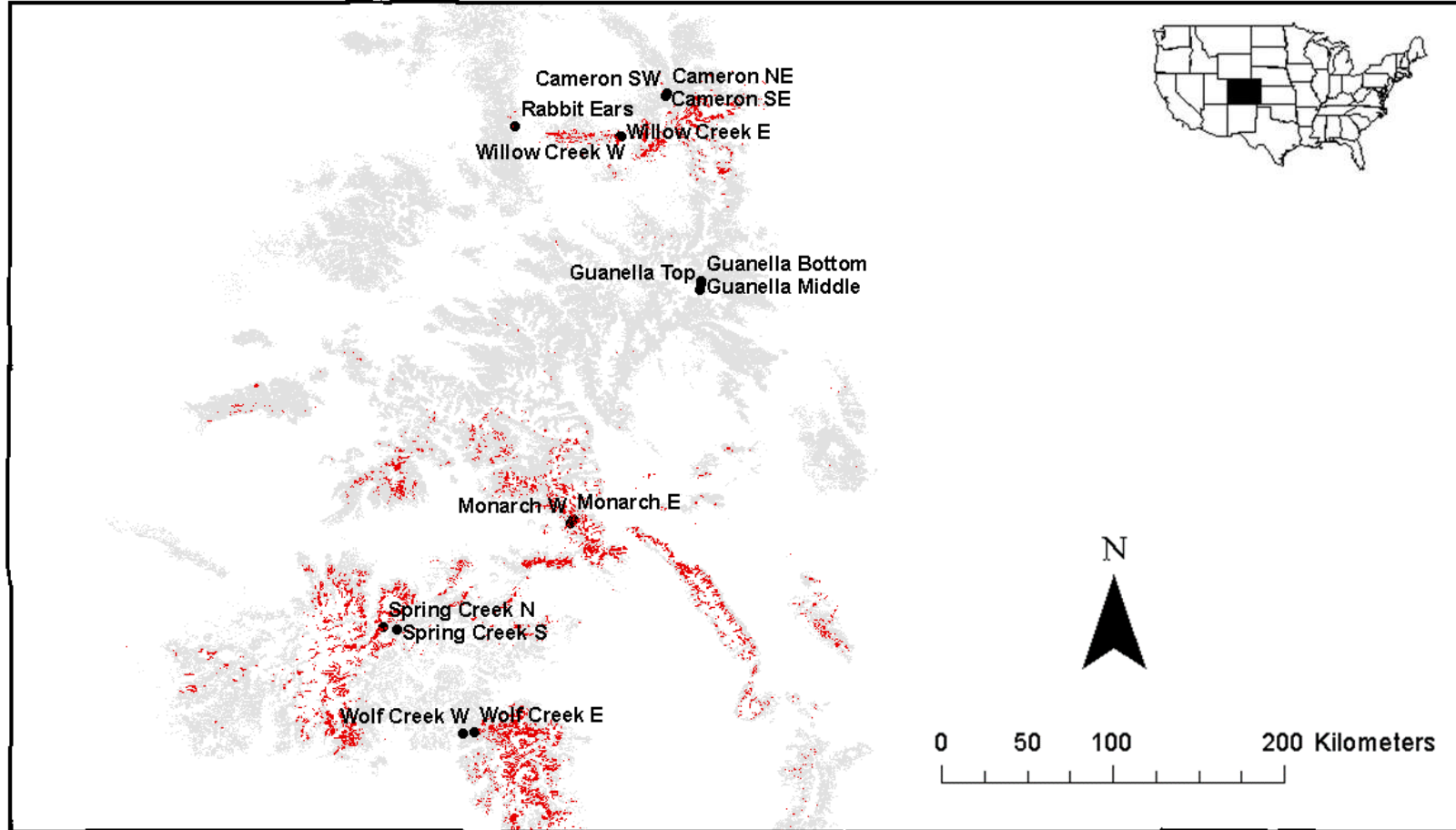


Figure 1. Map of Colorado with all 15 study sites labeled, range of host Engelmann spruce *Picea engelmannii* Parry (in grey) and 2015-2016 aerial survey polygons of *Dendroctonus rufipennis* (Kirby) caused mortality (in red).

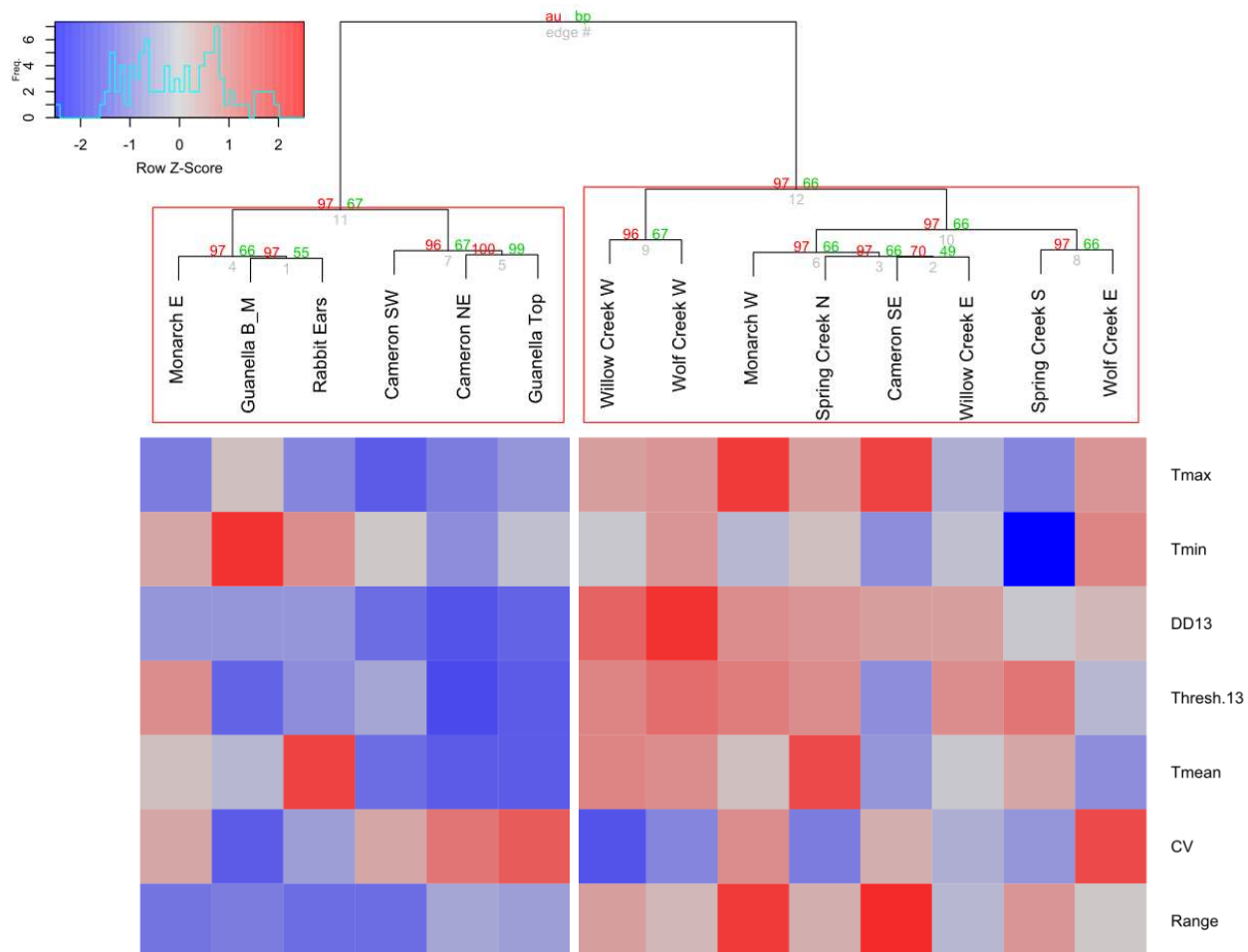


Figure 2. Bootstrap-resampled hierarchical cluster dendrogram (Ward's method using Euclidian distance) displaying approximately unbiased (AU) p-values and bootstrap probability (BP) values. Red rectangles indicate statistically supported groupings of sites based on mean site-level thermal variables recorded over two growing seasons (April-September): maximum, minimum, and average temperature, total degree days base 13°C, total number of days exceeding 13°C (threshold days), the coefficient of variation in mean temperature and the temperature range. Heatmap values are z-scores, the number of standard deviations from the mean, for each thermal variable. Grey represents values near the mean, blue less than the mean, and red greater than the mean (see legend in upper left corner).

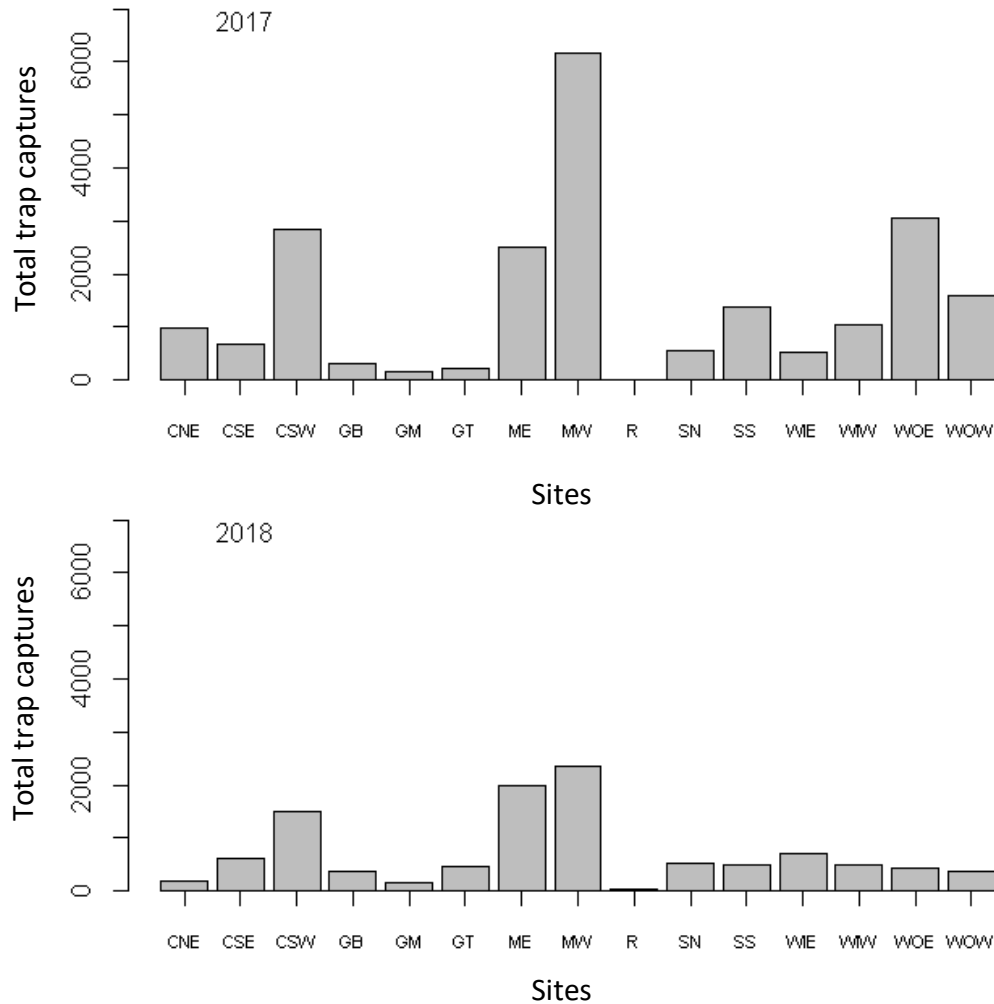


Figure 3. Total trap captures (y-axis) of *Dendroctonus rufipennis* at fifteen locations throughout Colorado in 2017 and 2018. Site names are abbreviated as follows: CNE-Cameron Pass Northeast, CSE-Cameron Pass Southeast, CSW-Cameron Pass Southwest, GB-Guanella Pass Bottom, GM:-Guanella Pass Middle, GT-Guanella Pass Top, ME-Monarch Pass East, MW-Monarch Pass West, R-Rabbit Ears Pass, SN-Spring Creek Pass North, SS-Spring Creek Pass South, WIE-Willow Creek Pass East, WIW-Willow Creek Pass West, WOE-Wolf Creek Pass East, WIW-Wolf Creek Pass West.

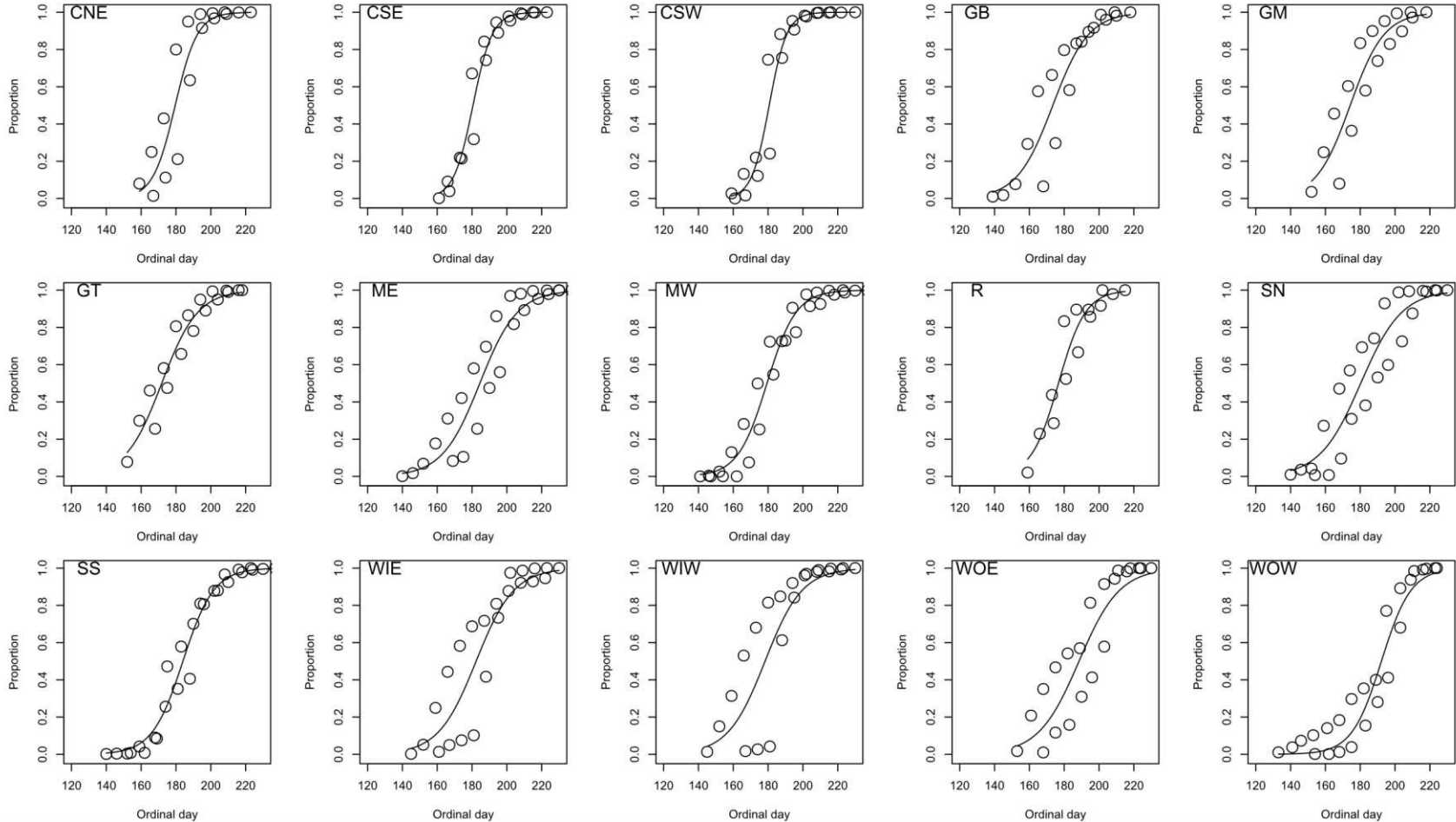


Figure 4. Cumulative trap capture data from each site fitted to the day of year using the two-parameter logistic function:

$$\frac{1}{(1+\text{Exp}(-a(\text{Ordinal day}-b)))}$$

where a = the growth rate and b = the inflection point; Logistic fits are to combined 2017 and 2018 datasets.

Colorado site names are abbreviated as follows: CNE-Cameron Pass Northeast, CSE-Cameron Pass Southeast, CSW-Cameron Pass Southwest, GB-Guanella Pass Bottom, GM:-Guanella Pass Middle, GT-Guanella Pass Top, ME-Monarch Pass East, MW-Monarch Pass West, R-Rabbit Ears Pass, SN-Spring Creek Pass North, SS-Spring Creek Pass South, WIE-Willow Creek Pass East, WIW-Willow Creek Pass West, WOE-Wolf Creek Pass East, WOW-Wolf Creek Pass West.

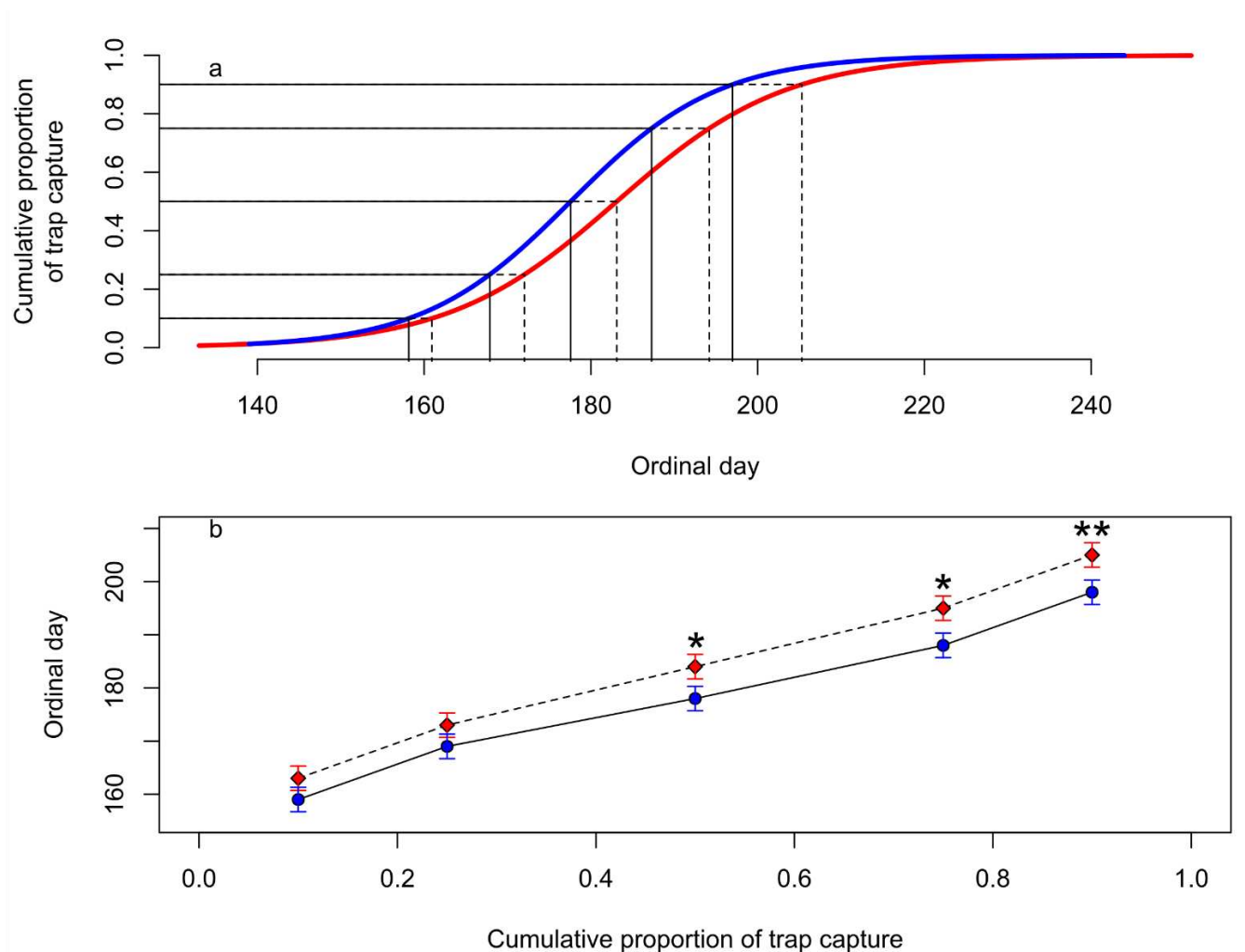


Figure 5. (a) Modeled spruce beetle flight phenology between ‘warm’ (red) and ‘cold’ (blue) sites using the following two-parameter logistic function: $\frac{1}{(1+\text{Exp}(-a(\text{Ordinal day}-b)))}$ where a = the growth rate and b = the inflection point; Logistic fits are to combined 2017 and 2018 datasets. (b) Comparison of the average day-of-year on which 10, 25, 50, 75, and 90% trap capture occurred between ‘warm’ and ‘cold’ sites. Asterisks indicate significant differences between means as identified from contrast tests; * $p < 0.1$, ** $p < 0.05$.

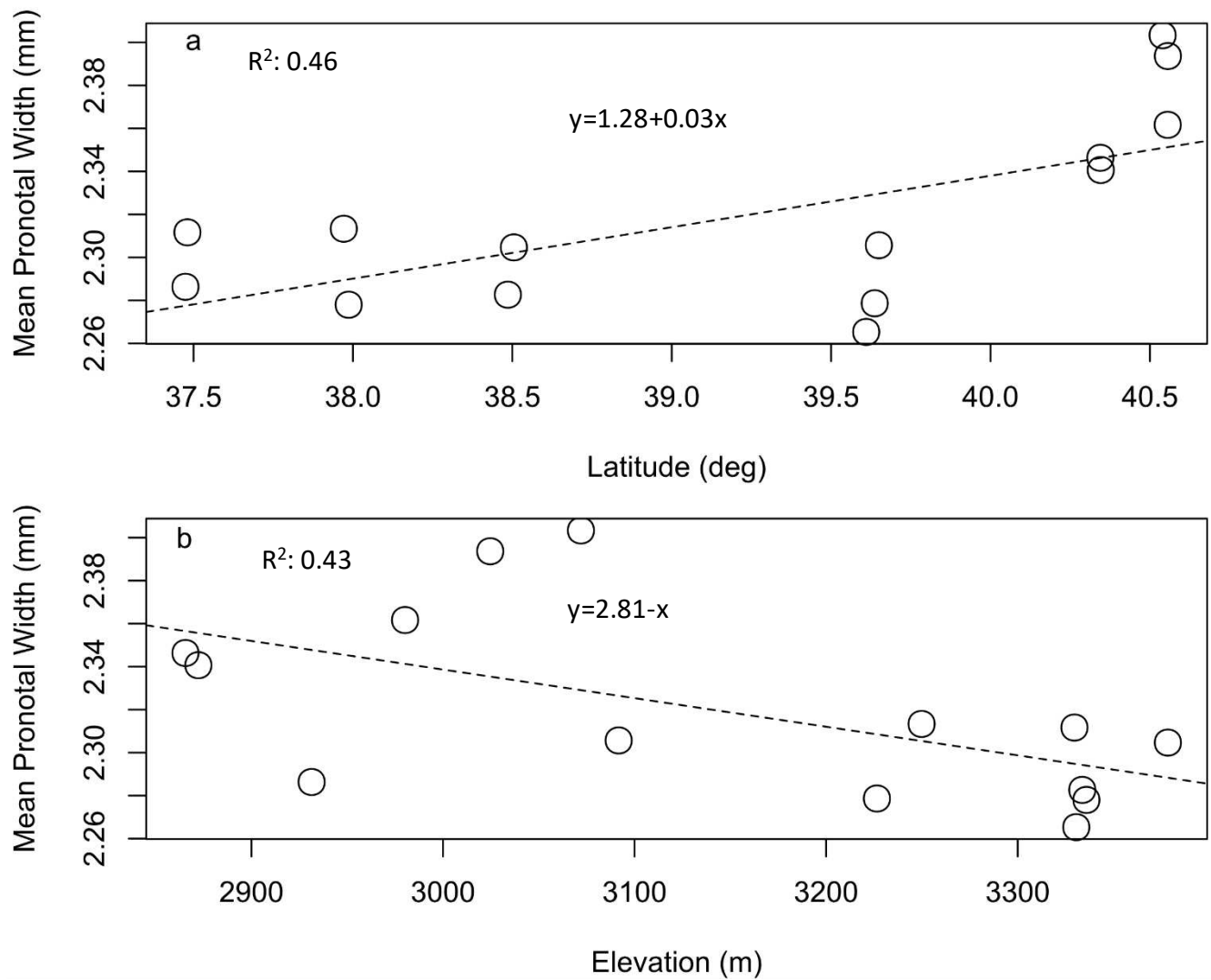


Figure 6. The relationship between mean spruce beetle pronotal width and (a) latitude and (b) elevation. There were no differences in pronotal width due to sex.

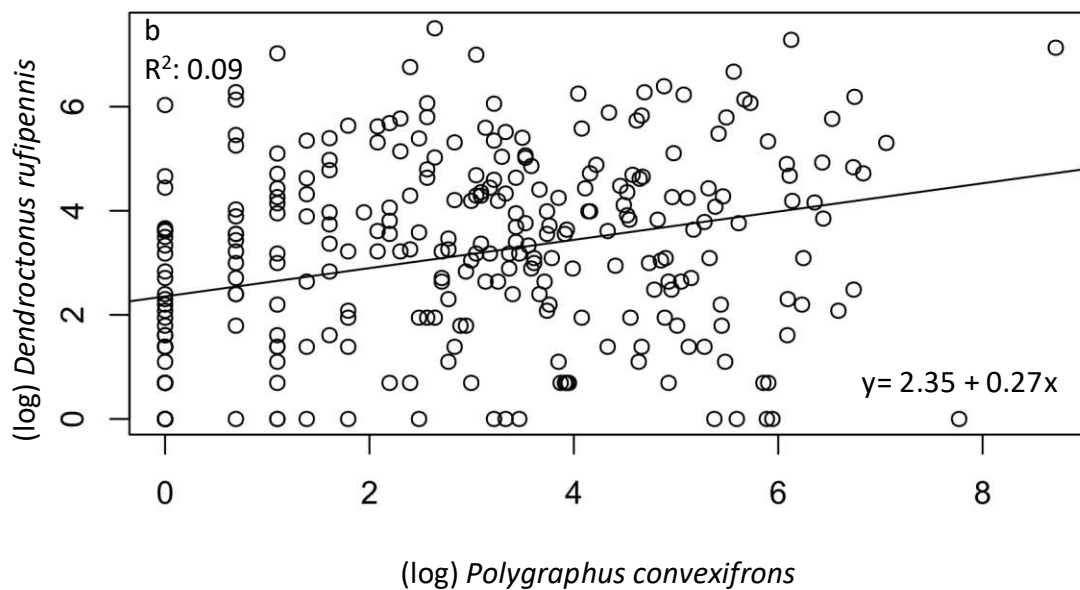
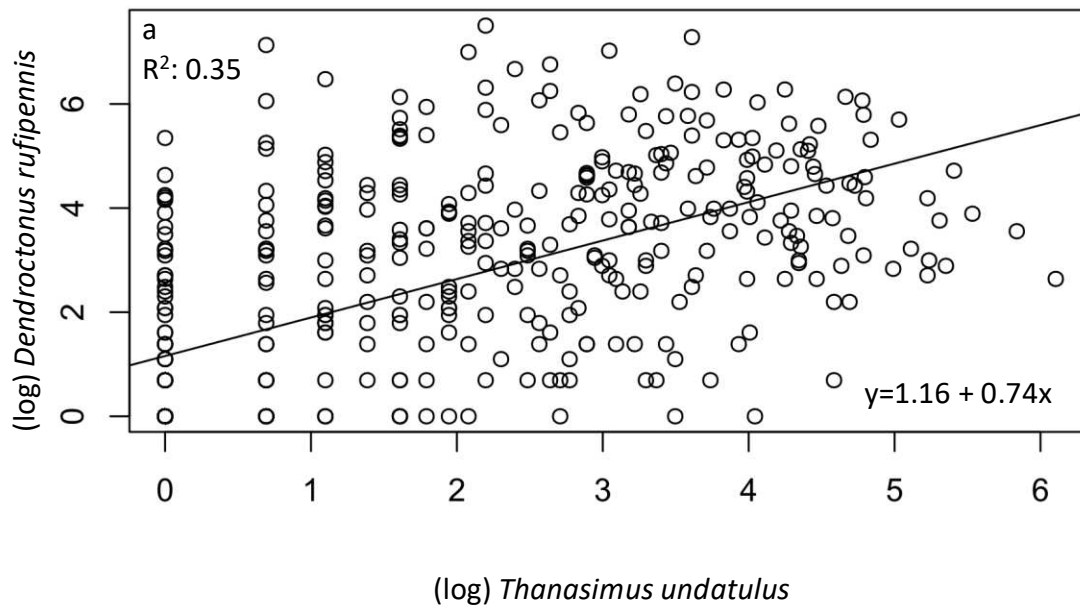


Figure 7. Correlation between spruce beetle abundance and the abundance of (a) predatory beetles (*Thanasimus undatulus*) and (b) competitors (*Polygraphus convexifrons*).

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