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Effectiveness of ambient control on invertebrate pest management in a botanical collection in the Galapagos

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Abstract

Herbaria are natural history collections that host a vast amount of information on plant taxonomy, biology, distribution, and genetic diversity, and are therefore are a key resource for scientific research. However, changes in environmental conditions can make these collections highly susceptible to pest infestations. Maintaining relative humidity (RH) and temperature control within herbaria can help preserve plant specimens. The role of these variables has not been properly studied in tropical regions, especially in relation to the abundance of invertebrates that can infest collections. In this study we use daily temperature and RH measurements, and data from invertebrate pest traps collected quarterly between 2017-2021 in the CDS herbarium of the Charles Darwin Research Station. With these data, we test for 1) the effect of ambient conditions on invertebrate abundance in the herbarium, 2) the effect of surpassing the recommended temperature and RH thresholds on invertebrate abundance, and 3) the correlation between herbarium ambient conditions and outdoor weather data, in order to evaluate the effectiveness of environmental controls. Our results show a significant positive correlation between periods of high temperature and the abundance of invertebrates, increasing the number of individuals by 32.4% per 1°C (±12.7 S.E., $p = 0.02$), but no significant effects on potential pests. We also found a significant correlation between outdoor and indoor environmental conditions. These results suggest that despite imperfect environmental controls, best practice recommendations of 40-55% humidity and temperature of 21-23°C are most appropriate for maintaining invertebrate pest control. In this case, work is needed to ensure temperature is maintained below 23°C to prevent growth and spread of invertebrates in collections. Altogether, this study shows the direct relationship between environmental conditions and the abundance of invertebrates, and stresses the importance of maintaining ambient control in natural history collections in tropical climactic regions.

Keywords: Herbaria; environmental control; invertebrates; pest management; temperature; humidity; IPM

Introduction

Natural history collections such as herbaria are essential for hosting the biological and genetic resources necessary for botanical research (e.g., studies in morphology, taxonomy, genetics), environmental monitoring, and scientific education (Suarez and Tsutsui, 2004; Bradley *et al.,* 2014).

Being such an important tool and resource, collections need to be curated and preserved correctly to ensure their long-term preservation and use (Fosberg, 1946; Giberti, 1998). Among the biggest threats to herbarium collections are pests, particularly invertebrates, due to the damage they can cause to a collection (Bridson and Forman, 1998; Guarino *et al*, 2019). The CDS herbarium in the Charles Darwin Research Station in the Galapagos Islands is the only herbarium on the archipelago. It is the most significant collection of botanical specimens from the Galapagos Islands (Jaramillo *et al.*, 2020; Mauchamp and Aldaz, 1997) containing approximately 46k specimens of plants and fungi found in the Galapagos (CDF Collections dataZone, 2021). The CDS has played a pivotal role in botanical research for Ecuador and the Galapagos Islands over the past few decades. The main collection of vascular plants and ancillary collections of pollen and seeds has supported projects on plant-animal interactions (Blake *et al*., 2012; Traveset *et al*., 2015), palynology (Van Leeuwen *et al*., 2008), Galapagos plant taxonomy (Darwin *et al.,* 2003; Weeks and Tye, 2009), species descriptions and identification guides (Bungartz *et al.,* 2020; Jaramillo *et al*., 2021), and ecological restoration and urban restoration (Atkinson *et al*., 2017; Tapia *et al*., 2019).

Historically, methods to protect herbarium collections from infestations have included chemical pest control by fumigation, or 'protecting' specimens with pesticides (Querner *et al.,* 2013; Thacker, 2002). These methods, however, are often costly and toxic for users (Hall, 1988; Drobnik, 2008), and leave chemical residue on the specimens. This has caused certain agents to be made illegal (for example, ethylene oxide and carbon disulphide) due to health and safety concerns (Pinniger and Harmon, 1999; Querner *et al.,* 2013). It is for this reason that successful integrated pest management programs (IPM) focus mostly on prevention and monitoring, with pest control and treatment being only one part of the process (Querner *et al*., 2013; Querner 2015). Alternative solutions such as those implemented in the CDS herbarium focus on preventative actions, such as controlling the environmental factors in collection storage that promote pest growth; establishing strict quarantine and sterile entry procedures for incoming specimens; and ensuring proper sealing of both entrances to collections and of containers and cabinets. These have been suggested to be just as effective while being much safer for use in an environment where students and staff routinely work (Croat, 1978; Querner *et al*., 2013; Rojas *et al.,* 2020).

The most conservative environmental conditions recommended for preventing pest infestations in botanical collections are a stable relative humidity (RH) ranging between 30% and 55% and temperatures of 20-23°C (Dawson, 1987; Bridson and Forman, 1998; Szczepanowska *et al.,* 2013; Kirby-Atkinson, 2014). These limits are recommended to avoid the growth of mould, damage to binding glue, and discourage the presence of invertebrate pests (Rose and Hawks, 1995; Simmons and Muñoz-Saba, 2003; Giberti, 1998; Rojas *et al.,* 2020). Maintaining low humidity and a clean environment prevents the proliferation of dust and organic matter which helps inhibit the presence of paper-damaging pests (Querner, 2015), and maintaining RH below 43% in herbaria is recommended to inhibit growth of insect pests that damage herbarium specimens (Hall, 1988). These environmental conditions can be implemented with the use of air-conditioning units or heaters, and humidifiers or dehumidifiers, depending on the local climate (Linnie, 1996; Strang, 1997; Rojas *et al*., 2020). For example, a review by Kirby-Atkinson (2014) discusses how temperature and humidity ranges in a collection can be adjusted depending on local climate together with an institution's financial or carbon budget. The environmental conditions required for herbaria therefore need to be evaluated on a case-by-case basis, and while collections in temperate regions may be able to limit their use of air conditioning units and humidity regulators, tropical herbaria might not have this same flexibility (Rojas *et al.,* 2020).

Botanical collections in tropical regions are especially vulnerable to infestations due to the naturally higher temperature and relative humidity which encourage biological activity of invertebrate pests (Croat, 1978; Bridson and Forman, 1998; Jaramillo *et al*., 2005). Pests such as *Stegobium paniceum* (Linnaeus, 1758) and *Lasioderma serricorne* (Fabricius, 1792) develop faster at high temperatures (~30°C) and *Liposcelis* spp., silverfish, and cockroaches thrive at humidity levels above 60% (Pinniger and Harmon, 1999). In 2017, there was an infestation of *L. serricorne* (cigarette beetle) in the CDS herbarium which caused damage to approximately 18,000 specimens of 14 vascular plant families (Acurio *et al.,* 2018). A procedure involving pest control, fumigation, quarantine, and posterior cleaning of specimens was implemented after the incident. Following this, a pest management protocol was implemented, and the current study uses data collected since then to test its efficacy.

In temperate regions, insects do not survive outside heated areas all year round and infestation is therefore a less frequent problem (Kirby-Atkinson, 2014; Inuzuka, 2016). Most studies on the effects of environmental conditions in collections have been conducted in temperate regions (such as the UK, Western Europe or the US). This could suggest that these temperature and relative humidity best-practices might not be feasible for collections in other climates such as tropical or subtropical regions (Bickersteth, 2014; Kirby-Atkinson, 2014; Staniforth, 2014; Inuzuka, 2016). Multiple previous studies in agriculture have linked the effects of temperature and humidity to reproduction, growth, and development of invertebrate pests (Chang *et al.,* 2008; Norhisham *et al.,* 2013; Zulfiqar *et al.,* 2010). These studies suggest that surpassing certain levels of maximum temperature or relative humidity in a closed environment such as our herbarium could stimulate invertebrate growth. Studying the association between these environmental factors and invertebrate abundance is critical in helping determine ideal environmental control best-practices for tropical herbaria.

In this study we use four years of invertebrate and environmental data from the Charles Darwin Station Herbarium (CDS) in the Galapagos Islands (Ecuador) to test the association of environmental variables with invertebrate abundance. Specifically, our objectives were to:

1. test for any association of maximum temperature and/or maximum RH with the abundance of invertebrates and pests found inside the collections;

2. evaluate the effect of exceeding the current recommended environmental thresholds on the presence or abundance of pests, and

3. test the effect of the local climate on the temperature and humidity within the herbarium to evaluate the efficacy of environmental controls in a tropical herbarium.

Through these objectives we aim to find empirical recommendations for the IPM of tropical herbaria. Herbaria in the tropics face greater challenges in terms of invertebrate pest control due to natural climate differences (Jaramillo *et al.*, 2005), and this assessment of current pest management and control will serve as a guide and reference for the conservation of natural history collections in similar regions.

Methods

Location

The study took place in the CDS herbarium (henceforth CDS), one of four natural history collections of the Galapagos National Park, located in Charles Darwin Research Station (CDRS) in the Galapagos Islands, Ecuador (0°44'32.4"S 90° 18'13.4" W). Being the only botanical collection in the Galapagos Islands and the main point of reference for botanical studies, it is crucial that appropriate integrated pest management methods are employed to ensure long-term preservation of the collections. Controlling entry to the collections helps maintain and prevent accidental damage to collections (Rojas, 2011). Access to the collections is limited to research and scientific purposes only, and scientists may only access the collection with a research permit (Jaramillo *et al.,* 2013). This protects specimens from damage and reduces the risk of pests entering the collections, which could occur due to the herbarium's location right inside the Galapagos National Park.

Ambient control

Air conditioning units and dehumidifiers are used to maintain control of temperature and relative humidity on a 24-hour basis, and thermohygrometers are used to measure ambient temperature (°C) and relative humidity (%). Temperature and humidity levels were measured by taking readings every 24h from two BOE 330 thermohygrometers. The herbarium is of 75m^2 and is divided in two equally-sized areas, so each thermohygrometer was placed in the centre of each herbarium room. This daily monitoring informs of the atmospheric conditions within the herbarium environment to ensure they remain approximately between 20-23°C with a conservative maximum of 50% relative humidity. These controls are based on conservative guidelines from the literature as it is difficult to ensure constant environmental conditions throughout the rooms. Since 1995, air conditioners and dehumidifiers were installed in the collections with the purpose of maintaining atmospheric control (Jaramillo, 2002; Jaramillo and Tye, 2003). To do this there are currently two air conditioners and three dehumidifiers in place, set to 21°C and a maximum RH of 50% respectively. During this study, temperature and relative humidity data recorded in both collection rooms was analyzed for the period between 2017 and 2021.

Herbarium infrastructure

The CDS follows strict freezing and drying protocols for all specimens before entry into the collections. Botanical specimens are placed in a

heated chamber (light-bulb dryer) to dry any living materials (as suggested by Strang, 1995), and frozen at -18°C for at least 48h to kill any living organisms. Plant specimens, after being identified and mounted on herbarium sheets, are stored in sealed metal cabinets which protect plant material from dust, mechanical damage, light and also reduce the entry of invertebrate pests (Bridson and Forman, 1998). These herbarium cabinets are not completely sealed and also allow the passage of air, which assumes specimens are stored in similar atmospheric conditions as the rest of the collection room. The herbarium was built in 1994, and air conditioners were installed for temperature and humidity control in 1995 (Jaramillo and Tye, 2003). The collection room has since undergone some structural improvements such as permanent sealing of windows and other potential pest entry points, and, in 2017, was expanded to fit a growing collection. However, the foundations of the building are still several decades old, and there have been problems with water leaking into the collections during the rainy season.

Pest prevention and control

Through the IPM plan, CDS employs several measures of control. Physical control includes the use of metal cabinets mentioned earlier, as well as airtight containers, envelopes, and boxes to prevent the entry and spread of invertebrate pests in and around botanical specimens. There is also a room for quarantining specimens, where collected plants are frozen and kept for a week before they enter the main collection room to be identified. Chemical control in the herbarium includes an annual fumigation procedure using the insecticide Raid ® Multi, which contains fewer chemical agents than traditional insecticides, and is executed by the maintenance team for further pest prevention (Jaramillo *et al.,* 2013; Jaramillo *et al.,* 2020). The main method for monitoring and controlling invertebrate pests is the use of sticky traps, placed along wall skirtings, in corners and entry points of the collection where crawling invertebrates are likely to be found (Querner *et al.,* 2013; Windsor *et al.,* 2015; El-Hassan *et al.,* 2021). Pre-baited and non-poisonous sticky (blunder) traps ("Catchmaster 150MBGL Gluee Louee" brand) are used to attract and trap crawling invertebrates. Periodic identification of trapped organisms and replacement of traps allows for early detection of pests.

Invertebrate identification

As part of the IPM program (Jaramillo *et al*., 2020), the blunder traps were collected every 3 or 6 months from 2017 until 2021, and once between 2019 and 2020 due to staffing issues and climactic conditions (these time period differences are accounted for in the analysis). Invertebrates caught in each trap were identified to the lowest possible taxonomic level with assistance from the entomological team from the Terrestrial Invertebrate Collection of the Charles Darwin Research Station (ICCDRS). Organisms that could not be identified to species level were labelled as "*Incertae sedis"* along with the lowest possible taxonomic level of identification. A list of all invertebrates in taxonomic order is provided in Table 1. Species that are known to be pests, or individuals identified to order or family level of groups known to be herbarium pests, were tagged as "potential pests" for further analysis (based on Iverson *et al.* 1996; Hall, 1988, Pinniger and Harmon, 1999; Sun and Zhou, 2012; Alexander *et al*., 2015; GISD, 2015 and Pocklington, 2015).

Data analysis

Environmental data in the collections from the years 2017-2021 were compared to the invertebrates trapped in those years. Mean and maximum values of temperature and relative humidity (RH) were calculated for each monitoring period based on daily morning measurements (Supplementary Table 1). These data were used to assess the relationship between ambient conditions (using maximum values of temperature and humidity) and invertebrate presence while the mean values were modelled against the outdoor environmental data. Due to the difference lengths of monitoring periods, the total number of days between each monitoring event was accounted for in all models. Two random effects were also included in the model: invertebrate order accounts for differences in diversity between invertebrate taxonomic groups, and unique trap ID accounts for the non-independence of samples collected from the same trap. The R package *lme4* (Bates *et al.,* 2015) within RStudio v.1.0.136 (R Core Team, 2021) was used to fit generalized linear Poisson models (GLM) with invertebrate number as the dependent or response variable, and temperature and RH as effect variables, as well as length of monitoring period and the additional random effects. A table with the explanation of the models used to test each hypothesis explains the fixed and random effect variables in each model (Supplementary Table 2).

For objective 1 (testing association between invertebrate abundance and temperature or RH), *P*-values were obtained by performing likelihood ratio tests (LRT) of the full model with the effect in question (maximum temperature and RH) against models without each of those effects.

The same tests were also performed using a dataset of only potential pest taxa. Poisson coefficients from the full model of the effect of temperature and RH were converted to proportional effects on the response variable by exponentiating and subtracting one. Marginal effect plots using fixed effect errors were also plotted using the *ggeffects* package (Lüdecke, 2018) to visualize the predicted effects of temperature and humidity on insect abundance while keeping all other variables constant. For objective 2, the efficacy of recommended limits was tested using the conservative thresholds of 24°C and 50% RH. The number of days within a single monitoring period in which these thresholds were surpassed was modelled against invertebrate and pest abundance using the GLM described above. For objective 3, the mean daily air temperature and RH was compiled from weather data and compared to the ambient temperature and RH collected daily in the CDS herbarium for the years 2017-2021. Weather data from Puerto Ayora, the town in which CDS is located, was downloaded from the Charles Darwin Foundation dataZone website (https://www.darwinfoundation.org/en/datazone/climate/puerto-ayora). To test for an association, a correlation test using the Pearson method was computed for both temperature and RH. The R package *ggplot2* within *ggpubr* (Alboukadel, 2020) was used to produce a scatterplot with the correlation results.

Results

Effect of temperature and humidity on invertebrate abundance

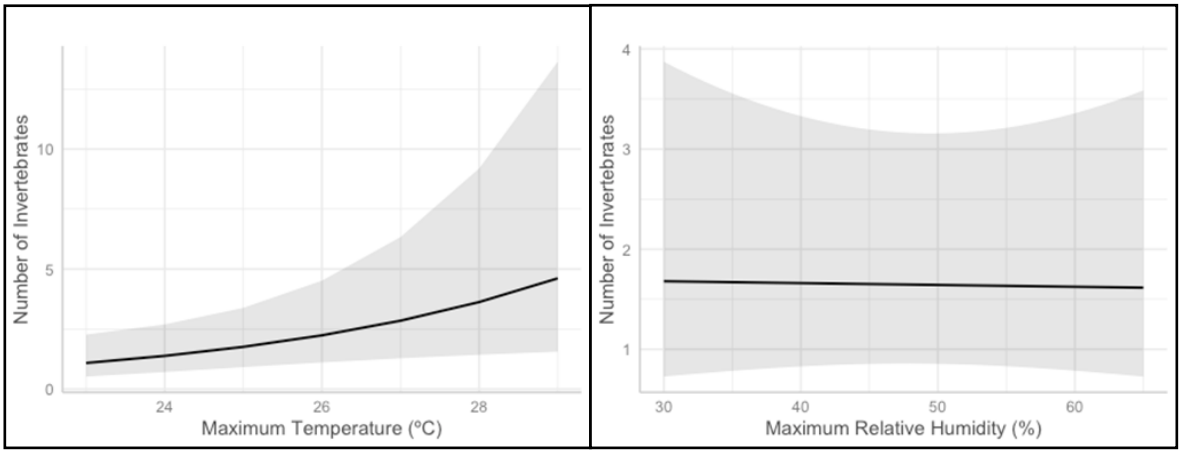
We found that although humidity does not significantly affect the abundance of invertebrates found in the herbarium ($\chi2$ (1) =0.0054, p=0.94), temperature does ($\chi2$ (1) =5.3193, p=0.02), increasing the number of individual invertebrates by 32.4% ± 12.7 S.E. per °C (Figures 1A and B). When focusing the analysis only on the potential pest species (Table 1), we found that neither temperature nor humidity had a significant effect on pest abundance.

Effect of exceeding the temperature or humidity threshold on the abundance of invertebrates

Neither the number of days on which temperature was over 24°C ($\chi2$ (1) =0.8967, p=N.S.) or the number of days on which humidity was greater than 50% ($\chi2$ (1) = 0.0087, p=N.S.) in a single monitoring period was significantly associated with the number of invertebrates found during that monitoring period. This was also tested using the dataset of only pest species, and there was no significant association between the variables of temperature ($\chi2$ (1) =2.529, p=N.S.) or humidity ($\chi2$ (1) =1.4685, p=N.S.).

Correlation between outside climate and internal herbarium environment

We found a significant correlation between average outdoor and indoor temperature (cor=0.355, p < 0.001) and average outdoor and indoor humidity (cor=0.258, p-value < 0.001) (Figures 2 and 3).



Figures 1A and B. Lineplot with confidence band showing the relationships between maximum values of temperature in °C per monitoring period (1A) and % relative humidity (1B) with number of invertebrates found in the CDS collections. Temperature had a positive significant effect on the number of invertebrates present in the collections (p = 0.02), while RH did not have any significant effect.

Table 1. Invertebrate species by taxonomic level and total number of individuals of each species collected per blunder trap, per monitoring event; as well as whether it has been cited as being a pest (based on Iverson et al. 1996; Hall, 1988, Pinniger and Harmon, 1999; Sun and Zhou, 2012; Alexander et al., 2015; GISD, 2015 and Pocklington, 2015).

Order	***Family***	***Species***	***Pest potential***	***Number of individuals***
Acari	NA	*Acari incertae sedis*	Yes	11
	Linyphiidae	*Linyphiidae incertae sedis*	Unknown	10
	NA	*Araneae incertae sedis*	Unlikely	6
	Oecobiidae	*Oecobius concinnus* (Simon, 1893)	Unkown	2
	Oonopidae	*Gamasomorpha sp.*	Unkown	22
	Pholcidae	*Aymaria conica*	Unlikely	31
		Modisimus sp.	Unknown	4
		Physocyclus globosus (Taczanowski, 1874)	Unknown	16
	Selenopidae	*Selenops galapagoensis* (Banks, 1902)	Unknown	3
	Blaberidae	*Psycnoscelus surinamensis (*Linnaeus, 1758)	Yes	27
	Blattellidae	*Symploce pallens* (Stephens, 1835)	Yes	1
	Blattidae	*Blattidae incertae sedis*	Yes	1
		Periplaneta americana (Linnaeus, 1758)	Yes	3
		Periplaneta australasiae (Fabricius, 1775)	Yes	7
		Periplaneta sp.	Yes	3
	NA	*Blattodea incertae sedis*	Yes	5
	Polyphagidae	*Holocompsa nitidula (*Fabricius, 1781)	Yes	3
	Carabidae	*Calosoma granatense* (Géhin, 1885)	Unknown	2
	Curculionidae	*Xyleborus spinulosus* (Blandford, 1898)	Unknown	1
	Elateridae	*Dipropus puberulus* (Boheman, 1858)	Unknown	1
	Phalacridae	*Phalacrus darwini* (Waterhouse, 1877)	Yes	2
	Ptinidae	*Lasioderma serricorne* (Fabricius, 1792)	Yes	7
	Tenebrionidae	*Blapstinus sp.*	Unlikely	2
Collembola	NA	*Collembola incertae sedis*	Yes	21
Diptera	Culicidae	*Aedes taeniorhynchus* (Wiedemann, 1821)	Unlikely	3
	Phoridae	*Dohrniphora cornuta* (Bigot, 1857)	Unknown	1
		*Megaselia scalaris (*Loew, 1866)	Yes	2
		Megaselia sp.	Yes	57
	Psychodinae	*Clogmia sp.*	Unknown	3
		Psychodinae incertae sedis	Unknown	1
	Sarcophagidae	*Sarcophagidae incertae sedis*	Unknown	1
	Sciaridae	*Sciara sp.*	Yes	3

Table 1. ***(cont)***

Order	***Family***	***Species***	***Pest potential***	***Number of individuals***
Hymenoptera	Diapriidae	*Diapriidae incertae sedis*	Unknown	6
	Formicidae	*Camponotus conspicuus zonatus* (Emery, 1894)	Unlikely	37
		Camponotus sp.	Unlikely	2
		Monomorium floricola (Jerdon, 1851)	Yes	16
		Odontomachus bauri (Emery, 1892)	Possibly	1
		Tapinoma melanocephalum (Fabricius, 1793)	Yes	21
		Tetramorium bicarinatum (Nylander, 1846)	Unknown	1
Isopoda	Porcellionidae	*Metoponorthus pruinosus* (Brandt, 1833)	Unknown	3
		Porcellio laevis (Latreille, 1804)	Yes	1
		Porcellionides pruinosus (Brandt, 1833)	Yes	544
Lepidoptera	Geometridae	*Cyclophora impudens* (Warren, 1904)	Yes	1
N/A	NA	*Incertae sedis 2*	Uknown	2
Ortoptera	Gryllidae	*Cycloptilum erraticum* (Peck, 1996)	Unknown	6
		Cycloptilum sp.	Unknown	3
		Gryllus sp.	Unknown	13
Psocoptera	Epipsocidae	*Epipsocus sp*	Yes	2
	Lachesillidae	*Lachesilla sp.*	Yes	63
	Lepidopsocidae	*Lepidopsocidae incertae sedis*	Yes	9
	Liposcelididae	*Liposcelididae incertae sedis*	Yes	42
		Liposcelis entomophila (Enderlein, 1907)	Yes	15
	NA	*Psocoptera incertae sedis*	Yes	1
Scolopendromorpha	Scolopendridae	*Scolopendra galapagoensis* (Bollman, 1889)	Unknown	1
Solifugae	Ammotrechidae	*Neocleobis solitarius* (Banks, 1902)	Unknown	4

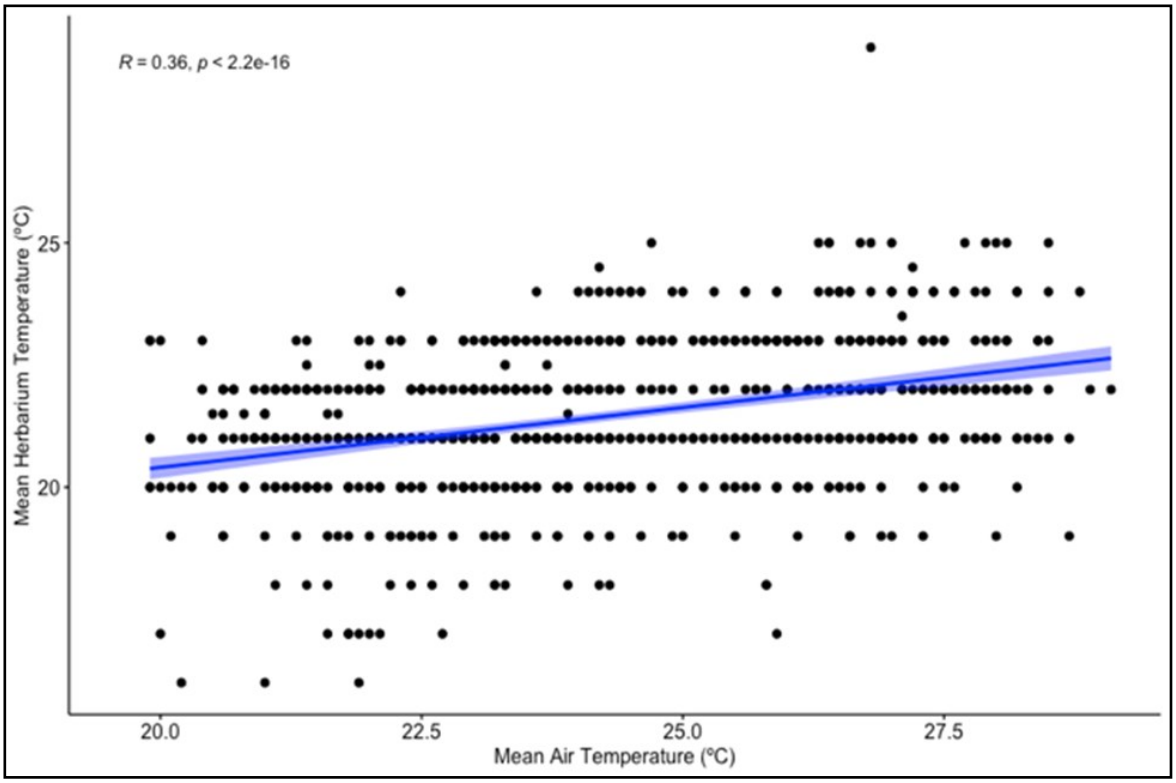


Figure 2. Scatterplot of the correlation between mean temperature (°C) inside and outside the CDS.

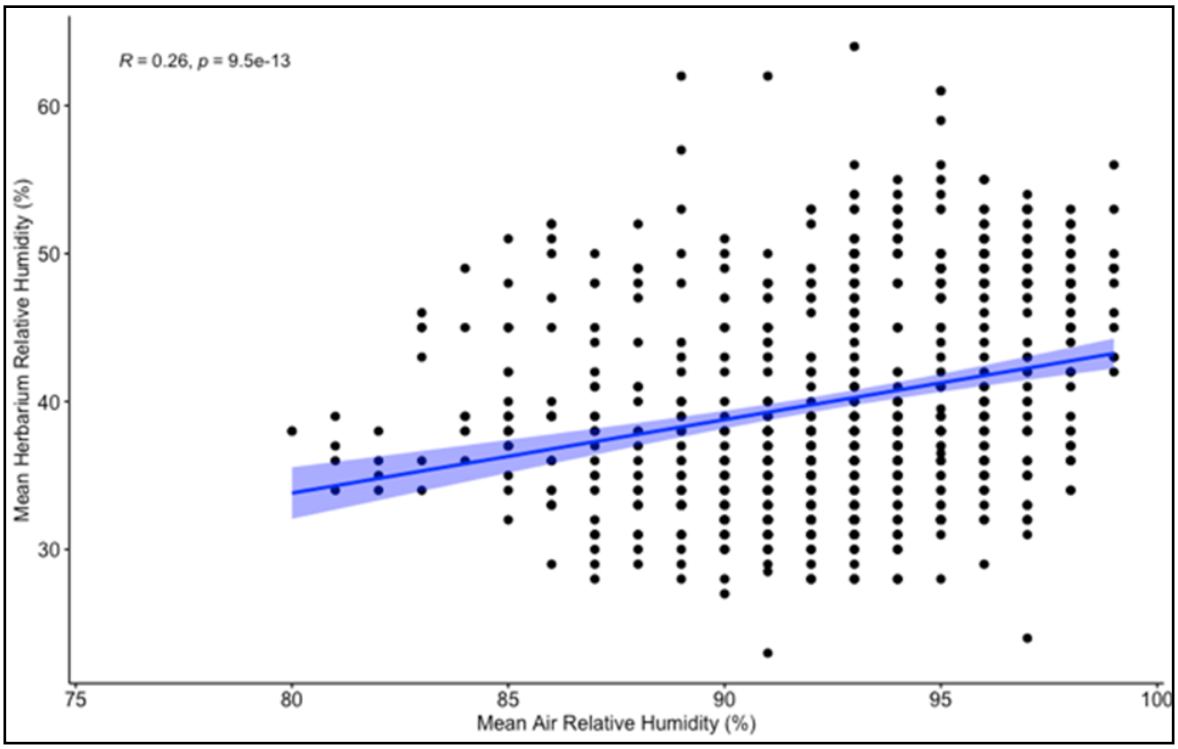


Figure 3. Scatterplot of the correlation between mean relative humidity (%) in and outside the CDS.

Discussion

To summarise the main results, firstly, maximum temperature values (per monitoring period) had an effect on the number of invertebrates found in the collection (32% increase for each 1°C) but not significantly on those that are pests, while maximum RH conditions did not have an influence. Secondly, the number of days in which temperature and RH exceeded the recommended thresholds did not significantly affect the presence or abundance of invertebrates (nor pests) found in the collections during that time. And, thirdly, there was a small but significant correlation between outdoor and indoor herbarium environments, with temperature being slightly more closely correlated.

While indoor humidity levels did not significantly influence herbarium invertebrates, temperature did, which is concerning as indoor and outdoor temperature were also more correlated to one another. This may suggest an indirect impact of outdoor temperature on herbarium invertebrates, which highlights the need for tighter temperature control or more protective infrastructure. This result, however, could also be explained by the fact that during the period of study, the maximum levels of RH (on average 49%) were also well within average recommended humidity levels (40-55%), while maximum temperatures averaged 25°C, above the 20-23°C suggested margin (Supplementary Table 1). Humidity-loving pests present in our traps such as *Clogmia* sp., individuals of the taxonomic group Collembola (springtails) which feed on mould and dry plant matter (Hopkin and Stephen, 1997) and cockroaches such as *Psynoscelis surinamensis, Holocompsa nitidula* and both *Periplaneta* species thrive at high humidities present in tropical countries (Pinniger and Harmon, 1999; Notton, 2018; El-Hassan *et al*., 2021). Species of Psocoptera (book lice) were found in nearly all monitoring events, and albeit in low numbers, these can become a problem if ambient conditions are not controlled, as they thrive under humid conditions of 60% RH and above. These insects are known to feed on mould and starch as well as paper and even plant specimens (Querner, 2015; Notton, 2018). While these invertebrates were identified among our trap data, analyses of pest data showed that their numbers were not significantly correlated with humidity levels in the herbarium. The current use of three dehumidifiers plus two air conditioning units in the herbarium may, then, be sufficient to provide optimal relative humidity levels in the collections and prevent population growth of these pests. This seems to hold coherence with the high number of individuals found of the hygrophyllic *Porcellionides pruinosus* (woodlouse) during the first few monitoring events (Table 1) which were greatly reduced after 2020, when environmental control became better managed in the herbarium. It appears that the relative air humidity levels of 30-40% currently maintained in the CDS herbarium are adequate to prevent an infestation.

Temperature, on the other hand, was found to be significantly associated with invertebrates overall, suggesting that there may be an important effect of temperature that should not be overlooked. Invertebrates are a large problem for natural history collections in the tropics, usually due to consistently higher levels of relative humidity, which encourages their development. The higher temperatures in these regions also play a role in providing the right habitat for their growth (Hall, 1988; Pinniger and Harmon, 1999). Maximum temperature met and/or exceeded 25°C during six of the nine monitoring events between 2017-2021 at CDS, even though the number of days this threshold was exceeded was not significantly associated with invertebrate numbers. Species of *Liposcelis* (an invertebrate found in CDS) are parthenogenetic, and at temperatures over 25°C populations grow rapidly, increasing the risk of infestation (Pinniger and Harmon, 1999). Temperature in the CDS was found to have a greater association with outdoor data than humidity, which may explain why this variable is also associated with more invertebrates in the herbarium. This suggests that in the CDS, there is a need to maintain a tighter control of temperature given these results, whereas humidity levels are appropriate at present levels (between 30-40% RH). The current environmental management protocol in the collections consists of daily emptying of dehumidifiers in order to consistently maintain low humidity, whereas temperature is set at 22°C on each air conditioning unit and that level is rarely modified. In the CDS, the ancillary collections play a crucial role in current research projects, and seed collections are known to be of particular risk of infestations due to the high nutritious content of seed heads (Pinniger and Harmon, 1999). As RH is mentioned as being a big factor in the presence of pests, it is tightly controlled at the CDS. As mentioned earlier, serious herbarium pests such as *Lasioderma serricorne* (cigarette beetles) can occur in conditions of high temperature.

The fact that there was no association between the number of days above the temperature or RH limits and invertebrate abundance, however, suggests our current IPM has been effective. This likely means that the limits did not reach

dangerous levels for too long before they were brought back down below 24°C and 50% RH. While temperature and humidity control aid this, other protective IPM measures such as specimen quarantining, insulated containers and fumigation also play a role. The results from the third objective showing that there is a correlation of environmental conditions with the outdoor conditions mean the environmental control in the CDS is not perfect, however, the results from testing the number of days exceeding these thresholds shows that despite this, the current conditions as a whole are effective at preventing the entry and population growth of pests. For instance, following the implementation of our current IPM, only 7 individuals of L. serricorne have been found in pest traps since, with none captured since 2019 (Supplementary Table 1). This shows that our IPM measures, coupled with strict environmental controls, can effectively halt the development of invertebrates and their population growth.

Future projections for climate change may have an impact on the way natural history collections are maintained. Puerto Ayora, where the CDS herbarium is based, has a yearly range of RH of approximately 85-90%, and a mean temperature of 30-30°C, although this area (the dry zone) is expected to become warmer and wetter (Trueman and D'Ozouville, 2010). Another study in the Galapagos showed predictions of increased seasonality (that is, an increase in mean warm season temperatures and a decrease in cool season temperatures), as well as increased annual rainfall in the islands over the coming decades (Wolff, 2010). This is another point to consider in terms of the effects of outdoor on indoor environments, as weather impacts may be stronger in future and possibly call for a need of more protective herbarium infrastructure and/or tighter ambient control within our IPM to prevent infestations. We recognise that the buildings natural history collections are housed in are never perfectly insulated, especially in the tropics, regions where weather can be more unpredictable, or institutions with less funding, as this is difficult and expensive. This shows coherence with recommending stricter monitoring and more conservative temperature and RH thresholds for indoor conditions, to provide a buffer for the impact of these variables. Ensuring preventative pest management through environmental control and physical insulation can also help prevent the need of using strong chemicals and pesticides. This is important to consider not only due to human impact but also its effect on local biodiversity, especially due to the herbarium's location within the Galapagos National Park.

Between January and March, the rainy season in the Galapagos islands often leads to leaks in some old buildings in the research station due to changes in humidity which can affect wood. There was one instance of a reported leak into the herbarium in February 2020 which was sealed immediately after being discovered. This could have caused an increase in humidity during this period, but the numbers suggest that the dehumidifying units were sufficient to curb this effect. It is important to address, however, that the thermohygrometers used throughout this study were not previously assessed or calibrated, so their absolute accuracy is a limiting factor in this study. That said, the measurements collected with these thermohygrometers still allow us to evaluate the relative association between temperature, RH and invertebrates in this study. There are herbarium cabinets that contain their own temperature and RH readers which would provide more precise measurements of environmental conditions of stored specimens, for instance, *ampfab* herbarium cabinets (https://ampfab.co.uk/herbarium-cabinets/).

Certain issues which should be mentioned regarding trapping and identification methodology were that many invertebrates, 12 of 55 species, were only identified to family or order level, and 1 was unidentifiable. The entomological team from the Terrestrial Invertebrate Collection of the Charles Darwin Research Station (ICCDRS) mentioned that sticky traps make genus or species -level identification difficult as individuals are often damaged when moved to examine body parts that are necessary for identification (*pers. comm*. Lenyn Betancourt, 2021). Correct identification of trapped invertebrates at every developmental stage is key in establishing the necessary protocols to prevent infestations. A potential solution to this issue could be to use different types of traps in future, such as UV-light traps and pheromone lures, which may attract other invertebrates depending on their size or biology that are also important to monitor in collections (Querner, 2015; Windsor *et al.,* 2015).

Conclusion

Our study of the CDS herbarium found that, in a plant collection in the tropics, 1) temperature had a significant effect on invertebrates found in the collection, 2) exceeding the thresholds for short periods of time (days) did not affect numbers, and 3) there is a correlation between outdoor and indoor environments, in particular temperature. Since buildings are not completely sealed, we suggest maintaining stricter control and monitoring

of indoor environmental conditions, particularly temperature. Sealing and securing entry points to the collections will also help with buffering the effect of outdoor climate and protecting specimens. In any IPM plan, prevention is key to evading pests, which includes controlling for collection surroundings, building entry zones, collection archiving, environmental conditions, and even staff habits. Herbarium pests such as the cigarette beetle, *Clogmia* sp., springtails, silverfish, woodlice and cockroaches are attracted by hot and humid environments due to their feeding or breeding activity (Pinniger and Harmon, 1999; Querner, 2015; Notton, 2018). Monitoring temperature and relative humidity range, as well as ensuring other protective barriers are held in place in natural history collections can help reduce the use of chemicals to prevent infestations in tropical regions of high biodiversity. Through our study we found that suggested best-practices of maintaining temperature in the range of 21-23°C and a relative humidity of 40-55% are sufficient for tropical herbaria without having protective controls strong enough to completely eliminate the effect of the outdoor climate.

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Supplementary Table 1. Values of maximum relative humidity and temperature, total number of species and individuals per monitoring period.

Monitoring period	***Total species***	***Total individuals***	***Maximum relative humidity (%)***	***Maximum temperature (°C)***
May 2017 - Dec 2017	23	159	61	24
Dec 2017 - Jul 2018	23	127	57	25
Jul 2018 - Feb 2019	18	59	53	25
Feb 2019 - Sep 2019	12	60	64	29
Feb 2019 - Jan 2020	44	466	47	24
Jan 2020 - Apr 2020	19	24	42	25
Apr 2020 - Jul 2020	23	33	41	25
Jul 2020 - Oct 2020	14	14	50	23
Oct 2020 - Jan 2021	12	15	31	24
Jan 2021 - Apr 2021	43	99	46	25

Supplementary Table 2. Table of models implemented to test the 3 objectives of the study, which were to: 1) test for any association of maximum temperature and/or maximum relative humidity with the abundance of invertebrates and pests found inside the collections; 2) evaluate the effect of exceeding the current recommended environmental thresholds on the presence or abundance of insect pests; and 3) evaluate the effect of the local climate on the temperature and humidity within the herbarium to test the limitation of maintaining an herbarium in tropical climates.

Objective Number	***Objective***	***Model***	***Variables***
1	Testing effect of maximum temperature on invertebrate abundance	glmer with poisson, compared to reduced model without maxtemp using LRT	invertebrate abundance, maximum temperature (scaled), maximum humidity (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
1	Testing effect of maximum humidity on invertebrate abundance	glmer with poisson, compared to reduced model without maxhum using LRT	invertebrate abundance, maximum humidity (scaled), maximum temperature (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
1	Testing effect of maximum temperature on pest abundance	glmer with poisson, compared to reduced model without maxtemp using LRT	pest abundance, maximum temperature (scaled), maximum humidity (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
1	Testing effect of maximum humidity on pest abundance	glmer with poisson, compared to reduced model without maxhum using LRT	pest abundance, maximum humidity (scaled), maximum temperature (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
2	Testing effect of number of days above temperature threshold on invertebrate abundance	glmer with poisson, compared to reduced model without days above temp threshold using LRT	invertebrate abundance, days above temperature threshold (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
2	Testing effect of number of days above humidity threshold on invertebrate abundance	glmer with poisson, compared to reduced model without days above hum threshold using LRT	invertebrate abundance, days above humidity threshold (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
2	Testing effect of number of days above temperature threshold on pest abundance	glmer with poisson, compared to reduced model without days above temp threshold using LRT	pest abundance, days above temperature threshold (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
2	Testing effect of number of days above humidity threshold on pest abundance	glmer with poisson, compared to reduced model without days above hum threshold using LRT	pest abundance, days above humidity threshold (scaled), days in monitoring period (scaled); random variables of order, unique trap ID
3	Testing correlation of outdoor humidity with herbarium RH	correlation test using Pearson method	mean outside air humidity and mean herbarium relative humidity
3	Testing correlation of outdoor with indoor herbarium temperature	correlation test using Pearson method	mean outside temperature and mean herbarium average