



THE  
SOCIETY  
SCIENTISTS

VOLUME III

# GUIDE TO THE ISCIENTIST

The *iScientist* publishes five types of articles

Article types are displayed at the top of the article



## Original Research

Articles in the format of original research publications in academic journals



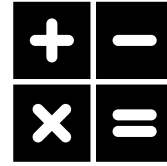
## Literature Review

A detailed review of previous research on a specific topic



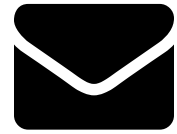
## History of Science

A historical perspective of a topic within science or technology



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Studies regarding theoretical considerations or applications of mathematical concepts

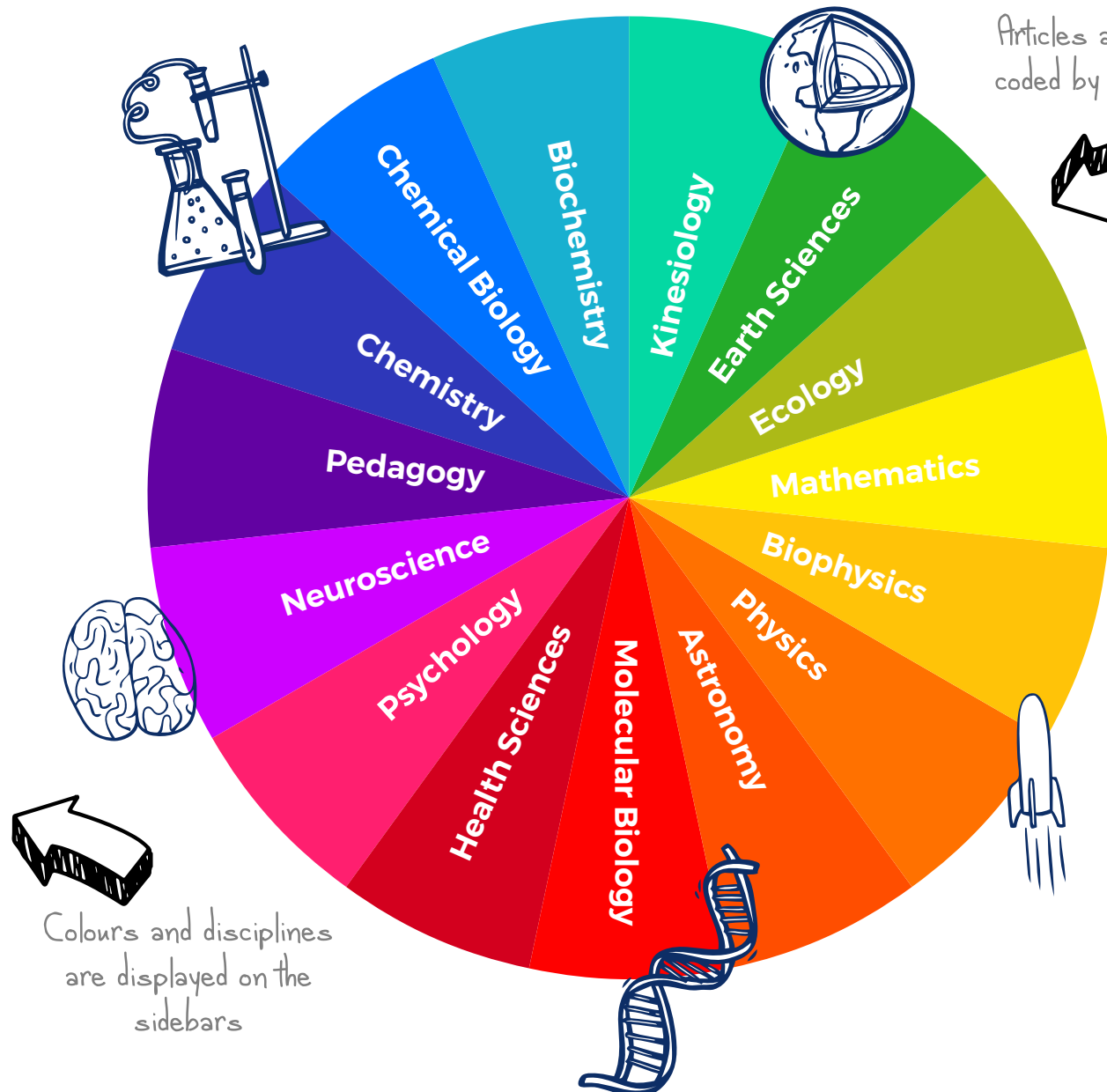


## Letter to the *iScientist*

Short pieces that discuss scientific research or argue an opinion

Our articles are divided into fifteen disciplines

Articles are colour coded by discipline



Colours and disciplines are displayed on the sidebars

# DEAR READERS

We are excited to present the third volume of McMaster University's undergraduate interdisciplinary science journal, *The iScientist*. Whether you decided to pick up this journal because you authored an article within its pages, are interested in interdisciplinary science, or are merely curious, we hope you will enjoy what you read and leave with a greater understanding and appreciation for the interdisciplinary nature of science. This year's edition features topics ranging from antibiotic resistance, to the ethics of clinical research, to star formation in far-off galaxies. Each article embodies the scientific curiosity and determination of our authors to expand the knowledge in their field and foster new connections with other disciplines.

We are proud to have expanded the reach of *The iScientist* this year by opening submissions to all students in the Faculty of Science and hosting science literacy workshops. Our first workshop, in partnership with the Student Success Centre, focused on effective communication within science. The second workshop, run by librarian Abeer Siddiqui, provided attendees with an approach to constructive peer review. We hope that our workshops provided a useful additional resource to students and are excited to run more workshops in the coming years.

None of this would have been possible without the help of many people at McMaster University. We would like to thank our advisors, Andrew Colgoni and Dr. Sarah Symons, for their continuous support and advocacy for *The iScientist*. Thank you as well to the Student Initiative Fund, which provided the funding to print the journal, run workshops, and run a website. Additionally, we are grateful for all the promotional help from the McMaster Science Society. Thank you to Mary McCaffery at the Student Success Centre and to Abeer Siddiqui, librarian extraordinaire, for helping us lead our first workshops. Thank you to all our peer reviewers, who provided thoughtful and valuable feedback on articles, even when schedules were tight and we all had too much on our plates.

Finally, thank you to the Integrated Science program, professors, students, and alumni, for supporting and promoting the journal, and entrusting us with your hard work. Your scientific curiosity, clarity of writing, and research prowess inspire and amaze us every time we receive a new article.

Thank you, and please enjoy Volume III of *The iScientist*!

All the best,

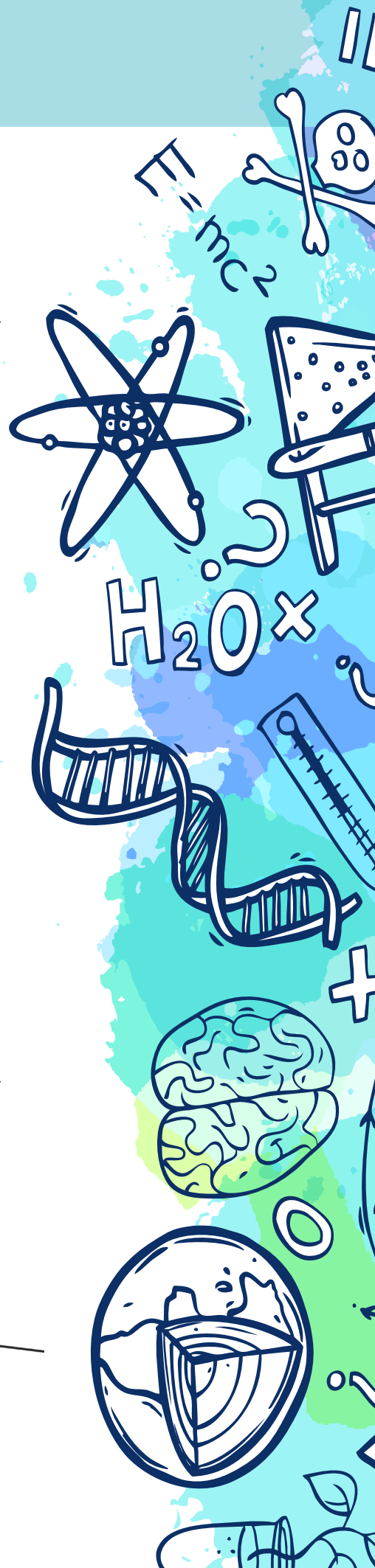


**Laura Green**

*The iScientist* Senior Editors, 2017-2018

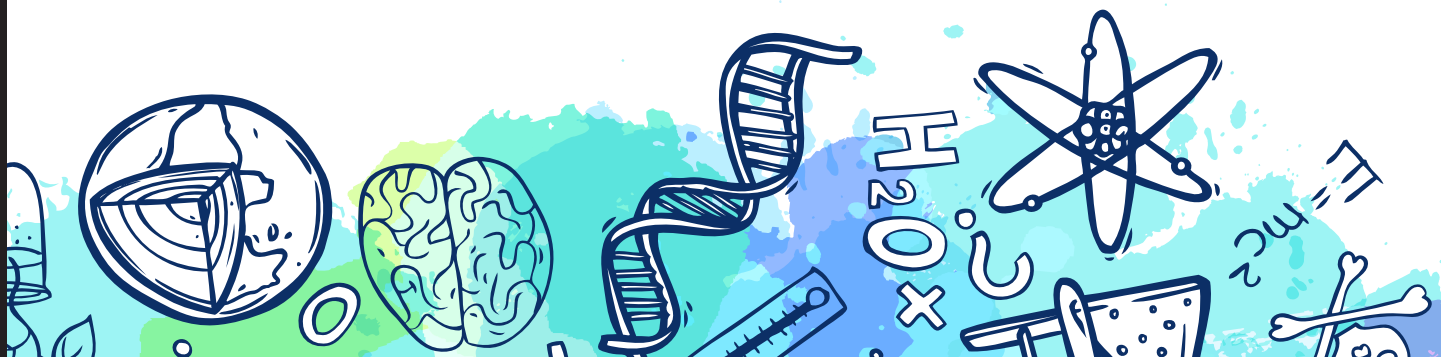


**Varsha Jayasankar**



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# Star formation and morphology of galaxies in local compact groups

JESSICA SPEEDIE<sup>1</sup>, LAURA PARKER<sup>2</sup>

1. Integrated Science Program, Class of 2020, McMaster University
2. Department of Physics and Astronomy, McMaster University

## SUMMARY

We present a study on the observed properties of galaxies in a large sample of compact groups identified in the Sloan Digital Sky Survey. Compact groups are extremely dense collections of galaxies, with 3 to 8 member galaxies within a very small volume. These systems are known to have frequent galaxy-galaxy interactions. In this work, we propose a new definition for the radius of a compact group and match our member galaxies to previous catalogues containing information about the masses, star formation rates and morphologies of these galaxies. We find that both star formation and morphology depend strongly on the galaxy's stellar mass but more weakly on their host environment: the member galaxy properties show very little radial dependence within their group, but they do depend on their group mass, as traced by the velocity dispersion. Galaxies in compact groups show similar properties to those in non-compact groups, despite being in fundamentally different environments.

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## INTRODUCTION

Large scale structure in the universe is comprised of aggregations of galaxies. Gravitational interactions assemble galaxies into groups (containing 10-100 members) and clusters (100-10,000 members). When in close proximity, galaxies can fall onto one another and affect the motion and formation rate of the stars within them. The environment in which a galaxy lives –group, cluster or field– has observable effects on its properties (Conselice, 2014).

A special class of galaxy group is the so-called compact group (Figure 1). Compact groups are comprised of 3-8 member galaxies situated very close together; typical galaxy-galaxy separation is on the order of kiloparsecs (kpc), similar to the size of the galaxies themselves (Sohn et al. 2016). Compared to “regular” groups, compact groups have low velocity dispersions ( 200 km/s, Hickson & P. 1982; McConnachie et al. 2009), meaning the deviation in the radial velocities of the constituent member galaxies is small.

High spatial densities and low velocity dispersions imply that compact groups have short crossing times,

and galaxy-galaxy interactions are slow. Compact groups are therefore environments in which many galaxy evolution processes occur, including mergers and other dynamical interactions (Brasseur et al. 2008; McConnachie et al. 2009). Previous work has shown that the observed properties of galaxies, such as star formation and morphology, are influenced by the group environment (Mcconnachie et al. 2008). Additionally, the extremity of the compact group environment puts constraints on galaxy evolution models, which aim to predict average global trends in galaxy properties, but that must reproduce the observations in all environments if they are to be successful.

The effect of mergers or gravitational interactions on star formation rate (SFR) has been explored for close pairs of galaxies and for regular groups (Carlberg et al. 1994; Ellison et al. 2008) as well as for clusters (Moore et al. 1996; Gomez et al. 2003; Lewis et al. 2002). As tidal interactions disturb and redistribute gas from which stars may form, enhanced SFR would be expected. The observations for close pairs are consistent with this picture, but decreased SFRs are found for galaxies in



**Figure 1:** Images of four example compact groups as identified by Sohn et al. (2016). Left to right: MLCG1428 ( $\pm = 37 \pm 8 \text{ km/s}$ ), MLCG1492 ( $\sigma = 49 \sigma 12 \text{ km/s}$ ), MLCG1569 ( $\sigma = 302 \sigma 57 \text{ km/s}$ ), MLCG1577 ( $\sigma = 273 \pm 69 \text{ km/s}$ ). Images are from the DR12 Science Archive Server (SAS).

clusters when local density is high (Brasseur et al. 2008). Where compact groups will fall on this spectrum is not immediately obvious.

There are special challenges involved in the study of compact groups, stemming from these groups containing very few member galaxies. Group velocity dispersion is difficult to quantify; the fewer galaxy velocities known, the less accurately their dispersion can be estimated. Additionally, the geometrical centre of the group is not a well-defined quantity for an aggregation of only a few galaxies. In larger groups and clusters, it is clear by their radial position in the group which galaxies live in the densest areas.

The goal of this work is to study the properties of galaxies in this dense environment - specifically, galaxy star formation rate and morphology. We explore whether galaxy properties exhibit dependence on their radial position in the group or on the group velocity dispersion (environmental metrics), as well on as stellar mass (a property internal to the galaxy).

The paper is structured as follows: In Section 2 we present the properties of the galaxies and compact groups within our data sample. In Section 3 we show our results for the dependence of galaxy properties (star formation and morphology) on environment (radial position and velocity dispersion) and on stellar mass. We discuss our results in Section 4 and present our conclusions in Section 5. Throughout, we adopt the flat  $\Lambda$ CDM cosmological parameters of  $\Omega_M = 0.3$ ,  $\Omega_\Lambda = 0.7$  and  $H_0 = 70 \text{ km s}^{-1} \text{ Mpc}^{-1}$ .

## DATA

In order to address questions related to galaxy evolution in compact groups we need a large population of these groups and the member galaxies within them. We take advantage of the largest ever survey of galaxies, the Sloan Digital Sky Survey (SDSS) (Alam et al., 2015). The SDSS is a wide-field multi-spectral imaging survey covering just over 35% of the sky. It contains photometric measurements for around 500 million objects, and spectral information (and thus redshifts) for more than 3 million. New SDSS data sets have

been released to the public every few years since data collection began in 1998.

From the galaxy properties provided by the SDSS (such as luminosity, colour, size, stellar mass and velocity dispersion), further information can be derived. Analysis of emission lines yields galaxy star formation rates, and galaxy morphology can be estimated directly from telescope images. Independent collaborations perform these analyses and publish them in separate catalogues. We use star formation data from the Max Planck for Astrophysics and Johns Hopkins University (MPA JHU) catalogue, which estimates star formation rates based on the technique discussed in Brinchmann et al. (2004) and Salim et al. (2007). We use the morphological data provided in the Galaxy Image 2D (GIM2D) catalogue by Simard et al. (2011).

## THE MAGNITUDE-LIMITED COMPACT GROUP CATALOGUE (MLCG)

Studying the properties of galaxies in compact groups requires reliable identification of these systems. Several algorithms exist for identifying groups in galaxy surveys, but they all involve linking together galaxies that are close together on the sky. The original criteria for identifying compact groups were specified by Hickson & P. (1982), who compiled the first collection (consisting of around 100 compact groupss) and started quantifying their properties. Hickson's approach involved three criteria based on the relative brightness, compactness and isolation of the galaxies. For our work, we employ the Magnitude-Limited Compact Group (MLCG) catalogue created by Sohn et al. (2016) from the enhanced SDSS Data Release 12 (DR12; Alam et al. 2015). Their sample was derived using the friends-of-friends (FoF) algorithm of Barton et al. (1996) and two of the three Hickson criteria. They omit the isolation criterion, arguing that it artificially selects against nearby groups.

The MLCG (column 1 of Table 1) consists of 1588 compact groups composed of a total of 5178 member galaxies, covering a range in redshift  $0.01 < z < 0.20$  (distances of 0.1 to 2.5 billion light years). The majority

of groups, 1276, have three member galaxies and the remaining 312 have four or more. Galaxies have absolute r-band magnitudes down to  $r < 17.77$ .

**Table 1: The number of galaxies contained in our sample and subsamples. We match the MLCC of Sohn et al. (2016) to the GIM2D morphology catalogue and the MPA JHU star formation catalogue.**

The MLCG	Match to GIM2D	Matched to MPA JHU
5178	4272	3656

## GALAXY PROPERTIES

The morphology of a galaxy ranges from disk-dominated spiral galaxies (such as the Milky Way) to bulge-dominated elliptical galaxies, whose structure is supported by stars undergoing random motion rather than rotational motion. Morphology can be described qualitatively by eye (using the Hubble “tuning fork”; Nair & Abraham 2010) or it can be quantified numerically using the generalized de Vaucouleurs profile (also known as the Sersic profile). We are interested in the latter. The GIM2D catalogue (Simard et al. 2011) provides this metric of galaxy morphology by fitting a surface brightness radial profile using a single parameter, the Sersic index  $n$ , as follows:

$$\mu(r) = \mu_e + 8.3268 \left\{ \left( \frac{r}{r_e} \right)^{1/n} - 1 \right\} \quad (1)$$

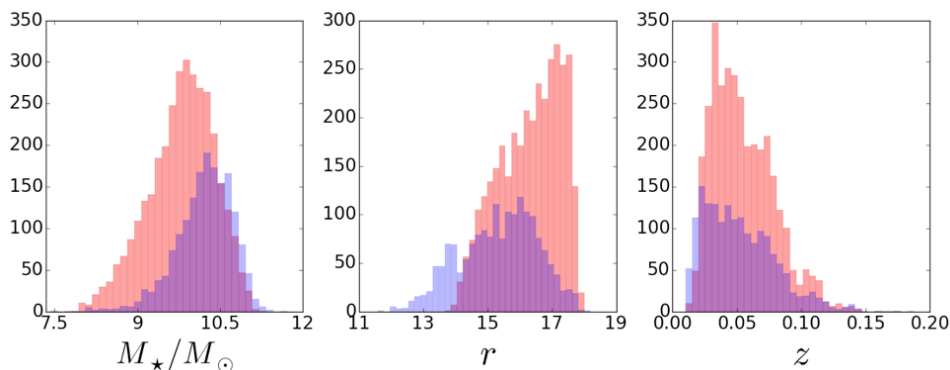
where  $r_e$  is the effective radius (the projected radius within which half of the galaxy’s light is emitted) and  $\mu_e$  is the surface brightness at  $r_e$ . A value of  $n = 1$  corresponds to a pure disc, and morphology becomes increasingly bulge-dominated with increasing  $n$ .

A second important observed property of a galaxy is its star formation rate. The star formation rate of a galaxy depends on how large a galaxy is and how much gas it has available to form stars. The SFR can be estimated in a number of ways; in the SDSS it is done using the

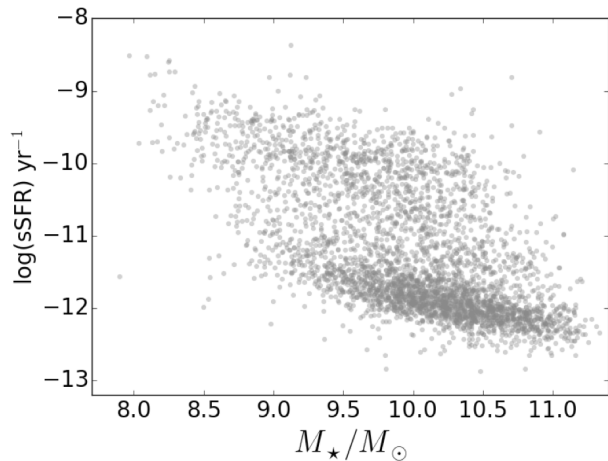
emission lines from hot gas around young stars. To control for the dependence of SFR on stellar mass ( $M_{\text{star}}$ ), we use the specific star formation rate,  $\text{yr}^{-1}$ .

To obtain star formation and morphological data on the galaxies in our sample of compact groups, we matched the MLCG to MPA JHU and GIM2D by the galaxies’ coordinates on the sky (Right Ascension, Declination). Not all galaxies in our compact group sample had matches in the other catalogues. This was partly due to the fact that MPA JHU and GIM2D originate from the SDSS Data Release (DR) 7 while the MLCG was constructed from DR12, although 1450 of the 1755 lost galaxies do appear in DR7. Figure 2 shows the relevant properties of the galaxies successfully matched and of those not. We find that the galaxies retained in our sample have lower stellar mass, lower luminosity in the r-band and are at slightly lower redshifts. It is likely that the unmatched galaxies suffer from fiber collisions (the inability to resolve objects that are close together on the sky due to the finite size of the fiber plugs in the telescope; Guo et al. 2011) or that they have low signal-to-noise ratios, making it difficult to determine star formation and morphology. Whenever possible, we conduct our analyses on the largest subsample with the required data; this means 3656 galaxies for plots with star formation data (column 3 in Table 1), 4272 with morphology (column 2), and 5178 galaxies otherwise (column 1).

In Figure 3, we show specific star formation rate plotted against galaxy stellar mass. Galaxies fall into one of two populations: star-forming or non star-forming. In the optical, these populations appear blue and red, respectively. The break in the bimodal distribution occurs at  $\log(\text{sSFR}) = 10^{-11} \text{ yr}^{-1}$ . A galaxy’s location on this plot is not static. Various processes can “quench” a galaxy (drive it off the star-forming sequence) or induce star formation activity. On a cosmological scale, the migration occurs rapidly (Wetzel et al. 2011). Processes



**Figure 2:** A histogram of three galaxy properties (Left panel: stellar mass; centre panel: magnitude in the r-band - note that brightness decreases with higher  $r$ ; right panel: redshift). Of the 5188 galaxies in the MLCG by Sohn et al. (2016), only 4372 of them are present in GIM2D morphology catalogue and 3656 in MPA JHU star formation catalogue. Shown in red is the sample remaining after matching to both, and in blue are the galaxies lost in the match.



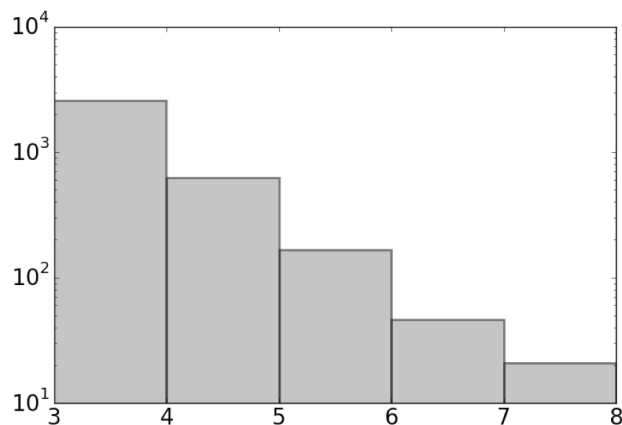
**Figure 3:** Specific star formation rate (SFR normalized by stellar mass) as a function of galaxy stellar mass for all 3656 galaxies in our sample with data in MPA JHU. Two galaxy populations are visible: star-forming and non star-forming (quenched). Like the total SDSS population, ours exhibits a bimodal distribution, with a break at  $sSFR \approx 10^{-11} \text{ yr}^{-1}$ .

are a function of the environment in which the galaxy lives in addition to the galaxy's intrinsic properties.

### GROUP PROPERTIES

As mentioned in the introduction, some properties of compact groups make them challenging to study: particularly, group richness (the number of member galaxies). As aforementioned, 1276 of 1588 compact groups have only 3 members, and in Figure 4 we show a histogram of group richness for our smallest subsample of the MLCG (3656 galaxies matched to MPA JHU). Reliable statistics cannot be run on a sample of so few member galaxies.

Low group richness has implications for another crucial group property: velocity dispersion. Similar to standard deviation, this is the statistical dispersion

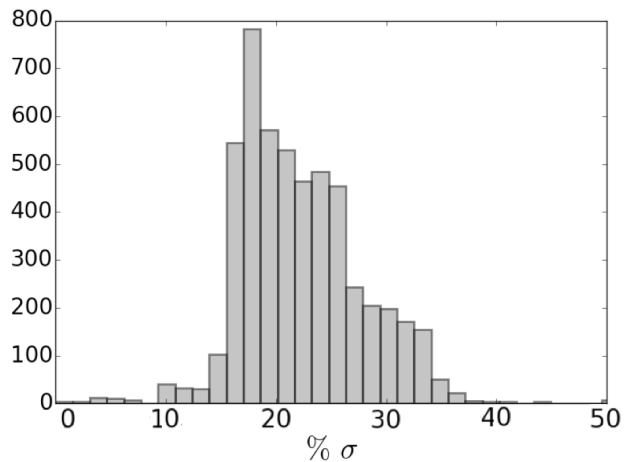


**Figure 4:** Log-histogram of compact group richness (the number of member galaxies) for the subset of our sample with data in MPAJHU and GIM2D (3656 galaxies). The vast majority of groups contain three galaxies, the minimum number to qualify as a group.

of individual galaxy velocities about their mean. It is derived for a group by measuring the line-of-sight velocities of the galaxies via spectroscopic redshifts. Observational incompleteness (due to the difficulty in resolving spectroscopic measurements for close-by galaxies) combines with the statistics to make velocity dispersion a very uncertain quantity for compact groups. Figure 5 shows the uncertainty in  $\sigma$  as a proportion of the reported value. The mean uncertainty for the whole MLCG catalogue is 22%. Two groups even have an uncertainty fraction greater than 1 (not shown).

### GALAXY AND GROUP RADII

We are interested in how the properties of galaxies are



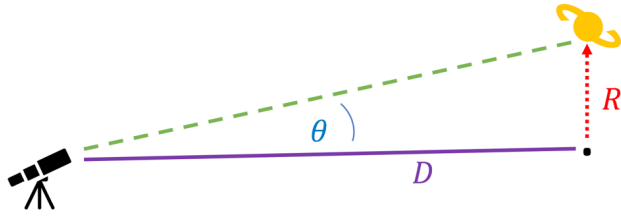
**Figure 5:** The distribution of observational uncertainty in compact group velocity dispersion ( $\sigma$ ) as a fraction of the reported value. Two groups (not shown) have an uncertainty fraction greater than 1 (185%, 511%). The mean for our sample of 5188 galaxies is 22%.

related to their distance from the centre of the group. However, investigations of radial trends cannot be done with simply the group radius as the independent variable. Compact galaxy groups come in different sizes (differing by a factor of 15), and so it is necessary to normalize the galaxy's radial distance  $R$  to a proportion of the group size,  $R_{Gr}$ .

The calculation of galaxy radial distance  $R$  involves three steps (refer to Figure 6) and was done as outlined in Hogg (2000). The distance to the group ( $D$ ) was calculated using the cosmological parameters assumed (described in the Introduction) and the group redshift ( $z$ ). By the small angle approximation, the distance between a galaxy and its group centre is simply  $D$  times the angular separation.

One commonly practiced way to define group radius is to fit a surface brightness or number density radial profile and identify where the curve falls to some chosen value; however, fitting radial profiles requires many galaxies, which compact groups do not have. The MLCG by Sohn et al. (2016) defines group radius  $R_{Gr}$





**Figure 6:** The radial distance of each galaxy ( $R$ ) from its group centre (black dot) is approximated as the angular separation ( $\theta$ ) times the angular distance ( $D$ ). The calculation of  $D$  involves cosmological parameters ( $\Omega_M, \Omega_\Lambda, \Omega_K$ ) and was done as outlined in Hogg (2000).

as the radius of smallest circle encompassing all group members. In other words, the group radius is the same as the largest galaxy radial distance. Although the motivation for this definition is understandable, it is not physical. An alternative way, often used in the literature, to calculate the radius of a system of galaxies is to assume that the system is spherical and in dynamical equilibrium. With these assumptions, we can relate group mass ( $M_{Gr}$ ), radius ( $R_{virGr}$ ) and velocity dispersion using Equation 2. Unfortunately, due to the large uncertainties in (Figure 5), we are not able to reliably estimate group radius in this way.

$$\frac{GM_{Gr}}{R_{virGr}} \approx \sigma^2 \quad (2)$$

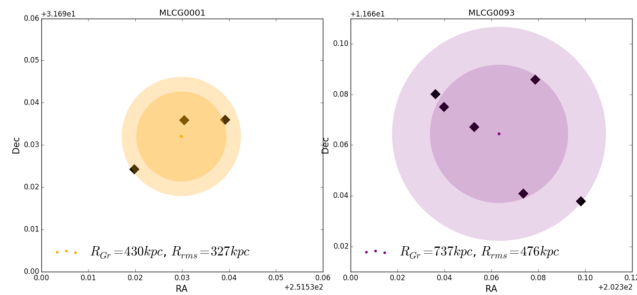
Instead, we follow the example of Mok et al. (2013) and define compact group radius as the root mean square of the constituent member galaxy radial distances:

$$R_{rms} = \sqrt{\frac{R_1^2 + R_2^2 + \dots + R_n^2}{n}} \quad (3)$$

where  $n$  is the number of member galaxies and  $R_n$  is the distance between the  $n^{\text{th}}$  galaxy and group centre (we do not redefine the centre). This definition is similar to a mean, but it takes into account the variation in the values of  $R_n$ . Because it uses the radial distances of all member galaxies, it is more physical than the  $R_{Gr}$  of Sohn et al. (2016). For comparison, we show these two definitions of group radius,  $R_{Gr}$  and  $R_{rms}$ , for two compact groups in Figure 7. The inner circle, representing  $R_{rms}$ , is necessarily smaller than that representing  $R_{Gr}$ , and does not necessarily have a galaxy located on its edge. Considering that galaxy sizes themselves are roughly on the scale of tens to hundreds of kpc, this figure also demonstrates the compactness of these groups.

## RESULTS

### CORRELATION OF GROUP RADIUS DEFINITIONS



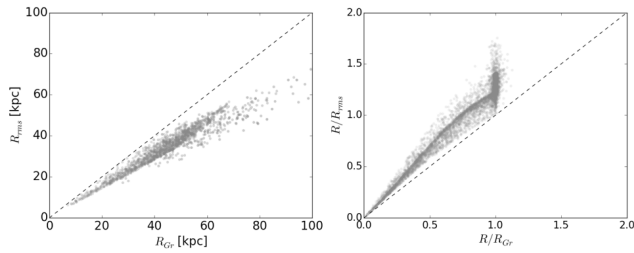
**Figure 7:** A visual comparison between two definitions of compact group radius. Points are coordinates on the sky (Right Ascension, Declination) representing the projected location of two groups and their member galaxies, as appear in the MLCG by Sohn et al. (2016). The group MLCG0001 (yellow) contains 3 members, the minimum number to be deemed a group; the group MLCG0093 (purple) contains 6, one of the richest groups in the catalogue. In both panels, the radius of the large circle corresponds to  $R_{Gr}$ . As a result, a galaxy always lies on the edge. The radius of the smaller inset circle corresponds to  $R_{rms}$ , the root mean square of all galaxies' radial distance from the geometric centre; a galaxy does not necessarily lie on the edge.

In the left panel of Figure 8 we plot our definition of compact group radius,  $R_{rms}$  against that of Sohn et al. (2016),  $R_{Gr}$ . As is to be expected based on how it is calculated,  $R_{rms}$  is always smaller than  $R_{Gr}$ , and all the points lie below the one-to-one line. For small, tight-knit groups, the two definitions do not differ greatly. However, for groups with a galaxy at a large radial distance (corresponding to a large  $R_{Gr}$ ), the root mean square  $R_{rms}$  is significantly smaller than  $R_{Gr}$ . We expect to see radial trends with  $R_{rms}$  that are similar but not identical to those of  $R_{Gr}$ .

In the right panel of Figure 8 we see what the two different definitions of group radius mean for the galaxies' normalized radial distance ("specific radius"). In this plot, the problem with the  $R_{Gr}$  definition is clearly visible. At  $R/R_{Gr} = 1$  is an artificial pile-up of galaxies that live on the edge of the group. The cut-off is not strictly at  $R/R_{Gr} = 1$  because we normalized to the  $R_{Gr}$  of the MLCG instead of to our own  $\max(R_N)$ ; the assumed cosmological parameters and details of numerical integration involved in our calculation of angular diameter distance likely differs slightly to that undertaken by Sohn et al. There is nothing physically significant about a specific radius of 1, and  $R_{rms}$  rightfully extends past 1 on its axis.

### MASS SEGREGATION

Previous work has shown that galaxy properties depend most strongly on their own stellar mass, therefore any trend in galaxy properties needs to be independent of radial trends in stellar mass in order to be significant. We explore trends of stellar mass with specific radius



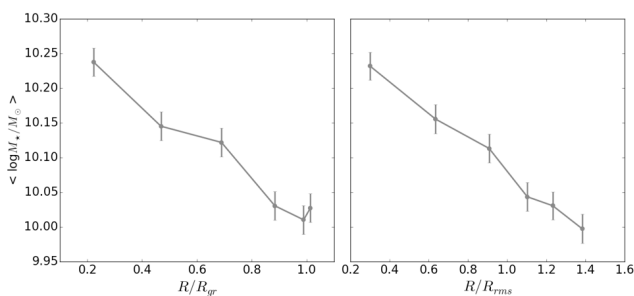
**Figure 8:** A comparison between two definitions of compact group radius. *Left panel:*  $R_{Gr}$  is the radius of the smallest circle enclosing all group member galaxies, as used in the MLCG by Sohn et al. (2016). In other words, the projected distance to the member farthest from the centre defines the radius of the group.  $R_{rms}$  is the root mean square of the projected distance of all members from their geometrical centre. *Right panel:* The specific radius of each member galaxy (distance from the group centre as a proportion of its group's radius) is plotted for the two group radius definitions,  $R_{Gr}$  and  $R_{rms}$ .

in Figure 9. Galaxy stellar mass is plotted as a function of normalized radial distance from the group centre for the two group definitions.

In both cases the trend is clear: galaxies with higher stellar mass tend to live in the centre of the group, and those with lower stellar mass on the outskirts. The range on the y-axis, however, is small ( $\log(M_{star}/M_{dot})$  0.25). We have binned the x-axis by frequency, making apparent the pile-up at  $R/R_{Gr} = 1$ , which does not occur in the right panel where specific radius is  $R/R_{rms}$ . For the remainder of the paper we fully adopt the  $R_{rms}$  definition of group radius for use in exploring radial trends.

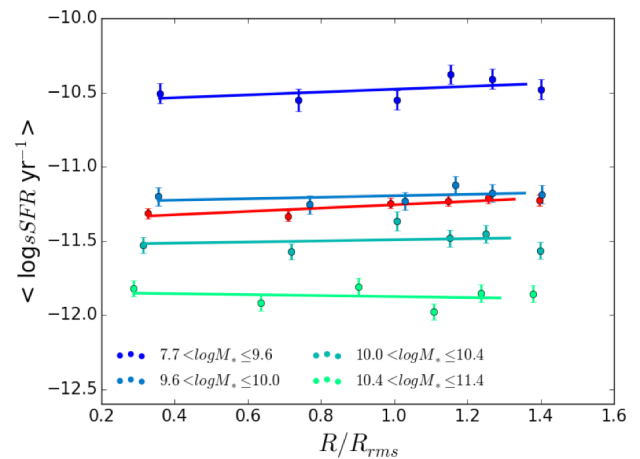
## RADIAL TRENDS IN STAR FORMATION

Figure 10 shows how the rate at which a galaxy forms stars is related to its radial position in the group. As described in Figure 3, specific star formation rate



**Figure 9:** Mean galaxy stellar mass as a function of specific radius (normalized radial distance from group centre) for all 5178 galaxies in our sample. The x-axis is binned by frequency and error bars correspond to 1 SE. *Left panel:* Galaxy radial distance normalized by  $R_{Gr}$ , the group radius definition used in the MLCG by Sohn et. al (2016). *Right panel:* Galaxy radial distance normalized by  $R_{rms}$ , the root mean square of all galaxy radii of the group.

(sSFR) is strongly dependent on stellar mass, and so we have divided our sample into four stellar mass bins. We also show the trend for the total sample (in red). We find a strong dependence of sSFR on stellar mass, with the lowest mass galaxies exhibiting the highest specific star formation rates. However, we see little to no trend with specific radius; the lines are consistent with being flat for all bins. In Figure 11 we differentiate compact groups based on their velocity dispersion. Since  $\sigma$  is such an uncertain quantity (Figure 5), we make cuts for only the highest (300km/s) and lowest (100km/s), instead of dividing the sample in half. Furthermore, we control for mixing of galaxies with different stellar masses by taking only galaxies with  $9.5 < \log(M_{star}/M_{dot}) < 10.5$



**Figure 10:** Mean galaxy specific star formation rate as a function of specific radius (normalized radial distance from group centre) for all 3656 galaxies in our sample with data in MPA JHU. In greens and blues are four stellar mass bins, and in red is the total unbinned mean. The x-axis is binned by frequency and error bars correspond to 1 SE.

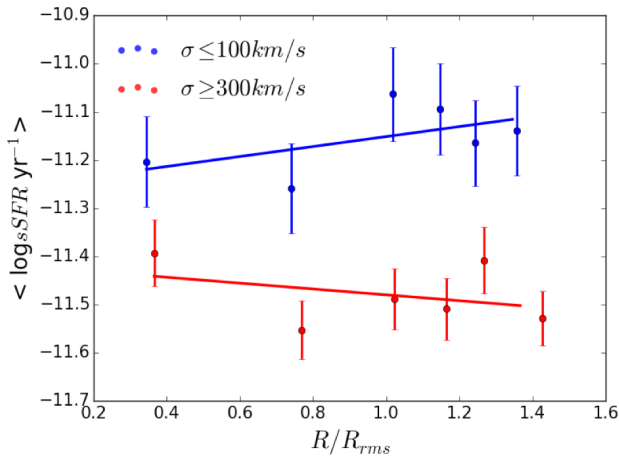
(Figure 9 estimates the issue of mass segregation). The stellar mass distributions of the two velocity dispersion samples are different (low,  $\log(M_{star}/M_{dot})$  of 9.8 compared to high,  $\log(M_{star}/M_{dot})$  of 10.1) and we found that neglecting to account for this yields a misleading radial trend in the low sample. The mean  $\log$  stellar mass of the resulting galaxies plotted in Figure 11 are different by a factor of only 0.04 dex. We find that there is a segregation of galaxies by group velocity dispersion. The galaxies in groups with low  $\sigma$  have consistently higher specific star formation rates than those with high  $\sigma$ . We find no significant trend with radial distance.

## RADIAL TRENDS IN MORPHOLOGY

Sersic index, our quantitative metric of galaxy morphology, is shown as a function of specific radius in

Figure 12. We find again a strong dependence on stellar mass, indicative of the known correlation between galaxy stellar mass and morphology: disks are lighter in stellar mass than elliptical galaxies.

The highest two stellar mass bins are consistent with being flat; high mass galaxies are more likely to be ellipticals and are found with equal probability at all radial positions. However, we do see a dependence of morphology on radial distance for galaxies low in stellar



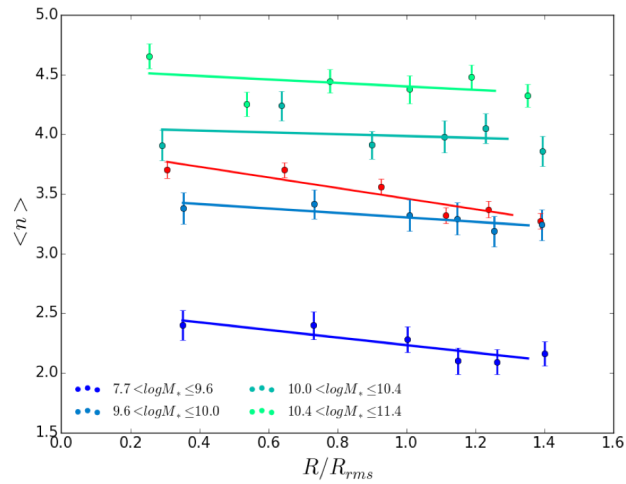
**Figure 11:** Mean galaxy sSFR as a function of specific radius (normalized radial distance from group centre) for the 2137 galaxies with  $9.5 \leq \log(M_{\text{star}}/M_{\text{dot}}) \leq 10.5$ . Galaxies are separated by group velocity dispersion: low,  $\sigma \leq 100 \text{ km/s}$  (479 galaxies); and high,  $\sigma \geq 300 \text{ km/s}$  (638 galaxies). Solid lines correspond to a least squares best fit to the means and both slopes are consistent with being flat. The x-axis is binned by frequency and error bars correspond to 1 SE.

mass. Statistically significant slopes exist for the lowest and second lowest bins, and for the unbinned samever, the radial dependence of the lowest two stellar mass bins is robust. The bulge component of low mass disk galaxies on the group outskirts is less than that of those closer to the group centre.

## DISCUSSION

Our work investigates two properties of galaxies (star formation and morphology) as functions of both stellar mass (another galaxy property) and environmental properties (radial position and velocity dispersion) in compact groups. In this section, we discuss and interpret our results in the context of previous astronomical work.

There are two quantities essential to our work whose limitations are important to keep in mind. Due to the nature of the process for calculating sSFR from the observed data, values of  $\log(\text{sSFR}) - 11.5$  have high uncertainty (Brinchmann et al. 2008); it is easy to calculate the SFR of a galaxy forming lots of stars but difficult to constrain that of a galaxy with very little



**Figure 12:** Mean galaxy Sersic index ( $n$ ) as a function of specific radius (normalized radial distance from group centre) for all 4372 galaxies in our sample with data in GIM2D. In greens and blues are four stellar mass bins, and in red is the total unbinned mean. The x-axis is binned by frequency and error bars correspond to 1 SE. Solid lines correspond to a least squares best fit to the means.)

star formation. Secondly, with respect to stellar mass, our sample is not complete. Data cannot be collected for the entire population of low mass galaxies; only the closest ones are bright enough to be measured. This introduces a potential bias with redshift, known as the Malmquist bias. In the future, we intend to estimate this incompleteness and weight our low stellar mass bins appropriately. It is important to note however that the sample galaxies with  $M_{\text{star}} > 10^{10}$  is complete.

## DEPENDENCE ON ENVIRONMENT

There are many ways to describe the nature of the environment in which a galaxy exists, although we focus on two common metrics. Equation 2 shows that group velocity dispersion is proportional to the mass of the group. A galaxy's mass takes two forms: ordinary matter (stars, gas) and dark matter. The mass of the combined group dark matter halo in which the visible mass sits,  $M_{\text{halo}}$ , can be proxied by the group velocity dispersion,  $\sigma$ . Because the value of  $\sigma$  is so uncertain (see Figure 5), we do not have a well-determined measure of this first environmental property, halo mass. Our second environmental measure is normalized galaxy radial position,  $R/R_{rms}$ . Galaxies living near the centre are in much denser environments. The problem with this metric is introduced by the necessary normalization of galaxy radial position by group radius, defined as  $R_{rms}$ , which again is affected by low  $N_{\text{Members}}$ . Controlling for stellar mass, a given galaxy has the same specific star formation rate regardless of where it lives

in its compact group (Figure 10). This is consistent with previous work by Ziparo et al. (2012), which finds a marginal dependence of sSFR on normalized radial distance in non-compact galaxy groups. The intergalactic separation of non-compact groups is on the order of hundreds of kpc and group richness  $10 N_{\text{Members}}$ , in contrast to the compact groups' mean separation of tens of kpc and  $3 N_{\text{Members}}$ . These two types of galaxy systems are very different, yet they exhibit similar radial trends with star formation.

The radial trend with morphology of Figure 12 exists only for galaxies of low stellar mass; their bulge component increases closer to the group centre. In addition to low mass, these galaxies have rotation-supported structure and tend to be gas-rich; they are easily disrupted by gravitational interactions. Our results suggest that low mass galaxies "feel" their environment more than high mass ellipticals. This is consistent with the work of Roberts et al. (2015), which finds that the effect of an X-ray-rich environment (which is to increase disk fraction) is strongest for low stellar mass galaxies.

It is common practice in astronomy to understand galaxy environment by considering the group's gravitational potential well. For an aggregation of a sufficient number of galaxies, it is possible to trace out the potential well with the velocities of the galaxies. It is unknown whether compact groups have similar dynamics and velocity distributions to regular groups and clusters. As shown in Figure 4 and 5, group velocity dispersion is very uncertain as compact groups have very few members. Understanding the precise shape of their potential well is challenging.

In this context, two main ideas can explain the lack of radial dependence for star formation rates. The first hinges on a relative scale concept: with the separation between galaxies in compact groups being comparable to the size of the galaxies themselves, the environmental gradient between the group centre and edge may too small to be noticed by whole galaxies. A second idea, perhaps more plausible (also suggested by Hickson, 1997), is simply that the centre of a compact group is not a well-defined quantity. We can speculate on what the observational work suggests, but a more complete understanding will come from theoretical work using simulated galaxy groups.

However, we can look at groups with the largest and smallest potential wells in our sample, which we effectively do with the 300km/s and 100km/s group samples in Figure 11. Our result that galaxies in groups with low  $\sigma$  have consistently higher specific star formation rates is consistent with previous work. Wetzel et al. (2011) find a strong dependence of galaxy sSFR on the mass of the dark matter halo in which the galaxy lives. Although their study looks directly at halo

mass and we look at velocity dispersion, because  $M_{\text{halo}}$  and  $\sigma$  are related (Equation 2), the agreement suggests that this is another aspect in which compact groups are similar to non-compact groups.

Considering the extreme differences between these two types of galaxy systems (namely, galaxy-galaxy separation), it would not be surprising if they exhibited very different galaxy properties. We find instead that they are in fact similar, in the ways we have outlined in this work.

## DEPENDENCE ON INTERNAL PROPERTIES

In addition to environmental dependencies, we also explore how star formation and morphology depend on stellar mass; we do this by binning our data by frequency into quartiles. In both Figure 10 and 12, there is clear segregation between the bins. That Sèrsic index correlates strongly with stellar mass is a well-known global trend (Conselice 2014). The result that sSFR depends strongly on stellar mass is consistent with previous work. In addition to finding a strong dependence on  $M_{\text{halo}}$ , Wetzel et al. (2011) also find a sSFR distribution predominantly more star-forming for galaxies of low stellar mass. It is not clear in the literature whether the mechanisms for quenching star formation seen in large galaxy clusters (such as ram-pressure stripping or strangulation) could apply to the smaller regime of groups or compact groups (Wetzel et al. 2011).

## GALAXY PAIR INTERACTIONS

The clear segregation in sSFR between galaxies in Figure 11 shows increased star formation in groups with lower group velocity dispersion. Although not exactly equivalent, the group velocity dispersion is an effective indicator for galaxy-galaxy flyby speeds. Within the constituent member galaxies, stars have internal velocities on the order of 100-200km/s. If the trajectories of two galaxies in a group bring them past each other at a speed comparable to their internal stellar velocities, gas dynamics are more affected. Interactions at these speeds are also generally longer in duration. Shock fronts (high density areas) are created, making for effective radiative cooling of intragalactic dust and gas. It is this material that collapses to form stars. Features indicative of galaxy pair interactions are visible in the images of four compact groups in Figure 1. Tidal tails, shock fronts and regions of high star formation are created as the galaxies slosh about in the potential well. Compact groups are ideal systems in which to study intergalaxy interactions.

## CONCLUSIONS

Our main findings are as follows.

- The star formation rates and morphologies of galaxies in compact groups depends strongly on their internal stellar mass. Low stellar mass galaxies are more star forming and diskier.
- Galaxies in compact groups with low velocity dispersion, and therefore lower halo masses, are more star forming.
- No radial trend exists for star formation rates of galaxies in compact groups. A weak radial trend exists for morphology at low stellar mass; the bulge component of low mass disk galaxies living on the outskirts of the group is less than that of those in the group centre.

In future work, we would like to explore galaxy X-ray profiles to define compact group centres based on a peak in that wavelength. Additionally, with simulations, we could trace out the potential well and quantify the effects of uncertain centres and test different definitions of group radius.

## ACKNOWLEDGEMENTS

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# The effects of used motor oil on *Myzus persicae* and *Arabidopsis thaliana*

YICHANG GE, DHVANIL JOSHI, CHRISTOPHER CHAN, SARA MAR, BEATRICE CO

Integrated Science Program, Class of 2020, McMaster University

## SUMMARY

Used motor oil (UMO) is a highly toxic substance that enters the environment as runoff from roadways and other urbanized impermeable surfaces. The effects of various runoff contaminants on the environment have been studied extensively, with research focusing on efficient and effective methods of clean-up. This study serves to examine the effects of varying volumes of UMO on plants and the subsequent impacts on herbivores. *Arabidopsis thaliana* was used as the model plant organism due to its rapid life cycle and *Myzus persicae* was used as the model herbivore organism due to its high proliferation rate. The results showed a statistically significant effect ( $F_{oil(3,64)}=3.3853$ ,  $P=0.023$ ) of UMO treatment on plant performance. There was also a statistically significant effect ( $F_{21,256}=2.4661$ ,  $P=0.00052$ ) due to the interaction of oil and day on the herbivore of the plant. The results indicate that contaminants can affect multiple trophic levels, which should be considered when looking at possible issues regarding the contamination of natural environments.

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**Keywords:** used motor oil, *A. thaliana*, *M. persicae*, block, plant stress, bioaccumulation, rosette diameter

## INTRODUCTION

Bioaccumulation of contaminants within an ecological community is a threat to urban biological systems (Tchounwou et al., 2012). Being sessile, plants are among the most vulnerable to these environmental effects. It is found concentrated primarily on roadways and other urbanized impermeable surfaces and enters the environment as runoff, potentially causing harmful effects on vegetation and biological diversity (Environment and Climate Change Canada, 2010). Used motor oil (UMO) is a highly toxic substance that contains heavy metals, polycyclic aromatic hydrocarbons, and dioxins, which are some of the most common ecosystem contaminants (Australian Government, 2010). When introduced to biological organisms, heavy metals can negatively impact cellular organelles (Tchounwou et al., 2012). The exposure of *Arabidopsis thaliana* to dioxins has been shown to result in a reduction in fresh weight, a drastic decrease in chlorophyll content, and a decline in seed germination (Hanano, Almously and Shaban,

2014). Polycyclic aromatic hydrocarbons have been shown to cause root growth reduction and trichome deformation in *A. thaliana* (Alkio et al., 2005). Therefore, UMO contamination can be used as a model to study the bioaccumulation of toxins within ecosystems. While previous studies show that UMO exposure leads to the overall reduction of plant growth in *A. thaliana*, no specific studies have looked at the resultant effects of this reduction on the subsequent trophic level. By monitoring the population of *Myzus persicae* on *A. thaliana* plants treated with varying amounts of UMO, this study creates a model for contamination effects on trophic levels. The experiment aims to answer (1) whether UMO contamination affects plant growth and (2) whether the oil contamination affects the herbivore of the plant.

## STUDY SPECIES: PLANT

*A. thaliana* (Col) has become a staple model organism for scientific research due to the ease of its cultivation

and success at plant transformation (Meinke et al., 1998). It is a small plant of the mustard family and is native to a broad distribution of regions consisting of Europe, Asia, and North America (Meinke et al., 1998). In 1987, the modern era of *A. thaliana* research surged into the frontlines (Meinke et al., 1998). This simple angiosperm has become a pivotal factor to the scientific advances in understanding plant development (Meinke et al., 1998).

### STUDY SPECIES: HERBIVORE

*M. persicae* is a polyphagous generalist aphid that feeds on over 40 families of plants (Blackman R.L. & Eastop V.F., 2000). The species is heteroecious holocyclic, but anholocyclic in tropical areas (Schoonhoven et al., 2005). A colony is mostly made up of females for the majority of the year, which results in rapid aphid reproduction (Blackman R.L. and Eastop V.F., 2000). The high proliferation rate validates the choice of *M. persicae* for this short 11-day study. Wingless aphids were specifically chosen because winged aphids have a lower individual fecundity (Mutti et al., 2008).

*M. persicae* and *A. thaliana* have been heavily involved in an evolutionary arms race leading to several different plant defenses and subsequent herbivore adaptations (Schoonhoven et al., 2005). Since *A. thaliana* is considered to be a generic plant and aphids are generalist insect herbivores, the host-attacker relationship can be used as a model in studying plant-animal interactions (Blackman R.L. & Eastop V.F., 2000). There is a plethora of applications to study from this model because of the large majority of plants and animals categorized under generalist species.

### USED MOTOR OIL

The UMO was obtained from Active Green + Ross Tire Automotive Centre located at 1289 Main St. W., Hamilton. The brand and the type of car the oil was extracted from is unknown.

### METHODS

#### EXPERIMENTAL DESIGN

Each sampling unit comprised of *A. thaliana* in the rosette life stage. The plants were placed in 5.5cm x 5.5cm x 10cm rectangular plastic pots filled with moist soil. 40 sampling units were split into two repeated tests, Block A and Block B. Both blocks consisted of a four by five arrangement of the sampling units. Four UMO treatment levels were used: 0mL, 1mL, 3mL, and 5mL. Each treatment level consisted of five plants, labelled 1-5, 6-10, 11-15, and 16-20 respectively.

**Table 1:** Assigned locations of plants for Block A based on a random stratified model created using Microsoft Excel software.

8	6	12	5
11	7	1	9
20	19	2	13
17	10	16	18
14	15	4	3

**Table 2:** Assigned locations of plants for Block B based on a random stratified model created using Microsoft Excel software.

12	15	20	18
9	1	8	17
14	4	5	11
7	3	2	6
13	10	16	19

The plant locations were determined using the Microsoft Excel software to produce a random stratified model, as shown in Table 1 and Table 2 (Microsoft, 2017). The RAND() and RANK() Excel functions were used to create a set of unique random numbers from 1 to 20 with each number corresponding to a location from the top left corner to the bottom right corner in the 4 by 5 grid, set up from left to right in each row. This process was repeated to create a new set of random numbers for Block B. This design minimized the effect of biases, causal claims, and accounted for external factors such as disproportionately distributed sunlight. The plants were placed so the plastic walls of the pots were touching, ensuring rosette leaves were not damaged.

The UMO was administered with 10 mL pipette pumps on the soil near the base of the plant. UMO contact with *A. thaliana* was carefully avoided. Each sampling unit was then inoculated with two adult female *M. persicae*. The aphids were placed at the lower base of the stem, avoiding the trichomes which could have affected the aphids. The sampling units were then placed on plastic trays with a transparent dome-shaped lid. This enclosed the plants with approximately 30cm of space between the top of the plant and the top of the lid. There was a mesh screen of about 100cm<sup>2</sup> that allowed for air exchange but prevented aphids from escaping. The individual plants were not removed from the design during data collection to ensure minimal disturbance to other sampling units. At the halfway point (day 5), 3mL of water was added near the corners of the sampling units with an eye dropper. Aphid population



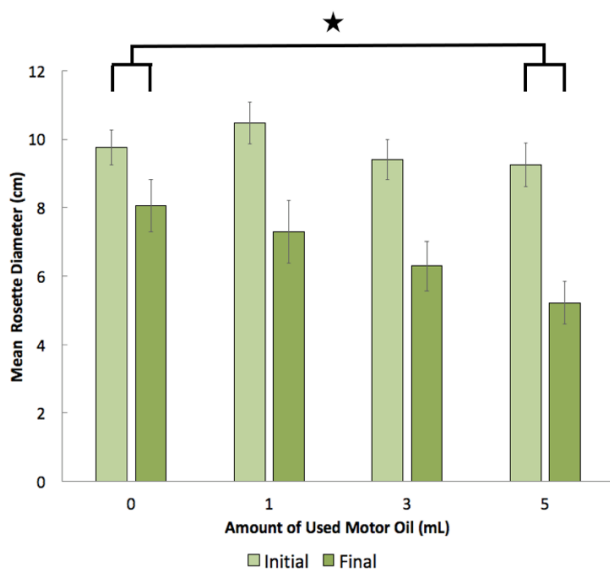
count was recorded on days 1, 4, 5, 6, 7, 8, and 11 to examine the effect of oil contamination on the herbivore of the plant. Rosette diameter was measured on days 0 and 11 to examine the effect of UMO on plant performance.

## STATISTICAL ANALYSIS

Statistical analysis was completed using R programming language, on version number 3.4.2. Analysis of variance (ANOVA) was used to investigate the independent and correlating effects of several variables (The R Foundation for Statistical Computing, 2016). These include: UMO treatment levels of 0 mL, 1mL, 3mL and 5mL, test days from Day 0 to Day 11 (with inconsistent days in between), blocks A or B. The dependent variables that were observed are mean aphid population per sampling unit and mean rosette diameter. Mean aphid population per day was quantified by taking the total number of aphids on sampling units within a treatment level per day and dividing it by the number of sampling units. Mean rosette diameter was defined as the sum of all rosette diameters divided by the number of sampling units. A Tukey Honestly Significant Difference post-hoc analysis was used to determine which UMO treatment levels and test days had a statistically significant effect on mean aphid population and mean rosette diameter (The R Foundation for Statistical Computing, 2016).

## RESULTS

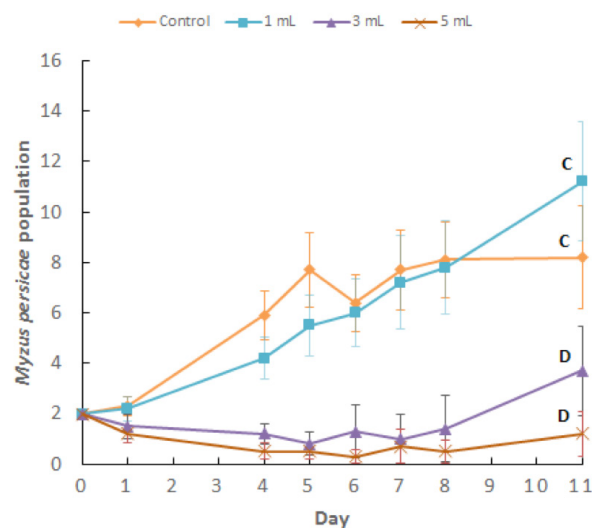
### UMO EFFECT ON PLANT GROWTH



**Figure 1:** Initial and final mean rosette diameter measured from the farthest tips of each rosette per treatment group for both Block A and Block B. Values are means  $\pm$ SD. The star denotes a difference between the control and 5mL treatment groups.

Initial and final mean rosette diameters were compared with the different treatment groups in order to investigate the effect of UMO on plant growth. It was determined that the UMO treatment levels had a statistically significant effect on the mean rosette diameter per treatment group (ANOVA,  $F_{3,64}=3.385$ ,  $P=0.023$ ). A statistically significant difference was determined between the rosette diameters of the control and 5mL treatment level (Tukey,  $P=0.048$ ), as denoted with the star in Figure 1. It is observed in Figure 1 that there is a suggested difference which supports these statistical findings.

### UMO EFFECT ON HERBIVORE

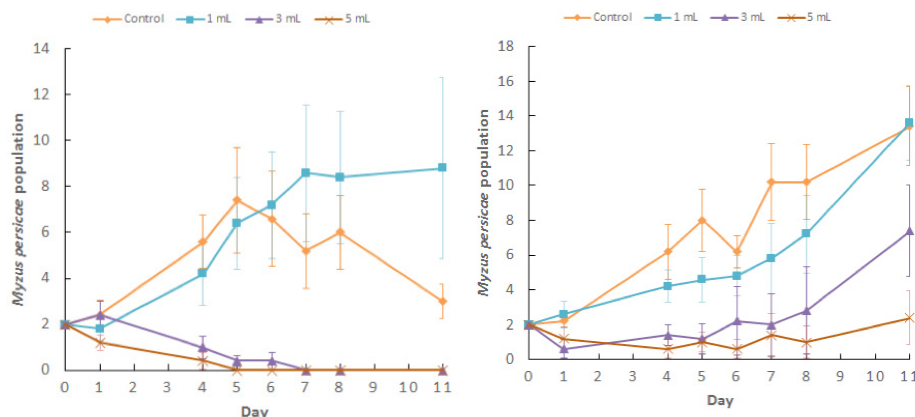


**Figure 2:** Daily mean aphid population per sampling unit for both Block A and Block B. Values are means  $\pm$ SD. It is suggested through the illustration that there is a significant effect between treatments of 0-1mL and 3-5mL denoted as treatments C and D respectively.

Mean aphid population was plotted over the data collection period in order to investigate the effect of oil on the herbivore of the plant. The interaction between UMO treatment levels and day had a statistically significant effect on the mean aphid population per sampling unit of both Block A and Block B (ANOVA,  $F_{21,256}=2.4661$ ,  $P=0.00052$ ). The interaction between UMO treatment and day demonstrated that the treatment levels had no significant difference within aphid count between the control and 1mL levels as well as the 3mL and 5mL treatment levels (Tukey,  $P=0.50$ ;  $P=0.98$ ). These treatment levels can therefore be grouped, denoted by C and D in Figure 2 and the shown graph supports this statistical significant difference.

### DIFFERENCE IN TREATMENT BLOCKS

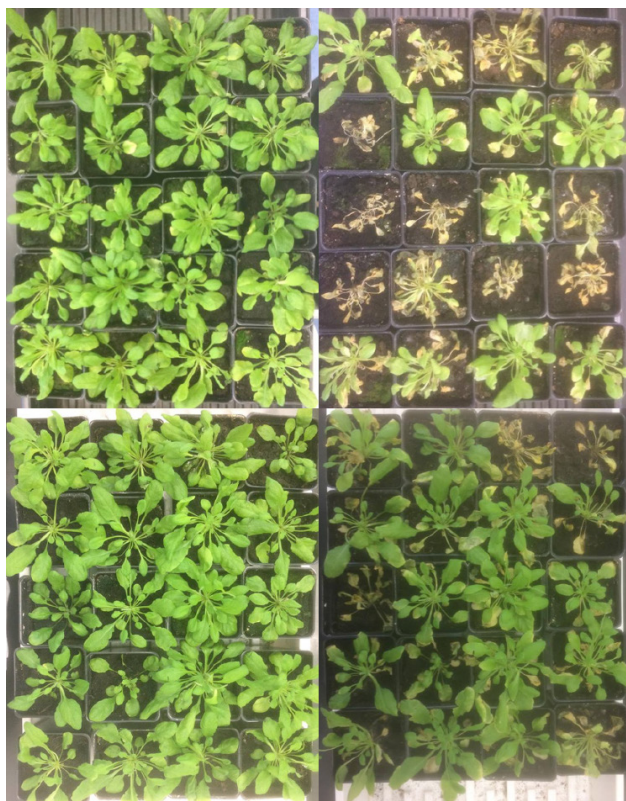
In the analysis of the differences between Block A and Block B, the same statistical conclusions were found



**Figure 3:** Mean *Myzus persicae* population per sampling unit over 11 days after being treated with various amounts of UMO for Block A (left) and B (right). Values are means  $\pm$ SD. These population means are calculated through the average aphid count at a varying time on each day, roughly from the times 10am to 4pm.

as if the data of the two blocks had been run together (ANOVA, Block A:  $F_{21, 288} = 2.286$ ,  $P = 0.0013$ ; Block B:  $F_{21, 288} = 2.286$ ,  $P = 0.0013$ ). On each both block A and B, there was no statistically significant difference between the control and 1mL treatment levels on aphid count (Tukey,  $P = 0.50$ ). Additionally, there was no statistically significant effect on count when comparing the 3mL and 5mL treatment levels (Tukey,  $P = 0.98$ ) (Figure 3). There was a statistically significant difference between the two sub-groups, previously denoted as C and D in Figure 2. Qualitative observations, taken of Block A and Block B at the beginning and end of the experiment, shown in Figure 4, supported this data.

## DISCUSSION



**Figure 4:** Block A (top left) and Block B (bottom left) on day 0 compared to Block A (top right) and Block B (bottom right) on day 11.

## UMO EFFECT ON PLANT GROWTH

The study investigated whether or not UMO was a significant stress factor in influencing plant performance. There was a statistically significant effect (ANOVA,  $F_{3, 64} = 3.3853$ ,  $P = 0.023$ , Tukey,  $P = 0.048$ ) of oil on rosette diameter for plants treated with 5mL of UMO, as shown in Figure 1. On day 5, the halfway point, 3mL of water was added to each plant. A small quantity of an odourless liquid, assumed to be water, seeped out onto the paper towel underneath. This may suggest that the roots of the plants became coated with oil which prevented root uptake of water. A study conducted by Abioye, Agamuthu and Abdul Aziz (2012) found that hydrocarbons could coat root surfaces, restricting gas and water exchange. Without water, plant growth and performance are stunted and the photosynthesis process is left without a vital resource (McElrone et al., 2013). Photosynthesis plays a large role in the growth of *A. thaliana*, and with the limited presence of water, the plant must actively reprogram its metabolism and growth (Claeys and Inzé, D., 2013). This means *A. thaliana* has shown to be flexible toward water limitations. A supporting example of this claim is shown through a study by Skiryecz and Inzé (2010), which showed sharp declines in leaf elongation, which was then followed by recovery to a steady growth rate known as acclimation. This could indicate that if given the opportunity to continue the experiment over time, rosette diameter could restabilize if allowed proper resources. In our plant environment with both biotic (aphids) and abiotic (oil) stress factors, it would be likely that plants focus their resources on surviving. Overall, this suggests that greater concentrated oil treatments damage plant growth in terms of rosette diameter which could be explained by *A. thaliana* allocating its resources towards survival rather than growth.

## UMO EFFECT ON APHID POPULATION

The ANOVA and Tukey post-hoc results indicate that the 3mL and 5mL treatment levels of UMO had a statistically significant effect on *M. persicae* population (Figure 2). The final aphid populations on the 3mL and 5mL

treated plants were much lower than the final aphid populations in the control and 1mL plants. The 1mL treatment had a greater positive effect on aphid population than all of the treatment levels including the control group. This supports the plant stress hypothesis, which states that environmental stress on plants decreases plant resistance (Joern and Mole, 2005). It claims that herbivores favour feeding on plants under biotic and abiotic stressors more than unstressed plants (Joern and Mole, 2005). The increased performance of *M. persicae* on the 1mL treated plants could then potentially be the result of the decrease in *A. thaliana* defense mechanisms. Plant defense could have been hindered in several ways: damaged morphological features, reduction in toxic chemicals present, weakly expressed volatiles, and change in foliar chemistry (War et al., 2012; Joern and Mole, 2005). Trichome deformation eliminates physical barriers to aphid movement and decreases secretion of poisonous secondary metabolites (War et al., 2012). Plant volatiles are weakly expressed resulting in a reduction of harmful signals (Gatehouse, 2002). With change in foliar chemistry, herbivores prefer to feed on stressed plants with greater nitrogen concentrations since it has a central role in all metabolic processes (Joern and Mole, 2005; William J. Mattson, 1980).

## BLOCK EFFECT

Initial ANOVA tests determined that there was a statistically significant effect on aphid count due to a possible block effect ( $F_{\text{block}(1,308)} = 7.7828$ ,  $P = 0.0056$ ). This idea may be supported by how qualitatively different Block A was compared to Block B after the testing period, as seen in Figure 4. This raised concerns about the conclusions made in response to the second research question since statistics were run on the combined Block A and B data. As such, individual ANOVA and Tukey post-hoc tests of Block A and Block B were taken; however, these tests resulted in the same conclusions and significant differences between UMO treatment levels that were previously indicated by the tests using the data from both blocks. It is possible that the block effect resulted from unequal averages of beginning rosette diameters between the two blocks. It is suggested that future experiments address this issue by having the two random stratified blocks begin with equal average rosette diameters. Another possible explanation is that the block effect resulted from the random clustering of the higher UMO treatment levels within Block A but not in Block B.

## LIMITATIONS AND NEXT STEPS

The study consisted of four oil treatment groups with only 10 individual plants in each group. This is quite a

small sample size, resulting in an increased influence of outliers on the data trends. A larger sample size of *A. thaliana* was not possible due to resource restrictions, but is necessary in order to further support any prevalent patterns identified. Improvement of the experimental design could potentially include increasing the number of plants to a sample size of 400. The increase by a factor of 10 allows for more specific results and increases the accuracy of any trends observed. The experiment suggested a point between 1mL and 3mL where significant negative effects occurred on the aphid count. Therefore increasing the number of treatment levels to 10 and using more specific dosage increments would allow this threshold to be further pinpointed. Resource limitations also resulted in a sample plant population with varying initial rosette diameters, meaning there was inherent error in rosette diameter change. An improved experiment would have a controlled initial rosette diameter for all plants used so the change in rosette diameter can be increasingly correlated to the treatment level.

Additionally, the experiment was carried out over a period of 11 days. Compared to the overall lifespan of *A. thaliana* of approximately six to eight weeks (Koornneef and Scheres, 2001), the experiment time frame was considerably shorter, potentially resulting in less significant observed changes in rosette diameter, leaf number and size, and stem length. An improved experiment would involve measuring results over a larger time period - potentially the entire lifespan of *A. thaliana*. Furthermore, contamination effects were studied on *A. thaliana* only in the rosette stage. In order to achieve more accurate results, contamination effects would need to be observed during several different plant life stages, accounting for factors such as variation in plant biomass, defense, and nutrient uptake.

The effects of UMO on plants and herbivores should be further examined in greater detail. Possible areas of future research include studying the effects of UMO contamination on higher trophic levels in an environment and the results of biomagnification in affected ecosystems, studying UMO quantity limits before negative impacts on fauna are distinguishable, and studying how plants in different life stages respond to UMO contamination.

## CONCLUSION

The exposure of *A. thaliana* to 5mL of UMO had a statistically significant negative effect on plant rosette diameter compared to the control, indicating that oil contamination negatively affects plant growth. The 3mL and 5mL treatments had a statistically significant negative effect on aphid population while the 1mL treatment was statistically equivalent to the control,

indicating that oil contamination negatively affects the herbivore of the plant once a critical point of contamination is reached. This study indicates that pollutants can influence multiple trophic levels, which should be taken into account when looking at future contamination of natural environments.

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## AUTHOR CONTRIBUTIONS

C.C., B.C., Y.G., D.J., and S.M., conceived and carried out the experiments. S.M. took the lead in running statistics, with Y.G. collaborating with C.C. to integrate the necessary statistics for figures. B.C. and D.J. had a primary focus experimental design and analysis of results. All authors contributed to the final writing and editing process of the manuscript.

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# Investigating the link between Elite-level gymnastics and short stature

VARSHA JAYASANKAR

Integrated Science Program, Class of 2018, McMaster University

## SUMMARY

Women's artistic gymnastics is one of the most popular spectator sports at the Olympic Games. Artistic gymnasts compete in two streams—elite, regulated at the international level with ~40 hours/week of training and interclub, regulated at the national level with ~20 hours/week of training. Noticeable physical features of gymnasts include short stature and short limbs. Height data of gymnasts from the 2012 Olympic Games, 2016 Olympic Games, and 2016 NCAA Gymnastics Championships were collected and analyzed to determine whether elite gymnasts were significantly shorter than interclub and non-gymnast counterparts. Elite gymnasts at both Olympic Games were significantly shorter than interclub gymnasts at the NCAA Championships ( $p < 0.0001$ ). Additionally, gymnasts from each nation were significantly shorter than the national average height from females ( $p < 0.05$ ). Furthermore, a literature review analyzed physiological and osteological mechanisms behind the differences in stature. Female gymnasts show reduced levels of 17- $\beta$ -estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), critical growth hormones in female development. High prevalence of growth plate injuries in the olecranal, patellar and tarsal regions of gymnasts suggest a potential mechanism for shortened limbs in gymnasts. Height data of gymnasts who competed at both the 2012 and 2016 Olympic Games determined that more than 50% of returning competitors displayed growth, and gymnasts who took a lengthy (1+ year) break from gymnastics demonstrated growth.

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## INTRODUCTION

Artistic gymnastics is one of five disciplines within the sport of gymnastics recognized by the Fédération Internationale de Gymnastique (FIG). It is one of the most popular spectator sports at the Olympic Games. In women's artistic gymnastics, athletes compete in four different apparatus—vault, uneven bars, balance beam and floor—performing short (30 to 90s) routines on each. These routines require a combination of strength, flexibility and agility to complete the mandatory movements (Girinov, Parry and Girinov, 2004).

A noticeable fact about Olympic gymnasts are their short statures. The most decorated gymnast at the 2016 Olympic games, Simone Biles, stands at 4'8" (142.24

cm). Past gymnastics stars Nadia Comaneci, gold medalist at the 1976 Olympic Games, and Mary Lou Retton, gold medalist at the 1984 Olympic Games, stood at 4'10" (147.32 cm) and 4'9" (144.78 cm), respectively (De Benito Sanz et al., 2016)

The petite heights of Olympic gymnasts have caused many controversies over the past 30 years. A gymnast must be 16 years of age by the end of the calendar year to compete at international competitions such as World Championships or Olympic Games. The small statures of some gymnasts cause them to appear younger than 16, casting doubts on whether age falsification has occurred. At the 2008 Olympics, the ages of four Chinese gymnasts was brought into questions, after all were said to be 16 but were less than 145 cm in height. After

investigation, it was determined that all gymnasts were of their reported age (Macur, 2008).

Not all gymnasts are of short stature. Two-time Olympic champion Svetlana Khorkina was 165 cm tall at the peak of her career. Five gymnasts at the 2016 Olympic games were Khorkina's height or taller (International Olympic Committee, 2016). This study investigates whether elite female artistic gymnasts are significantly shorter than their non-elite and non-gymnast counterparts. Furthermore, an analysis of relevant literature will determine the ecological, hormonal and physiological causes behind short stature in female artistic gymnasts.

## BACKGROUND

### FEMALE GROWTH AND MATURATION

Puberty is the process by which hormonal changes signal a child's body to undergo physical transformations to mature into an adult with sexual reproduction capabilities. Females begin puberty at approximately age 10; the process is completed around age 16 (Kail and Cavanaugh, 2008). Puberty is regulated by hypothalamic-pituitary-gonadal axis and is initiated in the hypothalamus through the secretion of gonadotropin-releasing hormone (GnRH) at increasing levels (Millar et al., 2004). A possible mechanism from GnRH increase is through the increase of leptin, the satiety hormone.

In addition to the characteristic physical changes such as large growth spurts and development of feminine or masculine body types, puberty is also defined by behavioural changes—one such change in increased nutritional and food intake. Increased intake, especially with regards to fats, results in an increase in leptin release (Clavien, Theintz, Rizzoli and Bonjour, 1996). Leptin receptors that initiate the production of GnRH are located in the hypothalamus (Meister and Hakansson, 2001). Via the hypophyseal portal system, a network of blood vessels, GnRH travels from the hypothalamus to the anterior pituitary and binds to receptors on secretory cells, which in turn release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Charlton, 2008).

LH and FSH are two critical hormones in female development. LH supports the theca folliculi, a layer of the ovarian follicle, which are responsible for the production of 17- $\beta$ -estradiol precursors. FSH initiates follicular growth especially in oocyte precursors (granulosa cells). Both of these actions are part of a positive feedback loop and result in an increase in estrogens. This in turn allows for greater expression of LH, allowing for more 17- $\beta$ -estradiol. The development of secondary sex characteristics, vertical growth and changes in body shape are driven by the estradiols. This positive feed-

back loop continues until the follicle has fully matured, at which point, 17 $\alpha$ -hydroxyprogesterone inhibits the production of estrogens (Mahesh, 2011).

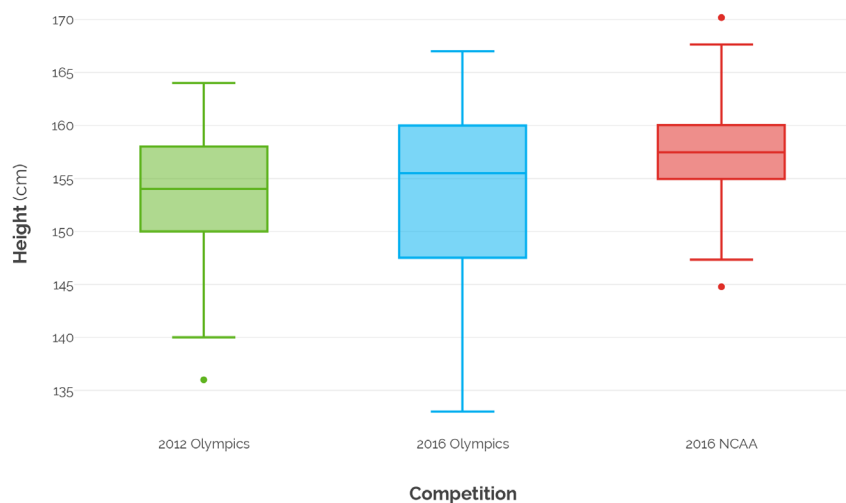
Another hormone that plays an important role in childhood growth is Insulin-like growth factor-1 (IGF-1), a hormone with similar structural features as insulin. Newborns diagnosed with IGF-1 deficiency are 2-10cm smaller than healthy babies, and present growth-related defects such as smaller extremities and heads (Lar-on, 2001). Additionally, the use of IGF-1 as a growth promoter resulted in an increase of in mean growth velocity from 3-4.7 cm/year to 8.2-9.1 cm/year. IGF-1 binds to its specific receptor (IGF1R), which initiates intracellular signaling and activates the AKT signaling pathway. This pathway is a stimulator of cell growth and proliferation. Specifically, with regards with human growth, IGF-1 is responsible for the large growth spurts during puberty. It allows long bones, such as the femur and, and soft muscle tissues to grow (Soliman et al., 2014). Production of IGF-1 is controlled by human growth hormone (hGH). hGH, also known as somatotropin, is produced in the anterior pituitary gland and released into the bloodstream, reaching the liver for IGF-1 production (Yakar et al., 2002). Stress and reduced concentrations of sex hormones inhibit hGH, which in turn inhibits IGF-1 and reduces skeletal and muscular growth (Caine et al., 2001) especially from an auxological perspective. The objective of this review is to determine if gymnastics training inhibits growth of females.

Adrenarche is period of the growth that precedes puberty, occurring between ages 7 and 10. During this stage, the adrenal cortex secretes increased levels of androgens such as dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S) (Parker, 1993). These hormones are responsible for adolescent changes such as pubic and axillary hair, body odour and mild acne. DHEA has also been proven to be a partial agonist of estrogen receptors ER $\alpha$  and ER $\beta$ , causing similar growth effects as estradiol (Webb et al., 2006).

### LEVELS OF GYMNASTICS

Women's artistic gymnastics in the United States, Canada, Great Britain and Australia can be divided into two different streams—Elite and Interclub. Gymnasts in the Elite stream intend to compete internationally (including but not limited to world championships and Olympic Games), while Interclub gymnasts limit their competition to the national level. The two streams differ in numerous ways, including but not limited to regulatory body, hours of training, scoring systems and routine composition.

Elite gymnastics is regulated by the FIG. Therefore,



**Figure 1:** Heights of female artistic gymnasts in elite (2012 and 2016 Olympics, n=60 for each) and interclub (2016 NCAA Championships, n=66) level competitions. All three sets of heights are normally distributed, as per the Shapiro-Wilk test. At the 2012 Olympic Games, the mean was 153.45 cm, with a standard deviation of 6.09. At the 2016 Olympic Games the mean was 153.75 cm, with a standard deviation of 7.82. The heights of gymnasts at the 2016 NCAA Gymnastics Championships had a mean of 158.07 cm with standard deviation of 5.24.

there is uniformity in the rules, regulations and requirements that these gymnasts are held to at an international level. Gymnasts at this level, are scored using an open-ended system, regulated by the Code of Points. There are two components to this scoring system—an open-ended difficulty score and an execution score out of 10. Because of this scoring system, elite gymnastics gives preference to difficult skills (Fédération Internationale de Gymnastique, 2016a). A recommended training plan for elite gymnasts consists of two workouts per day (2-3 hours in the morning, 3-4 hours in the afternoon), 6 days week. This results in 30-42 hours/week of training (Georgopoulos, 2002).

Interclub gymnastics typically consists of multi-level program, regulated by national governing bodies. Scoring in this stream is usually out of 10, with the maximum score assigned to a perfect routine. Because of the difference in scoring in this stream, quality of gymnastics is emphasized over difficulty. Gymnasts competing at the maximum level of Interclub streams train 18-25 hours per week, on average (USA Gymnastics, 2017). In the United States, gymnasts at this level train with the goal of athletic scholarships and the possibility to compete in post-secondary competitions as a part of the National Collegiate Athletic Association (NCAA). At this level, training is regulated to a maximum of 20 hours/week (National Collegiate Athletic Association, 2015).

## HEIGHTS OF GYMNASTS

To determine if elite female artistic gymnasts are shorter than interclub and non-gymnast counterparts, height

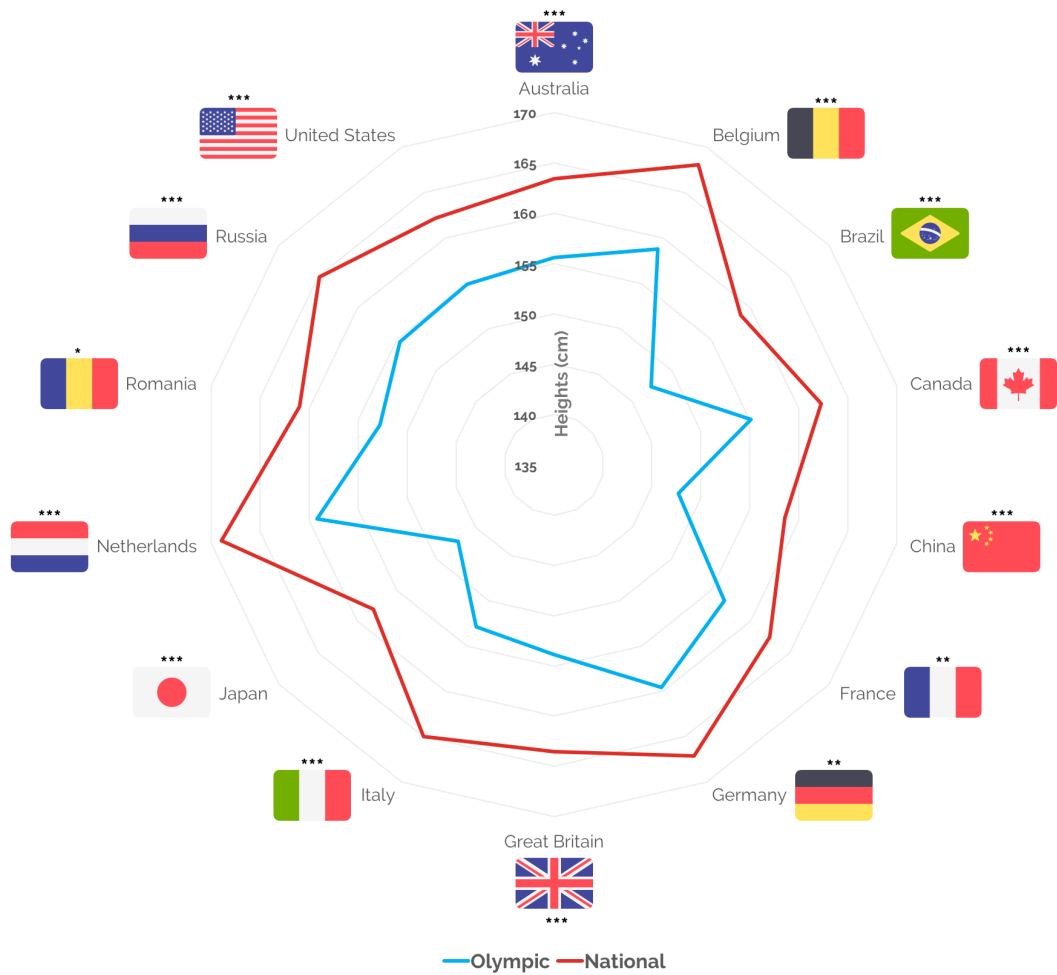
data of all gymnasts competing in the team event at the 2012 and 2016 Olympic Games were collected from the London 2012 and Rio 2016 websites (International Olympic Committee, 2012; 2016). The heights were separated by National Olympic Committee (NOC, the International Olympic Committee designation for nations or territories). Using the roster data from the 2016 NCAA Gymnastics Championships, height data for collegiate gymnasts were also collected, separated by school (National Collegiate Athletic Association, 2016). NCAA gymnast heights are representative

of interclub gymnast heights. Statistical analyses were completed using GraphPad InStat 3.1 (Motulsky et al., 2017). Normality for all three data sets were assessed using the Shapiro-Wilk test, with a 5% significance level. The heights of female artistic gymnasts at the 2012 Olympic Games (n=60) was normally distributed with mean 153.45 cm, and standard deviation of 6.09. The heights of female artistic gymnasts at the 2016 Olympic Games (n=60) was normally distributed with mean 153.75 cm and standard deviation of 7.82. Combining both Olympic Games (n=120), the mean was 153.6 cm with a standard deviation of 6.95. The heights of gymnasts at the 2016 NCAA Gymnastics Championships (n=66) was normally distributed with a mean of 158.07 cm and standard deviation of 5.24. An ANOVA was completed to determine significant difference between the three groups of gymnasts (Figure 1).

The heights of gymnasts from each NOC at both Olympic Games (n=5 for each NOC, 12 NOCs competed at each Olympic Games) were compared with the national average using one sample t-tests. All NOC means were significantly smaller than the national average (Figure 2). The heights of gymnasts at both Olympic Games were significantly smaller than 2016 NCAA gymnasts ( $p < 0.0001$ ). NCAA gymnasts are significantly smaller than the U.S. national average height ( $p < 0.0001$ ). There was no significant difference between the two Olympic Games ( $p > 0.05$ ).

## SELECTION BIAS

It is believed that elite gymnasts are smaller than their



**Figure 2:** Comparison of average Olympic gymnast and national female heights for all nations that participated in the team competition at the 2012 and/or 2016 Olympic Games. Australia, Belgium, Netherlands, and Romania competed at one Olympic Games ( $n=5$ ), while the other nations were represented at both ( $n=10$ ). All nations had significant differences between national and Olympic average heights. The asterisk above or below the national flags indicate the level of significance: \* ( $p<0.05$ ), \*\* ( $p<0.01$ ), \*\*\* ( $p<0.001$ ).

interclub counterparts due to a directional selection (Malina et al., 2013). In 2009, there were 4932 female gymnasts in the United States competing at the maximum interclub level (level 9 and 10). Only 79 (1.9%) of these gymnasts were classified as elite (USA Gymnastics, 2009).

Based on the data above, it was determined that there is a significant difference between the heights of NCAA and Olympic gymnasts, with the latter being smaller. Only 19% of NCAA gymnasts fall below the average height of Olympic gymnasts. Of the gymnasts that did fall below the Olympic average height, 50% were classified as and/or competed elite for a minimum of one season in their interclub career (USA Gymnastics, 2016). In contrast 77% of Olympic gymnasts fall below the average height of NCAA gymnasts. This data suggests that elite gymnastics, in a sense, selects for shorter gymnasts.

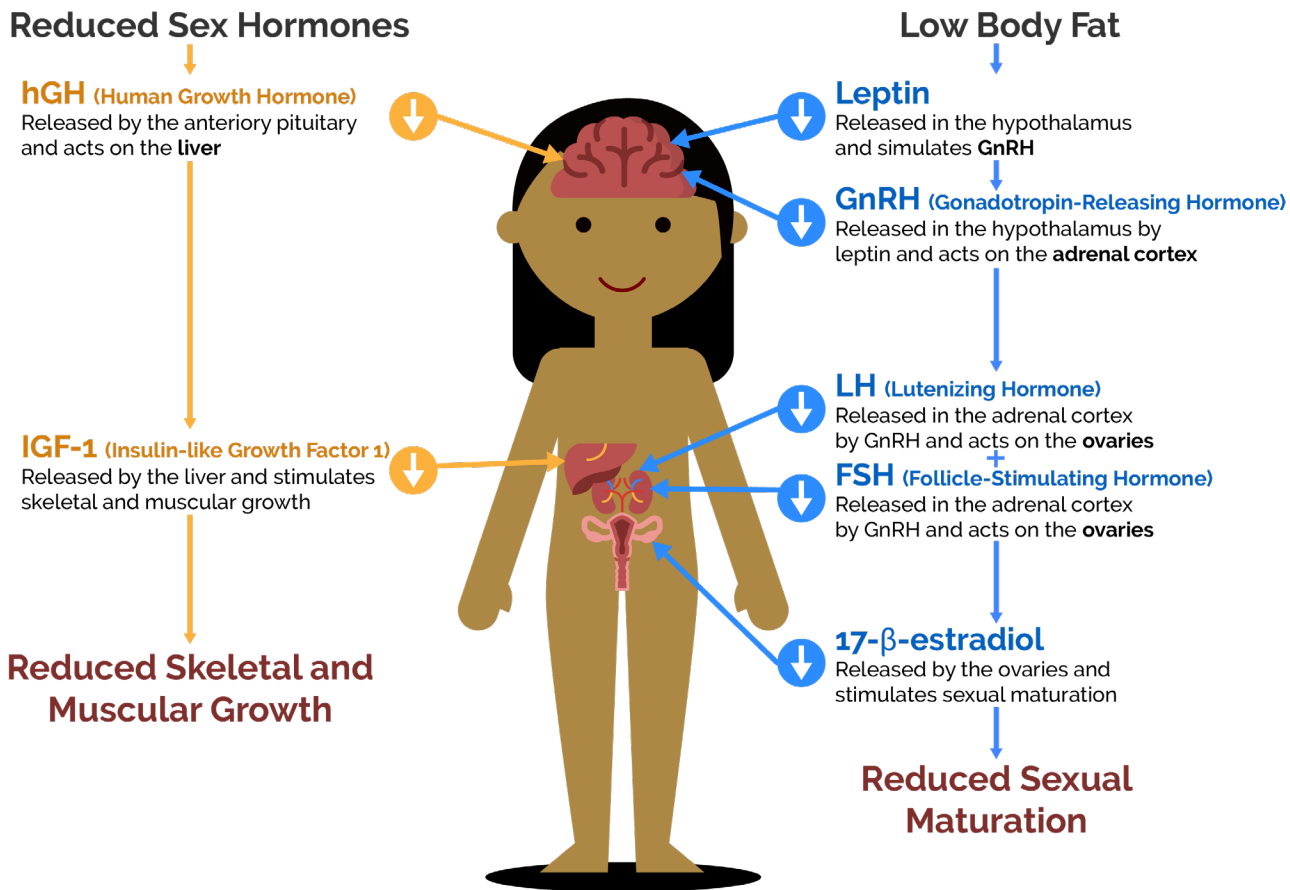
Studies in other countries have also determined a potential selection towards shorter heights in elite gym-

nasts. A sample of Polish elite gymnasts ( $n=5$ ) were, on average, shorter than their counterparts who dropped out ( $n=4$ ), although the difference was not significant. These height difference continued into late adolescence and full maturation, but were still not significant (Malina et al., 2013). In a study of Swiss elite gymnasts, dropouts ( $n=12$ ) were significantly taller than their elite counterparts ( $n=12$ ) (Tönz, Stronski and Gmeiner, 1990). A similar result was observed in a Belgian and Canadian study (Claessens and Lefevre, 1998; Lindner, Caine and Johns, 1991).

## ENDOCRINE AND NUTRITIONAL FACTORS

In addition to a selection pressure that results in overall short stature in gymnastics, endocrine and nutritional factors may also play a role in these physiological differences. Female athlete triad is disorder characterized by the presence of three conditions—reduced caloric intake (negative energy threshold), amenorrhea (absence





**Figure 3:** Endocrine pathways altered in female artistic gymnasts compared to non-athlete counterparts that affect stature and maturation. On the left is the hGH-IGF-1 system which overall reduces skeletal and muscular growth, resulting in reduced stature. The hypothalamic-pituitary-gonadal (HPG) axis is presented on the right; this results in reduced sexual hormones and maturation. The two pathways are interconnected as the reduction in sex hormones in the HPG-axis activates the hGH-IGF-1 system.

of menstruation), and low bone mineral density. The disorder is most prevalent in sports that emphasize small stature, low body weight, or leanness. When an athlete is diagnosed with one of the three characteristic conditions, it is highly likely that she is suffering from the other two (Weimann, 2002). The prevalence of this disorder is approximately 31% in female artistic gymnasts—much greater than the general population (<5%) (Buck, Bretz and Towns, 2008). These results are based on a self-reported survey of gymnasts—actual numbers could be much higher. Negative energy thresholds can delay growth and the onset of puberty, while amenorrhea alters gonadal hormone secretion, altering the levels of growth hormones including FSH and LH (American College of Sports Medicine, 2007).

Intensive gymnastics training, correlating to that of the elite level, in combination with a negative energy threshold has been determined to be inhibitory on the hypothalamic-pituitary-gonadal axis in female artistic gymnasts (Theintz, 1994). In menstruating adults, a negative energy threshold has been associated with decrease and disruption of LH release. Additionally, an experimental exercise program on post-menarcheal females increased LH disruption during periods of neg-

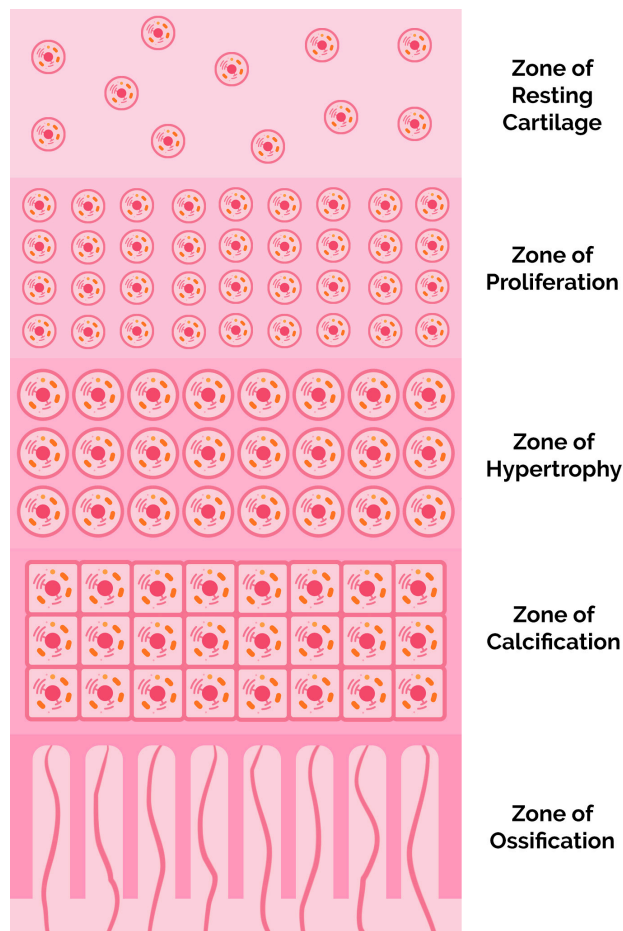
ative energy (Loucks, 2006). These trends however, have not yet been observed on maturing female athletes.

In a sample of 83 pre-pubescent athletes, 46 gymnasts and 37 swimmers, there were no differences in 17- $\beta$ -estradiol, DHEAS, LH, and FSH levels. A similar result was observed in early pubertal gymnasts. Comparing a sample of early pubertal girls ( $n=12$ ) with ectomorphic body types with gymnasts of the same age ( $n=12$ ), the latter group had lower LH and 17- $\beta$ -estradiol, but higher FSH levels (Peltenburg et al., 1984). Female artistic gymnasts typically have very low body fat levels, which in turn can reduced leptin levels. A decrease in leptin release reduces the production of GnRH in the hypothalamus (Meister and Hakansson, 2001), which in turn reduces the levels of FSH and LH in the body. Overall, a decrease in FSH and LH is associated with a lack of growth (Mahesh, 2011).

IGF-1 levels are also affected in female artistic gymnasts. In a sample of elite level gymnasts ( $n=16$ ), measured serum IGF-1 levels were lower than normal ranges for females of that age. These gymnasts participated in a 3-day period of intensive exercise consisting of ~5 hours of gymnastics training and ~3 hours of general athletic training. During this training period, the gymnasts

showed a decline in IGF-1 levels compared to the basal values. These measured levels were also lower than the controls (non-gymnasts of the same age) used in this study. Reduced levels of critical hormones in the hypothalamic-pituitary-gonadal axis suggest that puberty and adolescent development may be delayed female artistic gymnasts (Jahreis et al., 1991). Reduced IGF-1 levels result in reduced skeletal and muscular growth, which in turn could result in short stature (Caine et al., 2001) especially from an auxological perspective. The objective of this review is to determine if gymnastics training inhibits growth of females. (Figure 3).

## BONE DEVELOPMENT



**Figure 4:** The five zones of osteogenesis at an epiphyseal plate. The zone of resting cartilage is located closest to the head of the bone, while the zone of ossification is located closest to the diaphysis, or midsection of the bone.

Endocrine pathways such as the hGH-IGF-1 system described above are not the only physiological cause of short stature—skeletal impacts may also carry an important role. Long bones are one of five types of bones, characterized by the fact that they are more long than wide. In the human body, the long bones are located in the legs (femora, tibiae, and fibulae), the arms (humeri, radii, and ulnae), the hands (metacarpals and phalan-

ges), the feet (metatarsals and phalanges), and the collar bones (clavicles). These bones comprising half of human height—the other half comes from the vertebrae and the skull.

Osteogenesis, or bone growth, occurs through the addition of cells and tissue at the epiphyseal (growth) plates, a layer of hyaline cartilage at the ends or epiphysis of long bones. The epiphyseal plates have two sides—an epiphyseal side which is closer to the head or a diaphyseal side which is closer to the main body of diaphysis of the bone. Each plate contains five zones relating to growth. The zone of resting cartilage, which is nearest to the epiphyseal side of the plate, contains chondrocytes that do not rapidly divide, but bind to the epiphysis. The zone of proliferation is new cartilage is formed through rapid mitosis of the chondrocytes. These chondrocytes mature and enlarge in the zone of hypertrophy. The zone of calcification, which is nearest to the diaphyseal side contains ossified bones with blood vessels. As bones reach adult size, the epiphyseal plate is ossified and closes, ceasing growth. This occurs anywhere between ages 12 and 25 (Gilbert and Singer, 2003) (Figure 4).

## EFFECTS ON VERTICAL GROWTH

Injuries, especially fractures, to the epiphyseal plate can have a negative effect on bone growth. Epiphyseal fractures result in a joint cavity between the epiphysis and diaphysis, preventing osteogenesis from occurring. This can result in shorter than normal limbs. Fractures to epiphyseal plates are referred to as Salter-Harris fractures (Salter and Harris, 2001). Sever's Disease is a condition caused by inflammation of the epiphyseal plate of the calcaneus, typically as a result of activity that involves walking, running or jumping (Scharfbillig, Jones and Scutter, 2008).

Growth plate injuries are common in elite gymnasts. In a study of 349 Australian gymnasts (151 elite, 198 non-elite), over 50% reported injuries to long bone joints. While only 12.3% reported injuries to growth plates, it should be noted that nearly 25% of elite gymnasts reported this type of injury. Only 2.6% of non-elite gymnasts reported a growth plate injury. Growth plate injuries were the most commonly reported type of injury in elite gymnasts in this study (Kolt and Kirkby, 1999). A similar study was completed in the United States, where radiographic images of non-elite gymnasts' wrists were taken to assess the impact of gymnastics training on the distal radial epiphyseal plate. Of the 44 participants, 11 showed signs of stress injuries in the growth plates (DiFiori et al., 1997).

Female artistic gymnasts have been identified for having short limbs, relatively short legs for height or stunted growth of the legs (Buckler and Brodie, 1977).

**Table 1:** Height differences of gymnasts who competed at both the 2012 and 2016 Olympic Games

Gymnast	Height (2012, cm)	Height (2016, cm)	Change in Height	Age (in 2012)
<b>Black (CAN)</b>	155	155	0	17
<b>Bui (GER)</b>	155	155	0	19
<b>Douglas (USA)</b>	150	157	7	17
<b>Fasana (ITA)</b>	152	155	3	16
<b>Ferlito (ITA)</b>	157	160	3	17
<b>Ferrari (ITA)</b>	146	146	0	21
<b>Hypólito (BRA)</b>	147	147	0	28
<b>Mustafina (RUS)</b>	161	162	1	18
<b>Paseka (RUS)</b>	159	161	2	17
<b>Raisman (USA)</b>	157	157	0	18
<b>Seitz (GER)</b>	162	162	0	19
<b>Rogers (CAN)</b>	157	165	8	19
<b>Teramoto (JPN)</b>	136	141	5	17
<b>Average</b>	<b>153.38</b>	<b>155.62</b>	<b>2.23</b>	<b>18.54</b>

Growth plate injuries cause a temporary detachment of epiphysis from the diaphysis which prevents bone growth and can result in shorter limbs (Gilbert and Singer, 2003). These types of injuries are prevalent in elite and non-elite gymnasts. Therefore, it is possible that the short limbs and subsequent shorter heights of female artistic gymnasts can be caused by growth plate stress and/or injury.

## DELAYED GROWTH

Elite gymnastics training may impact female height through hormonal and physiological changes. However, numerous studies indicate that elite gymnastics training delays the onset of puberty, and still allow normal pubertal growth. In a longitudinal study of female gymnasts, concentrations of 17- $\beta$ -estradiol and LH were lower than the reference sample for 9-13 year olds, but showed later increases which were consistent with delayed maturation (Tönz, Stronski and Gmeiner, 1990).

Of the 120 gymnasts that competed in the team event at the 2012 and 2016 Olympic Games, 13 competed at both (Table 1). All but one of these gymnasts were past the normal age of puberty (16 years), and all but one were below the maximum age of epiphyseal plate ossification (25 years). Seven of these 13 gymnasts increased in height between the two Olympic Games, with the average increase in height of 4.14 cm. All but 2 gymnasts under the age of 19 showed growth between the two Olympic Games.

Research has also determined that cessation of gymnastics training for prolonged period of time can trigger the onset of puberty and/or growth spurts. In a longitudinal study of 21 Australian female gymnasts, it was

determined that a lower than normal growth rate could be associated with gymnastics training. Among with sample, 4 gymnasts retired from the sport at approximately age 11. Height velocities in these former gymnasts accelerated substantially, which was interpreted by the authors as catch-up growth caused by the cessation of training (Bass et al., 2000).

Of the 13 gymnasts that competed at both Olympic Games, three have publically stated that they had ceased elite gymnastics training for more than 1 year—Gabrielle Douglas (USA), Alexandra Raisman (USA) and Brittany Rogers (CAN). Both Douglas and Raisman took a break from elite competition between the 2012 Olympic Games and the 2015 competition season, completely ceasing gymnastics training between August 2012 and January 2015. Rogers did not fully cease gymnastics training—she trained and competed at the NCAA level with lesser hours than her elite regimen. Raisman showed no change in height between the two Olympic Games. Douglas and Rogers however, showed an increase of 7 and 8 cm, respectively, the highest of the 13 gymnasts.

Another case of delayed growth in elite gymnasts can be seen in Kyla Ross (USA). Ross, then 15 years old, competed at the 2012 Olympic Games, measuring 157 cm. Ross continued to compete at the elite level until 2016, when she switched to training at the interclub level in preparation for NCAA competition. During the time between her retirement from elite gymnastics in January 2016 to her enrollment as a freshman at University of California Los Angeles (UCLA) in September 2016, Ross experienced a large growth spurt. According to UCLA's roster, Ross now stand 5'7" (170.2 cm), a 13-cm growth between the two Olympic Games (National Collegiate Athletic Association, 2016). Over-

all, the high prevalence for delayed growth spurts in elite gymnasts, and larger growth spurts during periods of suspended training, suggest that training at the elite level may postpone normal pubertal developments in female gymnasts.

## ADVANTAGES OF SHORT STATURE

Overall elite gymnasts are indeed shorter than their interclub and non-athlete counterparts—this difference in stature provides them with a biomechanical advantage. A short stature provides gymnasts with increased rotation power. In a longitudinal study of 37 gymnasts over 3.3 years, it was determined that gymnasts with a lower growth rate have a higher strength to mass ratio, increasing their potential to perform full-body rotations. Gymnasts with higher growth rates have better performance on back rotation skills, but are lacking in side and front rotational skills, as well as vertical jumps. Skills in gymnastics emphasize rotation around the all body axes—frontal, longitudinal and sagittal. Elements of increasing difficulty involved rotation around multiple axes at the same time (Ackland, Elliott and Richards, 2003).

A gymnast's balance is also impacted by stature. A person's centre of gravity is approximately below the navel. A shorter gymnast, therefore has a centre of gravity that is closer to the ground. In a 2007 study of collegiate gymnasts and basketball players, gymnasts had superior static and dynamic balance abilities than basketball players. For reference, the average height of a collegiate basketball player is 6'6" (198 cm), while the average collegiate gymnast stands at 5'2" (158 cm). While centre of gravity has some effect on this result, the focus of the individual athlete's training can also have an impact. For example, basketball players rarely balance motionless on one leg, while this is quite common for gymnasts (Bressel et al., 2007).

A short stature also helps gymnasts navigate and manipulate the four pieces apparatus in women's artistic

gymnastics. As per FIG regulations the diagonal distance between the uneven bars must be between 130 and 180 cm. For a taller gymnast, a 180 cm gap may interfere with their ability to complete giant swings, 360° rotations around the high bar; a smaller gap can exaggerate this disadvantage further. Should their feet hit the low bar during rotation, it can cause injury and the gymnast will receive a mandatory deduction during competition. On the floor exercise, the diagonal distance is 1697 cm; a shorter gymnast can complete more somersaults and handsprings within this distance, while a taller gymnast has a higher chance of landing out-of-bounds and incurring a deduction. Similarly, the length of the balance beam (500 cm) provides the smaller gymnast the advantage, as she will be able to complete more saltos (Fédération Internationale de Gymnastique, 2016b).

## CONCLUSION

Across the world, elite female artistic gymnasts are significantly shorter than their non-elite and non-gymnast counterparts. Gymnasts at the 2012 and 2016 Olympic Games were on average, shorter than gymnasts that competed at the 2016 NCAA Gymnastics Championships. Between interclub and elite gymnasts, a directional selection towards smaller heights may be present, as a larger percentage of elite gymnasts are shorter than their interclub counterparts. Endocrine effects may explain the differences in height of elite gymnasts, as they showed reduced concentrations of hormones in the hypothalamic-pituitary-gonadal axis that control puberty and growth. Additionally, epiphyseal plate fractures, injuries and stress may stunt limb growth, affecting a gymnast's vertical height. While elite gymnasts at the Olympic Games are short, some show delayed growth compared to normal puberty standards, or growth during cessation of training regimens.

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## How a medical miracle evolved into a human hazard: The history of antibiotics

MEAGHAN CRAUGHWELL, AILEEN LIU

Integrated Science Program, Class of 2019, McMaster University

### SUMMARY

Antibiotics have saved millions of lives but the rapid development of antibiotic resistant bacteria is inevitably becoming the most dangerous public health threat. These miracle drugs are widely used in agriculture, medicine and common everyday products; however, the overuse of antibiotics is correlated with the development of drug resistance. This growing medical concern invokes fear in the public as it eliminates the ability to effectively treat infectious diseases. Starting from the serendipitous discovery of penicillin in 1928, scientific advancements and government policies have led to controversies between scientists and industries on the use of antibiotics as the development of resistance has accelerated. This review discusses significant events, people and causes involved and identifies the time in history when antibiotic resistance became a serious public health problem.

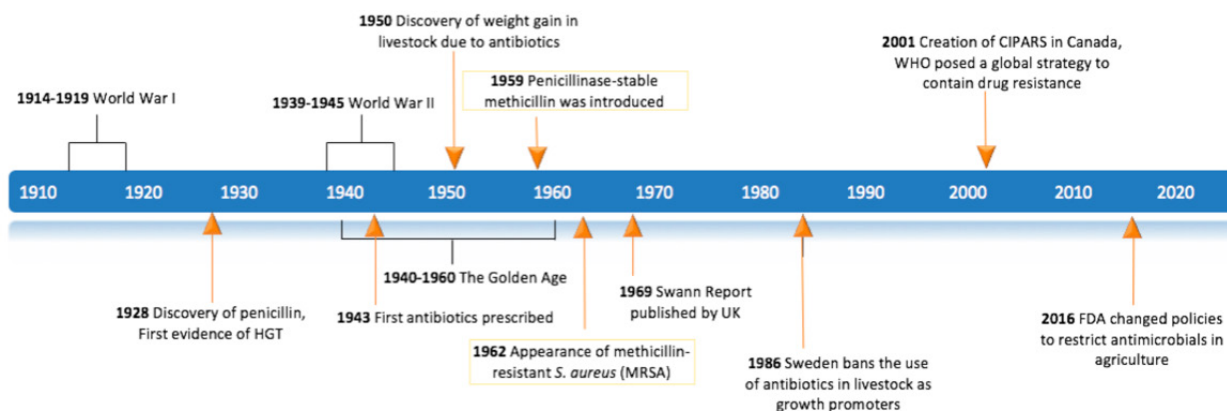
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**Keywords:** antibiotics, resistance, bacteria, public health, agriculture, Golden Age, Fleming, penicillin



**Figure 1:** A brief timeline focusing on the significant events and contributions of the antibiotic era, which ranges from 1910 to the present.

### INTRODUCTION

The use of antibiotics has been a controversial topic in recent years due to its ability to acquire resistance. However, resistance may not be a newly introduced phenomenon. It has been estimated that bacteria have been present on Earth for at least 3.5 billion years, thus it has been theorized that bacteria naturally developed genes that confer resistance to antibiotics billions of years ago, through their ability to evolve. Although re-

sistance may not be news to the environment, this has been a popular topic in the media for over a decade and has become a global threat to human health.

In 1941, Selman Waksman, a Ukrainian microbiologist, coined the term antibiotics and described these compounds to be molecules created by microorganisms that inhibit the growth of other microorganisms (Clardy, Fischbach and Walsh, 2006). Antibiotics are used to treat a variety of bacterial infections, and can

be created through either the modification of natural products with antibiotic properties or through the creation of synthetic compounds (Clardy, Fischbach and Walsh, 2006). Although most antibiotics currently prescribed have been isolated from microbes, there has been an overall decrease in the discovery of new antibiotics from natural sources (Peláez, 2006). This is partially due to the fact that less than 1% of bacteria can be cultured in the lab, which makes it difficult to investigate naturally occurring antibiotic compounds (Clardy, Fischbach and Walsh, 2006). Despite this difficulty, it is estimated that there is still a vast number of compounds with antibiotic properties from uncultured bacterial sources yet to be discovered. Thus, natural sources remain a promising area for the development of new antimicrobial drugs.

Antibiotic resistance occurs when a strain of bacteria can no longer be eliminated by a class of antibiotics that were previously successful in treating it. This means that if an individual is infected with a mutant bacterial strain, the bacteria may be multi-resistant and a required treatment no longer exists, which poses a serious public health threat. It has been determined that society has contributed to resistance through the misuse of antibacterial products. Scientists are in constant race between the discovery and development of novel antibiotics, versus the emergence of new resistant bacteria. If consumers continue to use antibiotics at the rate in which they are currently used, bacterial infections will cause more deaths than cancer by 2050 (Nothias, Knight and Dorrestein, 2016). It is crucial that governments, organizations, and hospitals address this issue to ensure that antibiotics are being used appropriately.

## BEHOLD THE MOULD

Dr. Alexander Fleming, a Scottish bacteriologist, worked on treating wound infections during World War I. During this time, he recognized the role of white blood cells in healing lacerations through its ability to kill bacteria (Bottcher, 1959). Astonished by his observations, Fleming performed further studies to identify the active substance in white blood cells (Bottcher, 1959). In 1922, he discovered that the substance was an enzyme, which he named lysozyme. Six years later, while working at St. Mary's Hospital in England, he studied the nature of *Staphylococcus aureus* (Fleming, 1929). One night, Fleming's assistant was putting away Petri dishes containing nutrient agar, however she neglected to cover the lids properly, allowing airborne fungal spores to contaminate the broth. The following day when Fleming observed the bacteria in the Petri dishes, he noticed that there was a fine lawn of grey-green mould and a transparent

halo, indicating an area of no growth (Fleming, 1929). This surprised Fleming as it appeared as though the bacteria had been dissolved by the fungus which had penetrated the broth. He identified the fungus to be *Penicillium notatum* and was intrigued by the idea of life inhibiting life, which motivated him to conduct further experiments over the next 10 years (Nicholas and Davies, 2012). Initially, he did not think of using penicillin to treat infections in humans, however reflecting on the research he conducted to identify lysozyme, Fleming proposed that the antimicrobial could be used against bacterial infections (Bottcher, 1959). Later, he proved the drug's ability to heal wounds in rabbits and its effectiveness in eliminating Gram positive pathogens (Page, 2012). However, ethical issues and Fleming's lack of biochemistry training presented him with challenges when attempting to isolate the active substance. Fleming eventually lost interest in penicillin and was reluctant to test it on humans (Page, 2012). Nevertheless, he published papers on his findings and hypotheses and allowed his fungal strains to be made available to the public.

Howard Florey, an Australian pathologist, and Ernst Chain, a German chemist, were fascinated by Fleming's work (Page, 2012). They expanded upon his research and successfully isolated penicillin from a related fungus, *Penicillium chrysogenum*, in 1939. This enabled them to conduct in vivo testing in mice to demonstrate how penicillin could treat bacterial infections (Page, 2012). Subsequently, they were granted the approval to conduct clinical trials in humans. After multiple failures leading to improvements of the flaws in their experiments, they were able to show that this miracle drug was effective in treating children who had severe streptococcal and staphylococcal infections (Page, 2012). To receive further funding and patents, they travelled from England to the United States in 1941 to produce the drug in large quantities, making it available as a medication in the early 1940s (Muniz, 2017). In 1945, Fleming, Florey and Chain received the Nobel Prize in Physiology or Medicine.

In Fleming's acceptance speech, he warned the public about something new and unheard of; antibiotic resistance. He cautioned the public to use antibiotics wisely as he understood that bacteria were able to develop resistance to these compounds (Fleming, 1945). Despite this, society disregarded his warning and the topic of antibiotic resistance was not investigated until decades later.

***"There is danger that the ignorant man may easily underdose himself by exposing his microbes to non-lethal quantities of the drug to make them resistant...Moral: if you use penicillin, use enough."*** – Alexander Fleming

## ANTIBACTERIAL ACTION

The development of penicillin antibiotics has allowed physicians to treat life-threatening infections, where patients show noticeable progress within hours after taking the drug. In the 1950s and 1960s, many scientists were dedicated to understanding the biochemical mode of action of penicillin (Nicholas and Davies, 2012). It was determined that beta-lactam antibiotics, such as penicillin, inhibit the synthesis of the bacterial cell wall, specifically peptidoglycan. Penicillin binding proteins (PBPs) are a group of proteins found in many types of bacteria that are essential for the cross-linking of peptidoglycan, which is necessary for the creation of a strong cell wall (Saga and Yamaguchi, 2009). The chemical structure of beta-lactam antibiotics resembles the C-terminus of peptidoglycan peptide chains; therefore, penicillin can bind to PBPs to form an enzyme complex (Page, 2012). Ultimately, this complex inhibits PBPs from performing cross-linking and the cell wall is not synthesized, which leads to the destruction of the bacterial cell.

Penicillinase is the enzyme responsible for inhibiting penicillin, thus contributing to the development of resistant bacteria (Ontario Medical Association, 2017). Resistant bacterial strains that produce penicillinase decrease the ability of penicillin to kill bacteria. These resistant bacterial strains were first discovered in the 1940s, but became more prevalent in the 1950s, signifying the spread of resistance (Saga and Yamaguchi, 2009). In response to this, another penicillin class antibiotic, penicillinase-stable methicillin, was developed in 1959. However, just three years after its introduction, methicillin-resistant *S. aureus* (MRSA) was discovered. The antibiotic streptomycin, another novel drug, was clinically introduced by Waksman in 1944 to treat tuberculosis (Davies and Davies, 2010). Similarly, resistant strains of *Mycobacterium tuberculosis* emerged rapidly after its use to treat the disease (Davies and Davies, 2010). Subsequently, the increased worldwide use of antibiotics after the 1940s acted as a selective pressure that led to the development of resistant bacteria (Read and Woods, 2014). This became regarded as an issue with the effectiveness of antibiotics and efforts were made to understand the spread of resistance. One mechanism was through spontaneous mutation and natural selection (Read and Woods, 2014). In 1928, British bacteriologist Frederick Griffith was conducting experiments using *Streptococcus pneumoniae* when he unknowingly discovered the process of horizontal gene transfer (HGT) (Arber, 2014). This was confirmed in the 1950s, when it was discovered that bacteria can transfer genetic material between individuals, including the genes coding for antibiotic resistance (Arber, 2014).

All in all, this trend of resistance was seen in all the an-

tibiotics introduced during this time period. To combat the mutant bacterial strains, other forms of antibiotics were needed to eliminate the resistant bacteria. However, the microorganisms continued to develop resistance to the new drugs, thus evolving into multi-resistant bacteria, or superbugs. Fortunately, many companies were on board with antibiotic discovery which advanced science into a triumphal period in antibiotic history.

## GOLDEN AGE

Most of the antimicrobial classes that are still administered today, such as tetracyclines and vancomycin, were discovered between 1940 and 1960 (Davies and Davies, 2010). This time period was known as the Golden Age of Antibiotics. There are various factors that contributed to the success as well as the eventual downfall of the Golden Age. The discovery of penicillin was the forefront of medical breakthroughs during this time, satisfying the high clinical need for these therapeutics (Mills and Dougherty, 2012). There was a major need for penicillin during World War II as death caused by bacterial infections were more common than casualties in battle. As a result, many drug companies, such as Pfizer, invested in antibiotic drug discovery. Additionally, during this period, there were great decreases in the death rates caused by infections. Once antibiotics were made available as treatments, the human life expectancy increased by 8 years between 1944 and 1972, as these common infections were no longer a leading cause of death (Mills and Dougherty, 2012).

Unfortunately, the 1960s marked the end of the Golden Age, where many pharmaceutical companies abandoned the search for new antibacterial classes. Some scientists thought that they had completely cured all infectious diseases, so there was no need for more antibiotics. Others believed that antibiotic resistance was not a concern, and in fact, they considered drug resistance to be rare and the likelihood of bacteria developing resistance to be very low. Furthermore, in the 1980s, companies that continued antibiotic research shifted their objectives in drug discovery (Page, 2012). Alternatively, they focused on improving the biochemical activities and properties of existing antibiotics rather than developing new classes. Meanwhile, many other drug businesses abandoned research in antimicrobials and began moving towards other areas of other therapeutic needs, where they had the potential to make large profits.

Overall, the lack of new antibacterial compounds was a factor that contributed to the threat of drug resistant bacteria. Additionally, investing in an antibacterial product was unappealing as it was very expensive and challenging to get approval by the FDA, and the



profits for medications to treat chronic diseases were much greater. Apart from the pharmaceutical industry, antibiotic resistance has also affected other industries.

## AGRICULTURE

Agriculture has been a necessary part of human survival and population growth for centuries, providing humans with food, clothing, heat and employment (Federico, 2005). As the global population has grown, there has been stress on the agricultural industry to produce greater amounts of food, and various technological advancements have allowed for more efficient production of food products (Federico, 2005). One of the advances in this sector was the discovery of antibiotics. In the late 1940s, Thomas Jukes and Robert Stokstad, two American scientists, were working at the University of California, attempting to find a source of vitamin B-12 (Wise, 2007). When studying chickens fed with a mix containing the fermentation products of *Streptomyces aureofaciens*, these scientists made an important discovery for the future of agriculture. They noticed that the poultry experienced a dramatic weight gain, which Jukes attributed this weight gain to the change of intestinal flora of the chickens (Stokstad and Jukes, 1950). Although its mechanism of action was not fully understood, the causative agent was determined to be a product of *S. aureofaciens*, called chlorotetracycline, which is an antibiotic of the class of tetracyclines (Wise, 2007). From this discovery, the benefits of antibiotics on livestock were realized and the worldwide agricultural industry began to change its practices.

The main purposes of adding antibiotics into animal feed include the promotion of growth and the prevention of infection in livestock populations, as this could have devastating effects on businesses (Khachatourians, 1998). In the 1950s, the recommended dose of antibiotics in animal feed was 10-20 ppm, however in the 1990s, this dosage had increased to 200-400 ppm (Khachatourians, 1998). With these increasing trends, the primary use of antibiotics became livestock feed. For instance, of the total production of antibiotics in the United States in 1998, 50% were used in the agricultural industry with only 5% being used to treat infectious disease (Khachatourians, 1998).

Although this discovery led to more efficient production of animal protein to sustain the ever-growing human population, problems with this new agricultural practice were soon discovered. The development of antibiotic resistance gradually became a concern in many different countries, especially in European countries. In 1969, the UK parliament created a committee to investigate the threat of antibiotic resistance in agriculture and provide recommendations for the future (Wise, 2007). This committee published the Swann

Report to advise the government and ease anxieties, which was then distributed to other countries in Europe and to the United States. Through their research, they concluded that the administration of antibiotics in agriculture posed a threat to human health due to the development of antibiotic resistance (Swann et al., 1969). They identified 1523 strains of bacteria that were resistant to certain antibiotics and concluded antibiotic use in agriculture was partially to blame (Swann et al., 1969). This committee recommended that antibiotics should be separated to be used either in feed or therapeutically and that drugs that are currently freely available should be limited so that they are only be available with a prescription.

Despite the recommendations of the Swann Report and further research on this emerging issue, the regulations and policies regarding antibiotic use in agriculture remained fairly unchanged. It wasn't until the 1990s when this issue became the subject of large debates, that more government action was taken (Wise, 2007). In 2001, the UK created a committee called the Specialist Advisory Committee on Antimicrobial Resistance that had representatives from human and veterinary medicine, public health, nursing, bacteriology, and virology (Wise, 2007). Similarly, a committee was created in Canada called the Canadian Integrated Program for Antimicrobial Resistance Surveillance (Government of Canada, 2007). The purpose of these committees is to monitor the development of resistant bacteria, including the contribution of resistance from antibiotic use in livestock (Government of Canada, 2007).

There are contrasting perspectives and lifestyles when it comes to antibiotic use between Western and European countries. Currently in Ontario, there are few regulations and little control regarding the types of antibiotics and dosage quantities that can be given to livestock (Ontario Medical Association, 2017). On the other hand, Sweden banned the administration of antibiotics to livestock as growth promoters in 1986 and Denmark followed their lead 9 years later (Ontario Medical Association, 2017). Antibiotic bans in Holland, Denmark, Germany and Sweden were shown to cause a decrease in resistance rates of one type of bacteria, called *Enterococcus* (Bogaard et al., 2000). As a result of these bans, the European economy has experienced deficits from the farming industry, which invoked fear in other countries and hesitations towards making drastic changes in their policies.

Currently, this overuse of antibiotics contributes to the development of resistance as the bacteria are exposed to the antibiotics more often. The drugs act as a selective pressure for resistance leading to increased development and spread of resistance genes through both HGT and random mutations, as previously mentioned

(Arber, 2014).

In the history of agriculture, the use of antibiotics is a fairly new development and the consequences of this practice are still being determined. Policies regarding the use of antibiotics as growth promoters are now being modified and implemented in many countries, yet agriculture is still considered a major contributor to antibiotic resistance.

## HEALTHCARE

Although there have been large advances in the field of public health care, antimicrobials are still one of the most important and successful developments in medicine (Aminov, 2010). Evidence of the use of antibiotics has been found from ancient human skeletons dating back to approximately 350 CE, but the concerns regarding resistance only began in the antibiotic era, in the late 20th century (Aminov, 2010). Similar to the use of antibiotics in agriculture, the overuse and misuse of antibiotics in the healthcare industry also contribute to resistance.

Antibiotics are used in healthcare to control infectious diseases that were previously some of the leading causes of death; however, the misuse of antibiotics can decrease their efficacy (Kardas et al., 2005). Misuse includes the failure of patients to complete antibiotic therapy, skipping doses, reuse of antibiotics and overprescription (Kardas et al., 2005). When antibiotics are used to treat an infection, the drug acts as a selective pressure which drives the selection of bacteria that are resistant and thus not killed by the drug (Kardas et al., 2005). In previous studies, it was determined that one third of patients did not comply with the therapy plan and one quarter had leftover antibiotics for later use (Kardas et al., 2005). The consequences of this misuse include the accelerated development of resistance.

Another practice in healthcare that contributes to antibiotic resistance occurs when physicians overprescribe these drugs. Antibacterial agents are commonly prescribed for respiratory tract infections which may be caused by viruses, instead of bacteria (Wang et al., 1999). A Canadian study that focused on data from preschool children from 1995 estimated that about half of the total cost of antibiotics in Canada was due to overprescribing the drugs (Wang et al., 1999). In Canada, it has been estimated that each year, there have been up to 26 million prescriptions from physicians that were unnecessary for treatment (Williams and Heymann, 1998). This means that the antibiotic acts more often as a selective pressure for bacterial evolution and does not actually treat the viral infection.

## THE FUTURE IS NOW

***“Antibiotic resistance is putting the achievements of modern medicine at risk.”*** – World Health Organization

Given that the consequences of antibiotic resistance were not understood when antimicrobials were introduced in the 1940s, regulations regarding the use and distribution of antibacterial products did not exist until recently. Since antibiotics are used on a global scale, the concern of antibiotic resistance is international. In 2001, the World Health Organization attempted to bring policies into action by posting recommendations and a global strategy on containment of drug resistance (WHO, 2017). Furthermore, federal governments have also tried to address the issue. The Canadian government intends on forming relations with animal agriculture providers by December 2019 to regulate the approval of veterinary medication and to ensure that these products are being used appropriately in animals and humans. Through these policies, they anticipate that there will be a decrease in the overall use of antibiotics. These are only a few examples of efforts that have been made by different organizations in recent years, however antibiotic resistance continues to be a large problem.

Aside from being an international issue, antibiotic resistance also involves affects different fields including but not limited to microbiology, healthcare, agriculture, ecology, education, legislative bodies, the pharmaceutical industry and the public (Aminov, 2010). Although efforts have been made to address this problem, more drastic global measures must be implemented, in order to prevent widespread resistance and loss of antibiotic efficacy. Since the spread of resistance is a naturally occurring process, it cannot be stopped, but the rate of this spread can be reduced.

Some recommendations to address this issue include changes in policies in both the agricultural and medicinal fields, which should be applied to many countries throughout the world. Prescription antibiotics should be used to treat bacterial infections and the use of these drugs for viral infections should be very limited as this misuse is a major driving force for bacterial evolution (Kardas et al., 2005). More recently in 2016, the FDA initiated a change in the marketing status of antibiotics used in livestock (Center for Veterinary Medicine, 2017). Antibiotics that were previously over-the-counter are now classified as either prescription or veterinary feed directive, which means that they can only be used under supervision of a licensed veterinarian. Since the majority of antibiotics are used in agriculture, a reduction or even an elimination of antibiotics in this industry would lead to a significant decrease in the spread of

resistance in bacteria. In addition to limiting use, the possibility of novel classes of antimicrobials should be explored as new technologies could lead to great discoveries (Aminov, 2010).

From the overview of the history of antibiotic era and studies regarding the spread of antibiotic resistance, we determined that antibiotic resistance first became a major concern in the 1970s (see Figure 1). When looking at the trends of antibiotic discovery, this time period was considered the end of the Golden Age and after this point, there have been few novel classes of antibiotics discovered. Additionally, there were few events promoting antibiotic discovery and few policies limiting antibiotic use in agriculture and healthcare as well as other products. With the combination of these factors as well as the increasing worldwide use of antibiotics, we propose that this marked the beginning of the antibiotic resistance crisis.

Ultimately, though antibiotics are considered relatively new in terms of human history, this revolutionary medicine could soon lose its value due to the spread and

further development of resistant bacteria. Compared to the billions of years of bacterial evolution, humans have only spent approximately the last 80 years in the antibiotic era. Although this time is only approximately equivalent to the current human life expectancy, these compounds have revolutionized human medicine with the ability to treat infectious diseases that were once considered leading causes of death and to produce enough food to support the growing worldwide population. Although resistance is inevitable, there are many measures that may be taken to slow this rate of resistance, some of which are stated in this article, however the fight against resistance requires global cooperation.

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## Ethics of clinical research: A historical review

**LIZ BALINT, ASHLYN LEUNG**

Integrated Science Program, Class of 2019, McMaster University

### SUMMARY

Research that contributes to significant advancements in healthcare is often achieved through the analysis of data from human participants. Many researchers in this field seek a diverse group of participants to study the effects of new drug treatments, diets, or other related therapies. Clinical trials and studies involving human participants are important for the progression of science, but require extensive ethical consideration. Ethics are the moral principles that govern a behaviour or activity, in this case, performing tests on humans. The understanding of ethical regulations outlines the key responsibilities of the investigator, and more importantly, ensures the protection of the participant's rights. This historical review examines the controversies that led to the development of ethical guidelines for human participation in research since the 1940s. It is through various major controversies that the documents containing these guidelines have continuously been shaped and edited. Analyzing the development of the Nuremberg Code, the Declaration of Helsinki, and the Belmont Report contributes to a stronger understanding of current regulations and modern ethical controversies. These documents carefully considered the issues in their predecessors and include key points that aim to protect the rights of those who participate in research.

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### INTRODUCTION

Besides their quality of instruction, many of the world's leading universities are well known for their research facilities and Nobel laureate affiliations. 40% of total Canadian research and development is performed by universities, accounting for approximately \$13 billion each year (Statistics Canada, 2015). Research from these institutions extend from innovative technology to advancements in healthcare. New drug treatments, diets, or other related therapies are often achieved by analyzing data from human participants.

The biggest challenges involved in healthcare research lie within the clinical trial phase and the ethical questions it poses. This has been recently brought to light in September of 2017 when a lawsuit was filed against Johns Hopkins University (Ome, 2017). The case tar-

geted the involvement of Hopkins' physicians, who reviewed and financed an unethical study in Guatemala in the 1940s. The study was conducted by the United States Government, who intentionally infected Guatemalans with diseases, including syphilis and gonorrhea, without their consent. Even though this occurred over 70 years ago, it is important to be reminded of these cases and how they can be prevented in the future. Ethical guidelines have not always existed in the past and reflecting upon the events that led to their establishment can provide better understanding of their importance in research.

It is necessary to understand the historical context upon which modern ethics are based in order to fully recognize the implications and significance of current guidelines and laws. Ethics can be defined as moral principles that govern one's actions. Before the 1940s, there were

**Table 1:** The ten principles that form the Nuremberg Code (Annas and Grodin, 1992).

#	Principle
1	The voluntary consent of the human subject is absolutely essential. This means that the person involved should have legal capacity to give consent; should be so situated as to be able to exercise free power of choice, without the intervention of any element of force, fraud, deceit, duress, over-reaching, or other ulterior form of constraint or coercion; and should have sufficient knowledge and comprehension of the elements of the subject matter involved as to enable him to make an understanding and enlightened decision. This latter element requires that before the acceptance of an affirmative decision by the experimental subject there should be made known to him the nature, duration, and purpose of the experiment; the method and means by which it is to be conducted; all inconveniences and hazards reasonably to be expected; and the effects upon his health or person which may possibly come from his participation in the experiment. The duty and responsibility for ascertaining the quality of the consent rests upon each individual who initiates, directs or engages in the experiment. It is a personal duty and responsibility which may not be delegated to another with impunity.
2	The experiment should be such as to yield fruitful results for the good of society, unprocurable by other methods or means of study, and not random and unnecessary in nature.
3	The experiment should be so designed and based on the results of animal experimentation and a knowledge of the natural history of the disease or other problem under study that the anticipated results will justify the performance of the experiment.
4	The experiment should be so conducted as to avoid all unnecessary physical and mental suffering and injury.
5	No experiment should be conducted where there is an a priori reason to believe that death or disabling injury will occur; except, perhaps, in those experiments where the experimental physicians also serve as subjects.
6	The degree of risk to be taken should never exceed that determined by the humanitarian importance of the problem to be solved by the experiment.
7	Proper preparations should be made and adequate facilities provided to protect the experimental subject against even remote possibilities of injury, disability, or death.
8	The experiment should be conducted only by scientifically qualified persons. The highest degree of skill and care should be required through all stages of the experiment of those who conduct or engage in the experiment.
9	During the course of the experiment the human subject should be at liberty to bring the experiment to an end if he has reached the physical or mental state where continuation of the experiment seems to him to be impossible.
10	During the course of the experiment the scientist in charge must be prepared to terminate the experiment at any stage, if he has probably cause to believe, in the exercise of the good faith, superior skill and careful judgment required of him that a continuation of the experiment is likely to result in injury, disability, or death to the experimental subject.

no universally accepted ethical guidelines for research involving human subjects. Clinical studies were formerly referred to as “human experimentation” and had little consideration for the rights of the research subjects. This led to many discrepancies in study protocols, and left the ethical responsibilities of researchers up to interpretation. The progression of ethical codes prior to the 1940s provides a clear indication of the foundational theories of modern ethics. Many scientists in the 1800s were familiar with the Hippocratic Oath, written between 470 and 360 BCE, which states, “I will follow that system of regimes which, according to my ability and judgement, I consider for the benefit of my patients, and abstain from whatever is deleterious and mischievous” (Freyhofer, 2004). These scientists built upon Hippocratic ethics to establish ethical guidelines for research involving human subjects, but it was not until the gruesome human experimentation during

World War II (WWII) that the first universal code of ethics, the Nuremberg Code, was formed.

## THE NUREMBERG CODE

The Nuremberg Code is considered to be the blueprint for modern ethics for studies involving human subjects. Current standards that protect subjects of medical research have their roots in the ten research principles presented in the Nuremberg Code (Table 1) (Shuster, 1997). The Code particularly focuses on the requirement of informed consent, described in the first principle (Shuster, 1997). This focus was a direct response to the tragic and perverse human experimentation conducted by Nazi physicians and scientists during WWII. After WWII, the victorious Allies held a series of military trials in Nuremberg, Germany, coined The Nuremberg Trials (Freyhofer, 2004). These trials were held to

prosecute Nazi officials who planned or participated in war crimes. The first of these was the International Military Tribunal, also known as the Major Trial, which tried the highest-ranking Nazi officials for war crimes and crimes against humanity (Freyhofer, 2004). This was followed by the Doctor's Trials, which tried physicians responsible for the abuse of human subjects to advance medical sciences. This revealed arguably some of the most horrific acts committed during WWII. The defendants argued that their acts were legal due to the lack of a universal standard of human research ethics, and the experiments' accordance to the law existing at that time (Freyhofer, 2004). The Nuremberg Code was developed during the Doctors' Trial in order to produce a set of medical standards with which to judge the physicians for their crimes (Annas and Grodin, 1992).

While the focus of the Doctor's Trial was the criminal nature of the experiments and the standards of human experimentation at the time, the broader concerns of medical research ethics were also examined and debated. The Code was ultimately based upon past literature, events, and philosophies (Annas and Grodin, 1992). Two primary medical expert witnesses who supported the prosecution, Andrew Ivy and Leo Alexander, were largely responsible for the development of the principles in the Code. Both scientists were familiar with the ethics of medical research proposed by Hippocrates, which ultimately formed the foundation of the principles within the Code. It is important to recognize that the ethical codes depicted by past influential scientists and physicians significantly impacted the development of the ten principles of the Nuremberg Code.

The Code has ultimately influenced global human-rights laws and medical ethics. It was not identified as simply a code of ethics, but rather a part of the final legal judgement in the Doctor's Trial. The physicians were judged according to the ten principles outlined in the Nuremberg Code (Annas and Grodin, 1992). Although it originates from the response to the tragic experiments at the hands of Nazi physicians and scientists, the Nuremberg Code emerged from the horrors of WWII to contribute to safer, more ethical practices in medical research involving human subjects. The Code has influenced the acceptance of informed consent in international law, and is used as a basis upon which many ethical guidelines are built, such as the Declaration of Helsinki and the current regulation, the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Shuster, 1997).

### **THE DECLARATION OF HELSINKI**

The Nuremberg Code provided important guidelines for the necessary measures to respect the research subject's wishes. However, little emphasis was placed on

the protection of their rights and welfare, and the responsibilities of doctors towards research subjects was overlooked. As a result, a new statement of principles for ethical research involving human subjects was put forth: The Declaration of Helsinki. The Declaration of Helsinki was first adopted by the World Medical Association (WMA) in 1964 and was largely based on the fundamentals of the Nuremberg Code, but addressed clinical research more directly (Goodyear, Krieza-Jeric and Lemmens, 2007). The main principles of the Declaration include that medical research can never take precedence over the rights and interests of the participant, the duty of the physician to protect the integrity and confidentiality of the participant, and that medical research must only be conducted by those with appropriate scientific qualifications (World Medical Association Declaration of Helsinki, 2013). The Declaration contains refinements and major advances to the principles outlined in the Nuremberg Code (Stone, 2004). The document was carefully examined by international organizations and critiqued by the world's preeminent physicians, scientists and ethics experts. From their evaluations, the Declaration has been revised seven times since its original publication. The most current version of the Declaration contains 37 principles, several of which may have been driven by the ethical analysis of controversial clinical studies.

An important outcome following the publication of the Declaration of Helsinki was the protection of vulnerable populations in research studies. Vulnerable populations, such as prisoners, are those whose individual freedom and ability to understand risks is affected due to various factors, including decreased freewill and inability to make informed decisions (Shivayogi, 2013). Principle 25 of the Declaration states that "participation by individuals capable of giving informed consent as subjects in medical research must be voluntary," which was controversial when it was implemented due to its ambiguity with regards to prisoners (World Medical Association Declaration of Helsinki, 2013). It was argued that prisoners were not in a legal state to express choice and therefore, should their consent be given, it should not be considered informed. One example of the exploitation of inmates is the study that occurred at the Holmesburg Prison in Philadelphia in the 1950s. In the studies conducted by dermatologist Dr. Albert Kligman, inmates were given experimental skin products, including creams, powders, and deodorants (Homblum, 2012). Although the inmates were paid for their participation, many suffered burns and scars as a result of the medical tests. Those who underwent the patch test, a 30-day trial, had up to 20 different products applied to their back and were exposed to heat from a sunlamp for a period of time (Homblum, 2012).

The patch test sometimes caused skin to peel, itch, and blister, however, the study was not deemed unethical due to the uncertainty of Principle 25. In addition, Dr. Kligman's research was not investigated by institutional personnel, nor was it supervised by the government, which allowed Holmesburg Prison to host one of the largest medical experimentation centres in the country for two decades. Holmesburg Prison became a key driving factor for the implementation of ethical guidelines and the importance of protecting research subjects. Although studies on federal prisoners ended in the mid 1970s, studies on other vulnerable populations continued until further revisions of the Declaration were effectuated. Among the seven revisions of the Declaration, the greatest changes occurred in the first, fourth and fifth revisions.

The first revision of the Declaration of Helsinki was published in 1975 after the realization that abuse of research subjects remained a common problem. Among the most important aspects of this revision was the elaboration of the requirements for informed consent and the rights of human subjects. This was specified in Principle four which read, "In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject" (Williams, 2008). To further protect the welfare of participants, it was also added that the research study must undergo advanced review by an independent committee prior to conducting the study in order to publish the results of the research (Williams, 2008). One of the factors that may have driven this revision was the controversy surrounding the Tuskegee Syphilis Study of 1932 to 1972.

A fourth revision of the Declaration of Helsinki occurred in 1996 to address placebo-controlled trials. The existing principle stated, "In any medical study, every patient – including those of a control group, if any – should be assured of the best proven diagnostic and therapeutic method," to which the fourth revision added "This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists" (Carlson, Boyd and Webb D, 2004). Motivation for this revision was driven by the increasing concerns regarding the use of placebo controls in prenatal HIV transmission in developing countries, specifically the trials for azidothymidine (AZT) in 1994 (Carlson, Boyd and Webb D, 2004). Prior to these trials, a study of HIV infected pregnant women, conducted by the USA and France, found that intensive treatment with AZT reduced maternal transmission of HIV in 70% of cases (Cohen, 1997). Researchers were aware that the incidence of HIV/AIDS was greatest in developing countries, but AZT treatment costs \$800-\$1000 per person, which is difficult for those women

to afford. Thus, researchers sought to test cheaper prevention strategies, such as shorter treatment regimens or HIV-antibody injections, and performed a study in several developing countries, involving over 17 000 pregnant women (Annas and Grodin, 1998). The question of including placebos in these study groups led to ethical debate since the original AZT treatment had already shown effective long-term results (McIntyre, 1998). The fourth revision of the Declaration hoped to address this situation by specifying that placebos should only be used where no other therapeutic method exists. Even though the study was approved by African national ethics committees, thus complying to that aspect of the Declaration, bioethicists were sceptical due to the reputations of said committees. Cases have been noted where materials for the studies were sent from abroad prior to study approval and bribery was used to gain ethical approval (McIntyre, 1998). These reports provide evidence for increasing the stringency of the principles outlined in the Declaration through constant revisions.

Questions surrounding the ethics of placebo-controlled trials arose again in 2000 after the fifth revision of the Declaration, where Principle 29 stated, "The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic and therapeutic methods. This does not exclude the use of placebo, or no treatment, where no proven prophylactic, diagnostic or therapeutic method exists" (Lewis et al., 2000). Although this principle does not appear to differ from that of the fourth revision, its importance was demonstrated through the controversy it generated. It's been argued that if Principle 29 was taken literally, all clinical trials should be barred because research subjects receiving the investigational treatment would not be getting the best proven treatment (Simon, 2000). Instead, they believe that placebo-controlled trials can be conducted ethically so long as the omission of the proven treatment would not be detrimental to the patient's health and patients are completely informed about the alternative treatments available.

Over 50 years after its initial development, the Declaration of Helsinki continues to be an important document that sets the ethical standards for modern clinical studies. The Declaration has remained dynamic and adapted to changes as ethical problems arose. Due to this document, physicians are more aware of their responsibilities and research subjects are more aware of their rights. It has brought substantial awareness to the importance of ethics in human research; however, a number of unethical studies are still being conducted despite the principles in the Declaration. Although seven revisions have been made in the past, it must con-

**Table 2:** The three fundamental principles of the Belmont Report (Annas and Grodin, 1992).

Principle	Responsibilities
<b>Respect for persons</b>	Protecting the autonomy of all people and treating them with courtesy and respect; this is applied in the informed consent process. Researchers must be truthful and conduct no deception
<b>Beneficence</b>	Incorporating the philosophy of “Do no harm” while maximizing benefits for the research project and minimizing risks to the research subjects is applied through risk/benefit assessments
<b>Justice</b>	Ensuring reasonable, non-exploitative, and well-considered procedures are administered fairly and equally and applied to the selection of research subjects

continue to change in the future to provide ongoing protection for human research subjects.

## THE BELMONT REPORT

Around the time of the first revision of the Declaration of Helsinki, the controversial conditions of the Long-term Study of Untreated Syphilis in the Negro Male, known as the Tuskegee Syphilis Study, were revealed. The study was conducted from 1932 to 1972 and was undertaken by the U.S Public Health Service which later became the Centers for Disease Control and Prevention (CDC). The study sought to document the course of the disease in 400 African Americans to determine racial differences in the manifestation of syphilis (Corbie-Smith, 1999). When the study first began, there was no effective treatment for the disease, which was the case until 1943, when researchers found that penicillin could be used to treat syphilis. Despite this discovery, the study was continued and the subjects were never notified of the existence of a treatment (Singer and Levine, 2003). In response to the ethical concerns that arose during the study, the Belmont Report was written to establish stricter regulations.

Even though the Declaration of Helsinki was formed in 1964, no changes were made to the Tuskegee Syphilis Study (Kim, 2012). In 1969, the CDC formed a panel to review the experiment, but they allowed the continuation of the study without modification to the protocol, for unknown reasons (Shovers, Lynch and Burmeister, 2000). In 1972, a story published in the New York Times and the Washington Star revealed the ethical concerns of the study, and the resulting public outcry led to hearings directed by Senator Edward Kennedy and closure of the study (Kim, 2012; Shovers, Lynch and Burmeister, 2000). At this time, participants were finally given the appropriate treatment, but it was too late for the approximately 100 men who died due to untreated syphilis or syphilis-related complications (Corbie-Smith, 1999).

Within two years of the termination of the Tuskegee Syphilis Study, the United States Congress passed the National Research Act. This established a human re-

search protection system to uphold the rights of human participants and prompted the development of regulations requiring the establishment of Institutional Review Boards at federally funded institutions (Singer and Levine, 2003). This led to the creation of the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research (Kim, 2012). The Commission was required to identify principles that underlie the ethical conduct of biomedical and behavioural research. Their main product, the Belmont Report, was published in 1979 and marked a key point in history for the development of ethical requirements involving the use of human subjects in research (Singer and Levine, 2003). The three fundamental principles of the Belmont Report (Table 2) influenced the criteria for the protection of human subjects and continues to be an important reference for Institutional Review Boards. The Belmont Report formed another base upon which medical research regulations are formed, and has widely influenced the standards that exist today. The controversies surrounding the Tuskegee Syphilis Study served not only as a representation of the exploitation of vulnerable populations through human experimentation, but also as an inciting incident to produce new regulations to protect the rights of future human volunteers. It can be argued that the most important consequence of the study was the creation of the National Research Act, the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, and ultimately, the formation of the Belmont Report. The lack of ethics surrounding the study have evidently led to the development of more protective guidelines to prevent these events from re-appearing in the future, and have powerfully impacted the ethics of current medical research.

## MODERN CONTROVERSIES

Although the regulations outlined in official documents such as the Nuremberg Code, the Declaration of Helsinki, and the Belmont Report drastically improved the ethical requirements surrounding clinical trials, there are still many controversies surrounding modern



clinical research.

One example of this is known as “pay to play” clinical trials. Most studies offer monetary compensation for their research participants, but a number of recent studies require payment for participation. Some researchers charge patients to be enrolled in their study as both a way to fund their research and for personal profits (Emanuel et al., 2015). Patient funded studies have been conducted for conditions including Parkinson’s disease, and Multiple Sclerosis. One particular case surrounds Novastem, a Mexican stem cell product distributor for the U.S company Stemmedica. Novastem reportedly charged their participants \$30 000 USD for enrollment in their trial which uses neural stem cells to treat stroke-related brain damage (Wenner, Kimmelman and London, 2015). This raised concerns regarding patient participation and regulation of the study.

First, selection of subjects for Novastem’s study were based on one’s ability to pay, thus prioritizing the needs of the wealthy. Not only does this pose an ethical dilemma, but this can affect the validity of the study, as the selection of participants should be primarily based on the goals of the research study. Issues then arise when considering the need for placebos because paying participants will be less willing to accept randomization when there is a possibility that they will not receive the treatment. Also, patient funded studies can cost thousands of dollars, causing patients to spend large amounts of their savings. Severe illnesses can compromise decision making when deciding to spend large funds on a potential treatment (Emanuel et al., 2015). This could allow researchers to exploit desperate patients, even though the majority of experimental agents used in early clinical trials fail.

Another concern regarding patient funded trials is the production of reliable medical evidence to ensure the safety of the study. New forms of treatment can be ineffective, and even dangerous, thus, several factors to oversee the process are typically required. Studies must produce adequate evidence for the safety, toxicity, and efficacy of their treatment prior to submitting a clinical trial application (Wenner, Kimmelman and London, 2015). Since trials are expensive, private sponsors may minimize the sample size and duration of the study to generate the required evidence. As a

result, patient risks increase, and consequences may remain unknown. Drug companies are mainly driven by commercial interests and may prioritize them over the interest of the patient. To accommodate this growing issue, bioethicists recommend that new policies be designed specifically for these trials (Wenner, Kimmelman and London, 2015). Such policies should promote research methods that minimize bias when collecting data and ensure that the paying patient is aware of all risks surrounding the trial. Should this type of trial continue in future studies, it is important to form new accommodating guidelines to ensure the safety of the participant.

## **CONCLUSION**

In the past century, various documents have been established which state that certain ethical principles must be incorporated in clinical studies to protect the research subject’s rights. It is necessary for researchers to understand how ethical regulations have been formed in order to understand their significance and potential for improvement. Knowledge of the biases, opinions, and controversies that have produced these regulations provides a better perspective with which these ethical codes can be understood. Since the 1940s, controversies surrounding experiments with human subjects have led to new guidelines that shape research ethics. The Nuremberg Code, the Declaration of Helsinki, and the Belmont Report were guidelines that formed as a result of controversial studies responsible for the improper treatment of human subjects. These regulatory ethical research codes have helped to shape the development of our current ethical standards, and will continue to be used as a basis for future ethical guidelines.

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## **AUTHOR CONTRIBUTIONS**

L.B. wrote the Nuremberg Code and Belmont Report sections and A.L wrote about the Declaration of Helsinki and modern controversies. Both authors contributed equally to the introduction and conclusion.

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# Adverse effects associated with monosodium glutamate consumption: A brief review

**JAMES LAI**

Michael G. DeGroote School of Medicine, Class of 2019, McMaster University

## SUMMARY

Monosodium glutamate (MSG) is a widely used flavour enhancer, first patented as a food additive in 1908. Although the compound has been recognized as safe for human consumption by regulatory bodies, there exist medical and anecdotal reports and beliefs that consuming MSG can elicit a variety of adverse effects. In this work, the known biologic effects of glutamate, the active compound in MSG, are first briefly discussed, followed by a summary of existing literature on adverse effects of human consumption of MSG. Overall, based on the current state of evidence, it is argued in this work that although the constellation of symptoms, now referred to as MSG symptom complex, have not reliably been reproduced in controlled study conditions and are thus often dismissed, some evidence points to possible effects of MSG on human physiology; gaps in the literature persist that warrant further study, such as better controlled studies involving MSG consumption with food.

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## REVIEW OF EXISTING LITERATURE

### INITIAL REPORTS OF ADVERSE EFFECTS OF MSG

Monosodium glutamate (MSG) is a food additive first patented as a flavour enhancer in 1908. The active component of MSG is glutamate, which produces the taste known as umami (Sano, 2009). Adverse effects of consuming monosodium glutamate were first notably reported by Kwok in 1968. In a letter to the editor in the *New England Journal of Medicine*, Kwok coined the term “Chinese restaurant syndrome” to describe the collection of symptoms he had repeatedly experienced after eating at a Chinese restaurant, including numbness at the back of the neck with radiation to the arms and neck, generalized weakness, and palpitations. He further described similar experiences from friends of Chinese descent after eating at some Chinese restaurants. Notably, Kwok did not specifically implicate MSG as

the responsible agent, but rather mentioned it in a list of speculative causes including soy sauce, cooking wine, and sodium (Kwok, 1968).

Following Kwok’s publication, many further anecdotal letters were published, and a variety of investigations were conducted into the cause of this constellation of symptoms (Anon., 1968). This included a double-blind study with three participants consuming wonton soup (Schaumburg and Byck, 1968), and a single-blind crossover study with thirty-five participants consuming tomato juice (Ambos, et al., 1968). These small initial studies concluded that MSG was the responsible agent by showing that participants reported experiencing adverse effects when they consumed test food items containing MSG, and not when they consumed the food item without MSG. However, both studies had a small sample size, were conducted solely on participants who self-reported as being susceptible to the symptoms, and neither included well-described protocols or detailed results.

## STUDIES BY REGULATORY AGENCIES

In the following years, several further studies were conducted. In particular, the issue was examined by various food regulatory groups, which conducted literature reviews to reach their conclusions. In 1980, a committee of the Federation of American Societies for Experimental Biology (FASEB) concluded that MSG may cause adverse reactions in a portion of the population that is susceptible to its effects, but that evidence was not strong because some studies showed that symptoms could be elicited without MSG. It further noted that MSG levels in use in processed foods were less than those used in the experiments that elicited symptoms. The average daily MSG intake was estimated at the time to be around 550 mg/day (He, et al., 2011), while experiments typically used amounts in the range of 1 to 12 grams in a single dose. Therefore, it was concluded that levels in use in processed foods were acceptable and posed no risk of harm. No comment was made regarding MSG levels in home- or restaurant-prepared foods, as this fell outside of the committee's scope (Federation of American Societies for Experimental Biology, 1980). A joint Food and Agriculture Organization and World Health Organization report in 1987 similarly concluded that levels required for the intended effect of flavour enhancement did not pose a risk, but suggested caution regarding taking large amounts in a single dose; it did not specify what constituted a "large" dose (World Health Organization, 1987). A further review of MSG by the FASEB in 1995 suggested the less pejorative name "MSG symptom complex" and concluded that evidence suggested that causality for MSG and reported side effects had been established for a subset of the general population, noting reactions as described by previous reports to oral MSG boluses  $\geq 3$  g without food, and more frequently if taken on an empty stomach. However, despite the abundance of reports on these symptoms, the quality of much of the literature available at the time was deemed to be poor (Federation of American Societies for Experimental Biology, 1995). Food Standards Australia and New Zealand reviewed the safety of MSG in 2003 and concluded that many earlier studies had methodological flaws. However, they agreed that the ingestion of large amounts of MSG ( $\geq 3$  g) without food did provoke symptoms in a small population of people. It was also noted that the literature did not adequately address whether consumption of MSG with food would alter the effect on participants (Food Standards Australia New Zealand, 2003).

## RECENT CLINICAL STUDIES

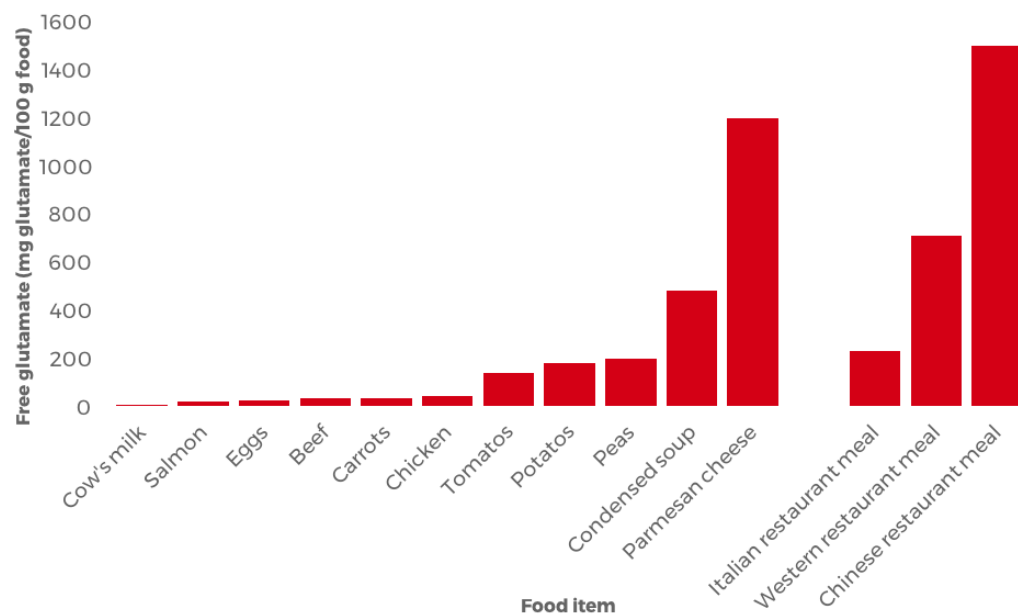
Various double-blind placebo-controlled (DBPC) trials have been conducted. One trial with 61 participants

suggested that statistically significant increases in headache ( $p < 0.023$ ), muscle tightness ( $p < 0.004$ ), numbness/tingling ( $p < 0.007$ ), general weakness ( $p < 0.040$ ), and flushing ( $p < 0.016$ ) could be elicited when participants consumed 5 g MSG disguised in a citrus-flavoured beverage (Yang, et al. 1997). A subsequent multicentre study with 130 participants used a similar protocol, but included further phases of testing on susceptible individuals, one of which included consumption of MSG with food (Geha, et al., 2000). As in the study by Yang and his colleagues, it was noted that subjects responded to higher doses of MSG; however, with later rounds of testing, it was found that subjects did not respond consistently to MSG challenge. More recently, Baad-Hansen, et al. (2010) found increases in headache, pericranial muscle tenderness, and systolic blood pressure with MSG intake in sugar-free soda versus placebo. Studies using a similar oral MSG-soda challenge have also concluded that MSG intake leads to sensitization of the masseter muscle and increased blood pressure (Shimada, et al., 2013; Shimada, et al. 2015), as well as increased susceptibility in individuals with temporomandibular disorder (Shimada, et al., 2016). However, these studies used a fairly high dose of 150 mg MSG/kg body weight.

## DISCUSSION

It is currently widely believed by the public that MSG does not cause adverse effects, and that studies have definitively shown that the MSG symptom complex is not reliably reproduced in laboratory settings. Based on the review of the current literature, it is argued in this work that a more nuanced and balanced position would be more appropriate. It is argued here that:

1. MSG consumption in large amounts without food have been shown to elicit uncomfortable but non-life-threatening symptoms
2. One well-conducted double-blinded placebo-controlled trial (Geha, et al., 2000) showed that the symptoms elicited were not consistently reproduced
3. There is a paucity of well-designed studies addressing MSG consumption in large amounts with food, and the fact that both food and the pattern of MSG consumption during a meal is expected to affect the dynamics glutamate absorption
4. Arguments asserting that the average daily consumption of MSG do not exceed recommended maximum acceptable doses have only addressed MSG consumption from processed food and home cooking, and not the MSG content of restaurant food; thus, further research should be conducted in this regard



**Figure 1:** Comparison of free glutamate levels in various food items and restaurant meals. Values obtained from Yamaguchi and Ninomiya (1998, cited in Food Standards Australia New Zealand, 2003) and Nicholas and Jones (1991, cited in Food Standards Australia New Zealand, 2003); maximum values were selected for those with ranges provided.

Of particular note, food regulatory agency reports have acknowledged that large doses of MSG consumed without food appear to elicit temporary adverse effects that are not life-threatening. Double-blinded placebo-controlled studies agree with this conclusion, but have shown that symptoms are not elicited consistently. The literature review conducted for this work did not find any major DBPC since that of Geha, et al. (2000); we argue it would be prudent to replicate this study given the relatively low number of participants that were tested in the later phases of their study, which precluded statistical analysis (12 participants in Protocol C, and 2 for Protocol D). Furthermore, following the work of Geha, et al. (2000), in which MSG consumption with food was assessed in Protocol D, no further DBPC with MSG with food were located. It has previously been asserted that consumption with food may affect absorption of glutamate; it has also been noted that consumption of MSG in food may occur in a variety of temporal patterns over the course of the meal. Studies should therefore evaluate MSG consumption with food to better elucidate possible adverse effects of consuming MSG in real-life situations.

The basis of several assertions of MSG's safety includes the argument that MSG is not consumed in amounts as high as those used in studies. However, these assertions are either based on the claim that high consumption of MSG does not occur because it is not required for its intended flavour-enhancing effect (World Health Organization, 1987), or based on calculations and surveys of processed foods and home cooking (Rhodes, et al., 1991; He, et al., 2011; Henry-Unaeze, 2017). Given that

MSG symptom complex was originally associated with eating restaurant food containing MSG, it is unclear whether MSG consumption amounts as previously estimated from processed food and home cooking, ranging from 0.6 to 1.5 g/day (Henry-Unaeze, 2017), apply to restaurant food. One estimate of MSG content in restaurant food reported up to 1500 mg free glutamate/100 g food (Nicholas and Jones, 1991 cited in Food Standards Australia New Zealand, 2003) (Figure 1). It has also been estimated (although, without justification or reference) that a highly seasoned restaurant meal may contain as much as 5 g MSG (Yang, et al., 1997), which falls within the range of MSG levels consumed in experiments. It may be reasonable to systematically survey restaurant food for its MSG content to more accurately inform MSG with food studies; new protocols have been developed that may facilitate this, such as the liquid chromatography-tandem mass spectrometry used by Cebi, et al. (2018).

In conclusion, evidence currently suggests that ingestion of large amounts of MSG without food may trigger symptoms consistent with MSG symptom complex in susceptible individuals. However, there is currently a lack of studies regarding the adverse effects of MSG consumption with food, and estimates of MSG consumption are currently based on processed and home-cooked foods, not restaurant foods. Therefore, it is argued that further DBPCs challenging participants with MSG in food, informed by better quantification of MSG in restaurant foods, should be conducted before dismissing the possibility that MSG may produce adverse effects when consumed.

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# Redefining invasive species using a stage-based process

PASCALE BIDER, TANYA DANIEL, ADAM TWEEDLE

Integrated Science Program, Class of 2019, McMaster University

## SUMMARY

Invasion ecology explores the impacts of new species entering established ecological niches and communities. While the effects of invasive species are frequently discussed in both industry and academia, the definition of invasion remains vague, arbitrary, and based on human perception. The objective of this project was to better define species invasion using a mechanistic, stage-based perspective. Several authors have attempted to develop a definition that focuses on the process of invasion, rather than on the characteristics of the (potentially) invasive species. This process encompasses three main stages: transport, establishment, and spread. The purpose of a stage-based definition is to classify a given species by its progression through the invasion process. By integrating existing definitions, we propose a novel version of the stage-based process of invasion. This approach incorporates extrinsic factors such as competition, predation, and parasitism in addition to contingent physical forces such as flooding, freezing, and fire. Therefore, a wide variety of species will be able to fit into this definition. To demonstrate its effectiveness, we test our definition with an agent-based model that simulates the influence of multiple ecological factors (propagule pressure, resource availability, energy consumption, reproductive capability), which can either inhibit or assist the invasion of a species. Adopting a more comprehensive method for defining invasive species will allow for better communication within invasion ecology research and throughout the scientific community. Ultimately, our definition helps to simplify the process of developing a practical invasion prevention policy that can be implemented at multiple stages for full effectiveness.

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## INTRODUCTION

Since the emergence of invasion ecology as a field, there have been many different words used to refer to a species that has travelled out of its native range. Terms such as “invasive”, “alien”, “introduced”, “exotic”, “imported”, “naturalized”, and “non-native” frequently appear in the literature, but generally remain poorly defined (Pereyra, 2016; Valéry et al., 2008). Authors will use some or all of these terms, but each with their own personal connotation. Despite ever-growing awareness of invasive species and invasion ecology, there has yet to be a consensus regarding how and when these terms

should be used. For a comprehensive comparison of the following definitions found in the literature and for the general public, see Table 1.

## DEFINITIONS BASED ON NEGATIVE IMPACTS

Many definitions of invasive species hinge on the idea that they are necessarily harmful. An example of such a definition comes from Russell and Blackburn (2016), who argue that an alien species becomes invasive only when it exceeds a given damage threshold. Another similar definition can be found in Richardson et al.’s

**Table 1:** Comparative table highlighting the various definitions of “invasive” used in invasion ecology (based on Pereyra’s 2016 methodology). Both academic and public sources were examined.

Citation	Terms used	Specific characteristics that must be met in order to be considered invasive		
		Negative Impact	Human Mediated	Other
Valery et al., 2008	Invasive	No	No	Must become dominant in new ecosystem
Russell and Blackburn, 2017	Invasive	Yes	No	-
Tassin et al., 2017	Invasive	No	No	-
Richardson et al., 2000	Invasive	No	Yes	Must be relocated beyond a specific distance
Hulme et al., 2016	Alien	No	No	-
Pycek et al., 2004	Invasive	No	Yes	Depends on specific term used
Heger and Trepl, 2003	Invasive	No	No	-
Pycek and Richardson, 2010	Invasive	No	Yes	Must produce reproductive offspring at locations far from the site of introduction
Powell, Chase, and Knight, 2013	Invasive	No	No	Must have high rates of population growth and become dominant in new ecosystem
Wikipedia, 2017	Invasive	Yes	Yes	Must directly threaten agriculture or biodiversity
USDA, 2016	Invasive	Yes	No	Must cause environmental harm or harm to human health
Ontario's Invasive Species Awareness Program, n.d.	Alien, Invasive Alien	Yes	Yes	-
Government of Canada, 2016	Alien, Invasive Alien	Yes	No	-

(2000) paper, who claim that alien species makes the shift from “naturalized” to “invasive” when it begins to have detectable consequences. This paper also proposes to label those alien species, once they are regarded to be harmful, as “pests” and “weeds”.

The method of classifying species based on how harmful they are to their surroundings has some obvious issues. To use such a definition, we must define what constitutes “invasive”, as well as what constitutes a “negative impact”. Our perception of what is “negative” is heavily based on social values (Tassin et al., 2017). Invasive species have a variety of consequences, ranging across ecological, biological, economic, or social impacts. The introduction of a new species may result in negative consequences within certain areas, but not in others. When using these types of definitions, some may choose to label a given species as invasive, while others will not. Differences in values could result in

confusing language and misuse of terms (Tassin et al., 2017). Additionally, defining “invasive species” based on their negative impacts makes it difficult to categorize species who have the potential for negative impacts in the future (Russell and Blackburn, 2016).

### DEFINITIONS BASED ON HUMAN FACILITATION

Another problem that is presented by many current definitions of the term “invasive” is that, by some standards, an invasive species must be introduced through human means. Gilroy et al. (2016) argue that, since we need different management practices for species introduced with human aid and species introduced without, any definition used should clearly differentiate the two. It has been proposed that terms such as “invasion” be reserved for the cases where species distribution is changed by human activity (Gilroy et al., 2016; Pysek



et al., 2004). Several legislative bodies have incorporated this school of thought in their definitions (Gilroy et al., 2016). For example, the European parliament stated in 2014 that an “alien” species is identified as “any live specimen introduced outside its natural past or present distribution” where introduction signifies “the movement by human intervention” (Gilroy et al., 2016). Additionally, it has been said that since non-human-mediated introductions are so rare, they should be classified as natural rather than invasive (Richardson et al., 2000). This type of definition is problematic for many reasons. First, organisms can travel to new regions without the aid of humans. For example, in one of the first works to ever describe invasive species, Charles Elton explains that since many migratory birds travel across the globe, they can easily transport seeds, small eggs, or even microscopic organisms far from their usual range (Elton, 1958). Secondly, classification is difficult when a species uses human-mediated processes only temporarily, or for only a part of their journey to a new habitat. For example, bats may temporarily rest on boats when travelling over water from one range to the next (Gilroy et al., 2016). There is ambiguity when a species travels to a new area by more than one pathway, some human-mediated and some not (Hulme et al., 2016). This approach can also complicate the labelling of a newly-arrived species whose vector of transport is still unknown. Some argue that once a species is introduced, it makes no difference from an ecological perspective whether the introduction was human-mediated or not (Heger and Trepl, 2003). Therefore, no distinction needs to be made between the two. Many texts have now adopted this point of view (Valéry et al., 2008; Colautti and MacIsaac, 2004; Vermeij, 1996).

## DEFINITIONS USING PEJORATIVE LANGUAGE

Texts describing species establishing themselves outside of their native range will often do so using unnecessarily pejorative or value-laden terms. The term “invasive” in itself can be associated with concepts such as “attack” or “aggression” (Richardson et al., 2000). However, this term has been used more often than any other in recent literature (Pereyra, 2016). Additionally, some papers may choose to use other pejorative terms such as “pest” and “weed” (Richardson et al., 2000; Pysek et al., 2004). This type of language is problematic as it may lead to biased attitudes towards non-native species (Pereyra, 2016). Viewing all invasive species as harmful attackers can cause us to overlook their potential uses and benefits (Geniusz, 2015). Some have proposed the replacement of biased terms by neutral ones, such as “alien”, “introduced” or “neobiota” (Pereyra, 2016). However, given how commonly some of these pejorative words

are used, it may seem unrealistic to remove them from our language altogether.

## DEFINITIONS THAT ARE EXCESSIVELY SPECIFIC

Throughout the literature, several definitions of “invasive” require the species to fit within highly specific, and relatively arbitrary, criteria. For example, Richardson et al. (2000) propose that, in order to be considered invasive, a plant must be carried further than 100 km from their native range. Then, plants spreading by seed must continue to expand in their new range at a rate of over 100 m in under 50 years (or over 6 m in under 3 years for plant spreading by root or rhizome). These minimum distances must be met in order for the plant to be considered invasive (Richardson et al., 2000). Another example can be found in the 2004 article by Pysek et al. where it is stated that a “naturalised alien” is a plant that has sustained a self-replicating population for at least 10 years without aid from humans. These overly precise definitions can be difficult to apply to a wide range of species. In addition, some feel that overly specific, academic language can complicate communication between invasion ecologists and the general public (Pereyra et al., 2016).

## THE IMPORTANCE OF A STANDARDIZED DEFINITION

This inconsistency present in invasion ecology vocabulary has arisen as a result of many factors. First, it has been speculated that since invasion biology has not been studied as a holistic field until recently, the separate disciplines previously concerned with species invasion have each established their own definitions (Pysek et al., 2004). Consequently, we have definitions that come from ecological, biogeographical, and anthropocentric perspectives, to name only a few (Pysek et al., 2004). Furthermore, invasion ecologists concerned with different taxonomic groups have generated definitions that are applicable only to their taxa and not others (Blackburn et al., 2011). In particular, there tends to be a divide between ecologists studying plants and animals (Blackburn et al., 2011). Some plant ecologists might use the term “invasion” to define the local establishment of new populations, whereas outside of this field, the same process might be defined as “colonisation” (Richardson et al., 2000). For example, *Taxillus tomentosus* (mistletoe) has been called invasive to certain forested areas in South India, even though the plant is native in this general region (Rist et al., 2010).

Another potential reason for misunderstanding regarding current terminology could be the result of different languages in which invasion ecology has been studied and written. Researchers from all over, particularly in

Central Europe, have contributed to the field of invasion ecology. Richardson et al. (2000) suggest the nuances with which terms are used in French, German, or other languages are not fully translated into English (Richardson et al., 2000). Furthermore, changes within the English language itself could also be responsible for this confusion (Richardson et al., 2000). Many of the terms used to talk about invasive species were defined before the complexities of the invasion process were fully understood. For example, the term “naturalized” appeared in biological literature in the mid-19th century, but invasion ecology as a field only began to emerge a century later (Richardson et al., 2000; Elton, 1958). Overall, this has led to terms such as “invasive”, “native”, “alien”, and “naturalised” being used throughout the literature with a variety of definitions and connotations. Imprecise language and definitions cause many problems for studying and writing about invasive species. Ultimately, it hampers comparison between studies (Blackburn et al., 2011). By its very nature, invasion ecology requires comparing data sets from geographically separate regions (Pysek et al., 2004). In order to detect trends, it is important to know what species are native to what region, and what species are invasive elsewhere (Pysek et al., 2004). If researchers do not use the same criterion when labelling their species under investigation, comparison of data becomes impossible (Hulme and Weser, 2011). Secondly, inconsistent labelling complicates communication with the general public. The use of multiple different terms to describe invasive species can lead to both the public and the media misinterpreting information (Russell and Blackburn, 2017). Difficulty in interpreting data surrounding invasive species can then lead to the implementation of inadequate policy and legislation (Gilroy et al., 2011). Since the term “invasive species” is connoted with negative impacts, the species thus labelled are more likely to be targets for eradication policy. Meanwhile, species labelled as “native” or a similar term might become pro-

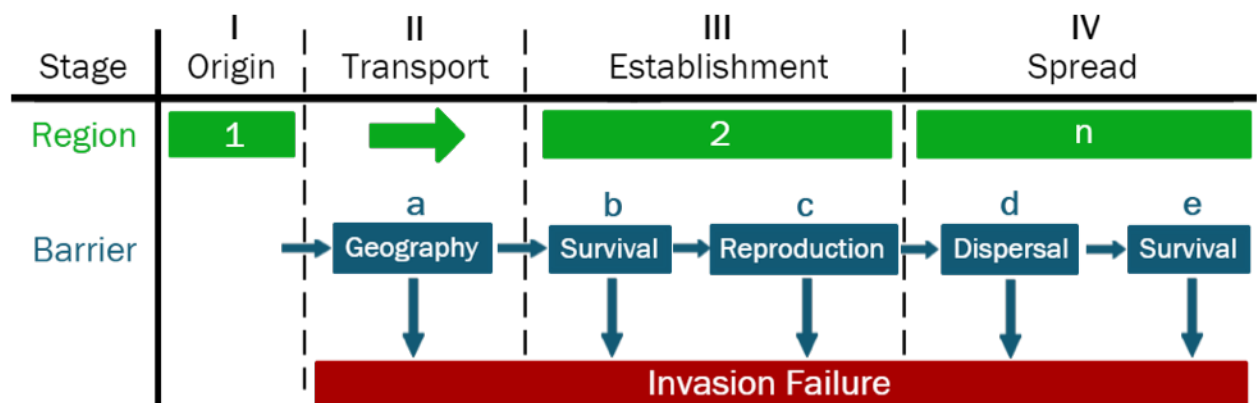
tected by legislation (Gilroy et al., 2011). The values we attach to the terms we use then inform environmental agendas (Pereyra, 2016). Evidently, it is vital to establish a clear-cut definition of “invasiveness” that can be applied to all species, while being separated from human values, and accounts for the entire invasion process.

## THE STAGE-BASED APPROACH

To properly distinguish a native species from a species currently undergoing the process of invasion, an alternative definition must be proposed. This improved definition must consider the process of invasion, rather than focusing solely on a species’ capacity to spread and reproduce. Thus, a stage-based definition studies the progression of the potentially invasive species’ ability to occupy a new environment. There are three fundamental stages within a stage-based definition: transport, establishment, and spread. Within each stage, there are several factors that either inhibit or assist the species when moving from one stage to the next. In our proposed definition of the stage-based process of invasion, these extrinsic and contingent factors are included.

A stage-based perspective is more beneficial as a definition because it can encompass multiple pathways by which a species can invade. Due to the definition’s broader perspective, the focus is now on the ecological logistics - of both the environment and the species - which allow for invasion to occur. Since the stage-based perspective looks at the process overall, the development of prevention techniques and policies can be created to stop the invasion of a species, if necessary. As Jeschke et al. (2014) state, impact can occur at any stage. Our definition is broad enough to show how changes to the environment can happen, but these effects are not implied to be decidedly positive or negative. Thus, it is not assumed that all invasive species are detrimental to an environment.

Much of a species’ ability to invade is based on circumstance and random factors (Heger and Trepl, 2003). Our



**Figure 1:** Our proposed model to demonstrate the process of invasion.

definition looks to include contingency and variability among species and the environment so that invasion can be generalized to a process. Furthermore, looking at invasion as a process allows investigation into the relationship and properties of both the invading species as well as the ecosystem itself. Invasion could be regarded as a temporally-dependent process rather than spatially-dependent. Overall, our proposed model builds on a more holistic perspective.

## THE PROPOSED INVASION PROCESS

Expanding from an invasion model proposed by Blackburn et al. (2011), we integrate previous versions of the invasion model to develop a broader stage-based definition (Figure 1).

**Stage 0: Origin**— Most models of invasion are fundamentally built around three main stages: transport, establishment, and spread (Blackburn et al., 2011; Lockwood et al., 2007; Colautti and MacIsaac, 2004; Heger and Trepl, 2003; Sakai et al., 2001). We have decided to keep these three stages as well as add a *Stage 0: Origin*. This stage represents a species in its natural habitat. Although several papers have varying definitions for the meaning of a native species and its home region (Lockwood et al., 2007; Colautti and MacIsaac, 2004; Heger and Trepl, 2003; Sakai et al., 2001), the general consensus is that a native habitat is wherever the invading species has travelled from. For our purposes, we will regard *Stage 0* as a relative region so that a change in habitat is evident. Thus, this could be regarded as the donor region. Species survive and thrive in this first region before entering *Stage I* of the invasion process. The *Region* numbers of our model are intended to quantify species movement as it progresses through the stages of invasion. To classify a species as being in *Stage 0*, the species must not inhabit more areas than those it is native to. However, once the species has commenced the invasion process, it will move to occupy increasingly more regions over time.

**Stage I: Transport**—In current literature, the first stage of transport (*Stage I*) is presented in a manner that is saturated with human bias. One source of this issue is that invasive species are believed to necessarily use human-mediated vectors. Our version of *Stage I* is broad enough to include human-facilitated transport as well as any other contributor to species movement that might arise. We primarily utilise Vermeij's (1996) definition of biotic interchange, which refers to the expansion of a species' range, as our definition for transport. More simply, transport is a broad term for a portion of a species' population leaving its original habitat and entering a novel environment. Our definition re-

gards transport of invasive species as any movement of a species, regardless if it was accomplished through a human-mediated vector or a non-human-mediated vector. Lockwood et al. (2007) and Sakai et al. (2001) distinguish several pathways of transport caused by humans which include but are not limited to: farming and hunting, environmental enhancement, biocontrol, conservation, and scientific pursuits. Examples of unintentional human-mediated transport include movement of species from airplane, automobile, and boat travel (Lockwood et al., 2007; Sakai et al., 2001). Although the transport of invasive species is usually due to human activity, natural transport can also occur, such as by wind and bird migrations (Lockwood et al., 2007). It should be noted that the success rate of a particular transport vector can change over time. For instance, the ability to travel across greater distances is increasingly becoming faster and more accessible. Additionally, transport is its own stage because it may take multiple trials and various new habitats before an invasive population can settle.

For survival through *Stage I*, there are multiple factors to take into account in order to overcome *Barrier a: Geography*. The vector itself must be sustainable to support the invasive population. For example, a greater distance to travel will likely result in fewer viable healthy individuals to establish in the new region. To counter this effect, a greater number of individuals could be transported to increase the chance of survivors. This is referred to as propagule pressure, which is defined as the size of the invading population as it enters the new habitat (Blackburn et al., 2011; Lockwood et al., 2007; Colautti and MacIsaac, 2004; Sakai et al., 2001). Another factor associated with propagule pressure is the frequency of the transport, and whether or not the invasive species population is being regularly replenished with healthy individuals to assist in mating, dealing with environmental stochasticity (unpredictable and stressful weather patterns), and maintaining competitive interactions with species in the novel region (Lockwood et al., 2007). As the density of the invasive population develops, it is more likely to establish.

Overall, the greater the propagule pressure, the more likely a species will succeed in entering *Stage II*. Our definition of *Stage II* resembles the description by Blackburn et al. (2011), which is that establishment refers to a single self-sustaining population over multiple generations in a localized region. Interestingly, there can be an intermediate period of time before an invasive species becomes self-sustaining in *Region 2*, which is denoted as The Lag Period (Lockwood et al., 2007; Sakai et al., 2001). This has been suggested to be the result of exponential population growth trends or a temporary timeframe used to increase propagule pres-

sure (Sakai et al., 2001). Since the time frame varies per species, has not been well-recorded, and would not be considered a barrier to overcome, it is not included in our model as a separate section, but rather as a factor within *Barrier b: Survival*.

**Stage II: Establishment**—Colautti and MacIsaac (2004) separate establishment as 1) widespread but rare or 2) localized but dominant. We chose not to separate establishment into two distinct types but rather focus on the process of establishment within invasion. *Stage II* is broken down into two barriers because success in surviving is separate from success in reproducing (Blackburn et al., 2011; Heger and Treppl, 2003). Within *Barrier b: Survival*, the species must be able to survive against predators as well as compete for resources among natural inhabitants. Essentially, basic physiological needs must be met. This ability to survive in the new environment stems from the interactions between the natural region's species and the invasive species. This includes competitive exclusion, predation (predator-prey), parasitism (parasite-host), herbivory (herbivore-plant), and mutualism (Lockwood et al., 2007; Sakai et al., 2001). Furthermore, *r*-selected species would have drastically different establishment and reproductive strategies than *k*-selected species. Since factors like fecundity, generation growth time, individual growth rates, and specialist versus generalist qualities depend on the particular species, they are not the sole reason for a species' ability to invade. As a result, it is more correct to look at ecological interactions. All species-species interactions may work in favour or against an invading species. For instance, the invasive species may find that access to resources is easier in the new environment due to lack of predators and similar niche-sharing species. On the other hand, the invasive species may not have the fitness to exploit or compete for nutritional resources with current inhabitants. Moreover, mutualism can have both positive or negative effects. A lack of mutualism can inhibit a species' population growth, while on the other hand, a newly established mutualism interaction can allow the species to thrive (Sakai et al., 2001). In addition to species-species interactions, *Barrier b* also includes the theory of disturbance and its role in allowing a species to invade (Lockwood et al., 2007; Sakai et al., 2001). This theory explains that environmental changes, as a result of human activity or natural activity, can lead to niche modifications which allow for invading species to enter (Lockwood et al., 2007; Sakai et al., 2001).

Within *Barrier c: Reproduction*, the invasive species must be near other viable mates. This proximity impacts an invading species' genetic variation, which can be both advantageous or detrimental to the population (Dick

et al., 2012; Lockwood et al., 2007; Sakai et al., 2001). Without genetic diversity, a population may not have the suitable characteristics to survive in the new habitat. This phenomenon is known as the founder effect, resulting in a bottleneck population. Limited genetic variation within a population leads to inbreeding depression and can limit population growth, and thus decreases establishment potential (Tsutsui et al., 2000; Sakai et al., 2001). However, the funneling of genetic diversity could also be beneficial to allow for adaptive evolution. The invading species may already have relatively pre-adapted traits that allow the species to grow in the new environment (Sakai et al., 2001). In summary, if the alleles that express the greatest fitness remain, the invasive species is more likely to establish. If the alleles express unsuitable traits, then the invasive species is more likely to fail. Furthermore, hybridization of native species with invasive species can be both beneficial or inhibiting (Sakai et al., 2001). The introduction of these new genes may lead to an increase in fitness or it can lead to genetic assimilation. In all of these cases, natural selection may favour the native species instead of the invasive species and vice versa. Evidently, genetic diversity and propagule pressure play an essential role in conjunction with species-species interactions to allow for an invasive species to establish.

**Stage III: Spread**—Once capacity of *Region 2* has been reached or enough individuals are present in the settled population, the invasive population can move onto *Stage III* with the proper transport. *Barrier d: Dispersal* is similar to *Stage I*, but the colonization outwards from *Region 2* tends to be more localized (Chapple et al., 2012; Sakai et al., 2001). In these newly introduced regions, the previously single, self-sustaining invasive population has now increased to two or more established communities. For *Barrier e: Survival*, this is similar to *Stage II* in terms of the factors that allow an invading species to live in the newest habitats. Genetic diversity, morphological traits, and reproductive rates are all key components in invasion. *Stage III* can theoretically occur in an infinite number of new regions for an infinite amount of time. In simple terms, *Stage III* is considered to be a range expansion of the initial invading population.

## SUMMARY

At any phase of the invasion process, a species can indefinitely stay at a particular stage or the species can fail to invade. The former occurs when the independent species population is able to remain self-sustaining. As discussed, there are many factors that could result in the latter, which include, but are not limited to, propagule pressure, species-species interactions, and genetic diversity. Thus, pressure from the new environment can

result in a lack of potential mates or inability to attain basic survival needs. As for the nomenclature, both stage and barrier are incorporated in a numeral-letter system. For example, a species that has established an invasive population in *Region 2*, but is not reproducing, would be in *Stage II-b* of the invasion process.

## NETLOGO AGENT-BASED MODEL

To visualize and observe trends in the stage-based model, an agent-based model was developed. The model was adapted and modified from the “Life Strategy and Invasion Biology Model”, designed by Kyra Simone (2016), in NetLogo (Wilensky, 1999). Parameters based on the interaction between a native ash borer species and an invasive ash borer species were set to demonstrate their roles within the invasion process.

The initial population size of the native and invasive specialist demonstrates how the population size of the native species will affect the fitness of the invasive species to progress through the model, as well as the effect of propagule pressure upon the native species.

The energy consumption of both species can be altered, helping to demonstrate how an organism’s ability to consume at a more efficient rate relative to another can provide a distinct advantage for population establishment and reproduction.

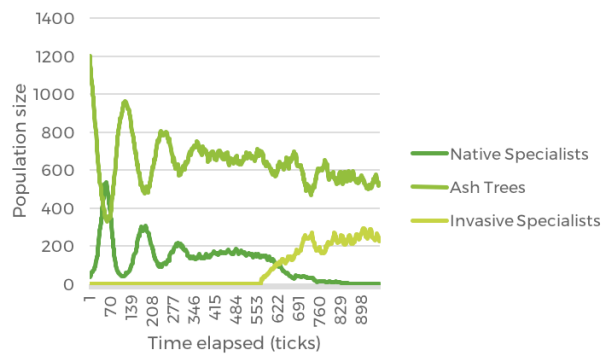
The minimum reproductive energy required of both the native and invasive borer species can be adjusted to demonstrate how high fecundity levels have significant impacts on propagation towards the surrounding region and throughout an environment.

The maximum number of offspring can be altered to simulate a species with a high fecundity rate and to aid in understanding its implications on the population dynamic in both the native and invasive borer species.

The final alterable parameter is the density of ash trees present within the environment. This demonstrates how a larger availability of hosts can significantly determine the ability of an ecosystem to support a native species as well as an invasive species simultaneously, or if the resources available will only support one species. Using these parameters, we hope to demonstrate the effect that these factors will have on the invasion process and how they will affect the progression of an invasive species through the stage-based model.

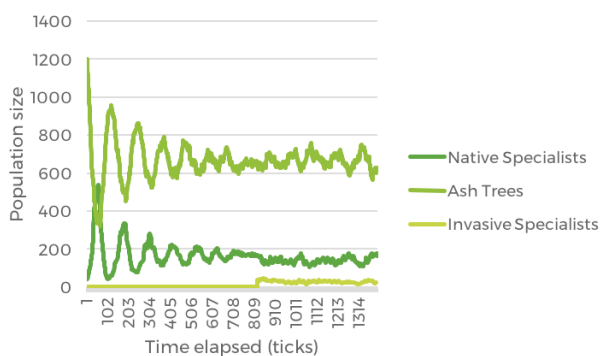
## RESULTS

Using the NetLogo code, various invasion situations were simulated. First, the code was used to re-create a situation with an abundance of food for the invading species, low competition, high reproductive ability, and high propagule pressure (Figure 2). When the new specialist was introduced, its frequency increased rapidly while the native specialist died out.



**Figure 2:** A new specialist species is introduced to the environment. The amount of native species individuals is shown in dark green, the amount of invasive species individuals is shown in light green, and the total energy of all ash trees, that both species eat, is displayed in medium green.

Next, an environment was created where the new specialist and the native specialist were similarly matched in terms of fitness (Figure 3). In this case, neither of the species died out and both appear to reach relatively stable population sizes.



**Figure 3:** Invasion where the native species and the invasive propagules are equal. The amount of native species individuals is shown in dark green, the amount of invasive species individuals is shown in light green, and the total energy of all ash trees, that both species eat, is displayed in medium green.

A third simulation was performed where the propagule pressure of the new specialist was low (Figure 4). In this scenario, the new specialist died out while the native specialist population size remained relatively unchanged.

Finally, an invasion scenario was created where the new specialist was not as well adapted to its new environment as it has a reduced ability to extract energy from its food source (Figure 5). In this situation, the size of the new specialist population initially increases, then drops back down to disappearance.

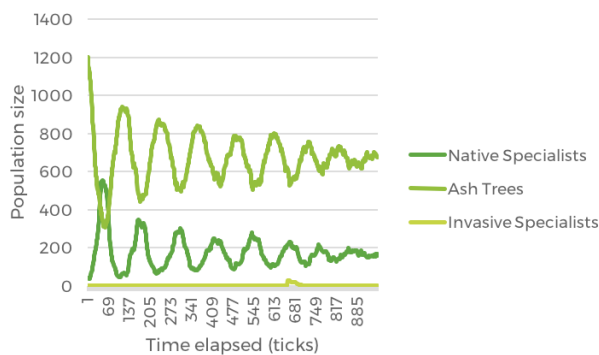
## DISCUSSION

With variation in starting conditions, it is possible to demonstrate how species in different situations will fit

the definition of our stage-based model with diverse results.

In a situation where an invasion is favoured, the invading species has a high fecundity rate, an abundance of food, few competitors and a large number of invading propagules that reach the new environment. As seen in Figure 2, the initial spike in invasive species individuals marks the introduction and beginning of establishment. Consequently, the invasive species will thrive and its numbers will continually rise, characterising it as a species in *Stage III-e*.

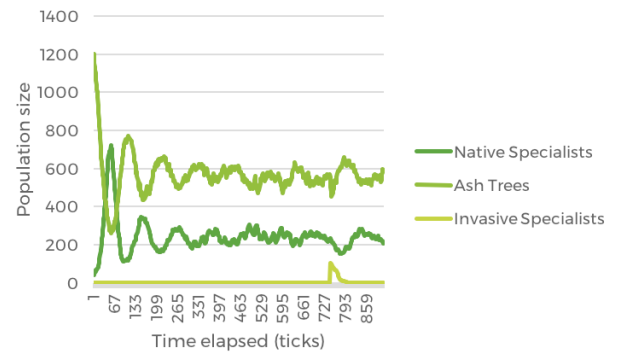
In a scenario where the native species and the invasive propagules are evenly matched in numbers, ability to acquire nutrition, and reproductive ability, the arrival of the invader modifies the environmental steady-state conditions and a new equilibrium is reached (Figure 3). As evidenced by the relative steady population size in both native and invasive specialists, it appears that the environment is able to sustain this equilibrium. The invader is able to establish itself and sustain its population, however its numbers do not increase. As such, it would be labelled as a *Stage II-c* species.



**Figure 4:** Failed invasion where the initial propagule pressure is relatively low. The amount of native species individuals is shown in dark green, the amount of invasive species individuals is shown in light green and the total energy of all ash trees, that both species eat, is displayed in medium green.

A third situation that may occur is when the potential invader fails (Figure 4). In this case, the initial number of propagules that reach *Region 2* is too low, and even though both species are equally able to feed themselves and reproduce, the invading species is not able to cross the barriers of survival and reproduction.

Similarly, invasion failure will also be induced if the invading species is ill-adapted to its new environment (Figure 5). In this scenario, the invasive species is not able to get sufficient nutrition once it is transported to the second region. Although the initial number of invasive propagules is almost double the initial amount of native specialists and they have equivalent reproductive capabilities, they have a lower likelihood to obtain



**Figure 5:** Failed invasion where the invading species is not well-adapted for the new region. The amount of native species individuals is shown in dark green, the amount of invasive species individuals is shown in light green, and the total energy of all ash trees, that both species eat, is displayed in medium green.

any nutrition from their environment. The species is not able to cross the barrier of survival and the invasion fails.

Due to the number of combinations that can be created and simulated using the slider functions of the Netlogo model, all results were not acknowledged. The combinations that were highlighted include two instances of a successful invasion and two examples in which invasion fails. Successful invasion is apparent when the invader is able to integrate a population into the new environment, whether or not the native borer species is affected. When the invasion process fails and the invading borer species is not able to establish a population, this is characteristic of invasion failure.

## CONCLUSION

Ultimately, it is suggested that previous invasion vocabulary should become umbrella terms, and that our stage-based, numeral and letter system be used to specifically refer to species in the invasion process. Our proposed novel definition is an improvement over existing definitions in several ways. First, it demonstrates minimal human bias, as it does not rely on human perception of supposedly damaging or harmful impacts. Further, it does not depend on arbitrary values of distance or spread. Third, our model focuses on the plant-animal-environment interactions, allowing for a holistic approach to defining the invasion process. Finally, it can be simply applied to a wide range of plant and animal species. Our NetLogo model provides a user-friendly reference point for understanding how to apply our definition. If this stage-based perspective is adopted into the literature, communication within scientific communities as well as conversation with the general public will be more readily understandable. Standardizing the language used when communicating with and across both groups will minimize confusion

and reduce the risk of mislabelling species. Clear language will allow for better legislation and prevention policies to be developed, as well as more effective outreach and education campaigns. Ideally, this will lead to greater ecological awareness in the community and a greater effort towards environmental stewardship.

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## AUTHOR CONTRIBUTIONS

P.B. conducted a literature review on the existing language used in invasion ecology for the introduction and assisted in observing and analyzing the results from the stage-based invasion simulation. T.D. conducted the research for several proposed stage-based approaches for invasive species. Afterwards, they were the main author to integrate these approaches to be used for our novel definition. A.T. developed the NetLogo modelling code for the stage-based invasion simulation as well as helped in writing the overall analysis and discussion of the outputs with P.B. All authors contributed in developing the proposed definition and were responsible for editing and revising one another's work.

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# The effects of water salinity on *Myzus persicae* and *Arabidopsis thaliana*

MONICA TAKAHASHI, PASCALE BIDER, ARIANA FRASCHETTI, CAMILLA GILLIS-ADELMAN, AUDREY TAM

Integrated Science Program, Class of 2019, McMaster University

## SUMMARY

Road salt is often used during the winter months as a cheap and efficient way to melt snow. However, it also poses some serious environmental issues. Runoff containing road salt can lead to soil salinization, which can impact plant health. This has detrimental effects on not only the organisms that feed on these plants, but also on the surrounding ecosystem. The purpose of this experiment was to study how salinity affects plants and the herbivores that feed on them. Wild type *Arabidopsis thaliana* plants and *Myzus persicae* (green peach aphid) were the model organisms used in the experiment. It was hypothesized that watering the plants with different concentrations of saline solutions would affect the health and growth of the plants as well as the *M. persicae* populations. Wild type *A. thaliana* plants were inoculated with *M. persicae* and watered with either regular tap water, a 20 mM saline solution, or a 60 mM saline solution. Rosette leaf surface area and aphid population were recorded for each plant over the course of the experiment and analyzed to determine the effect of salinized water on *A. thaliana* and *M. persicae*. The saline treatments did not have a significant effect on the aphid population growth, but did significantly affect the leaf surface area. There was no significant difference between the control and the high salt treatments or the control and low salt treatments. However, statistical significance was found between the low and high salt treatments, suggesting that salt did have some effect on plant fitness. The lack of effect on the aphids could be due to two main reasons. Firstly, the plant may have allocated its resources towards survival rather than defence against the aphids. Secondly, the regions of the plant preferred by the aphids may not have been affected by the saline solution. Since the aphids do not appear to be affected by salinization, plants will have to face the double burden of salt stress and herbivory.

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## INTRODUCTION

Road salt is one of the most widely-used environmental pollutants in Canada since it is a cheap and efficient way to melt snow and ice during the winter months. Road salt is usually made up of sodium chloride, and when used to de-ice roads and sidewalks, it significantly increases the concentrations of sodium in bodies of freshwater in North America (Godwin, Hafner and Buff, 2003). Moreover, studies have shown that the increase in sodium concentration has led to groundwater

contamination and plant toxicity issues (Forman and Alexander, 1998). Increasing soil salinity also has detrimental effects to plant health, affecting the plant's ability to uptake water and photosynthesize (Bryson and Barker, 2002). Consequently, it is important to study the impacts high salinity would have on not only the plants, but also on the ecosystem that the plants inhabit. While the negative effects of sodium on plant species may cause noticeable changes in plant health, any influence on the rest of the ecosystem and other species may be less apparent.



Although population size is regulated through both top-down and bottom-up interactions, the influence of either varies in each population. A discussion in the *American Naturalist* argues that community structure is controlled by bottom-up interactions, meaning every trophic level is “food-limited” (Hairston, Smith and Slobodkin, 1960). It is also argued that community structure is regulated by top-down interactions, whereby each trophic level limits the population size of the trophic level below it (Hillebrand, et al., 2007). In a community that operates via bottom-up interactions, an increase in soil salinity would likely negatively impact the rest of the ecosystem, which depends upon the affected plant species for energy. The influence of the salt would be seen first within the primary producers and then within herbivore populations such as insects. Any impact on the size of these insect populations would be sure to have repercussions elsewhere in the food chain. Therefore, it is important to determine whether road salt influences plant growth and the effect this has on herbivores.

To address this issue, a 13-day experiment was designed to observe the interactions between the plant species *Arabidopsis thaliana* and one of its predators, *Myzus persicae*, more commonly known as the green peach aphid. The study was conducted in order to answer the following set of questions: (1) is the growth rate of *A. thaliana* affected by an increase of soil salinity, and (2) how does the concentration of salinity affect the size and growth of the *M. persicae* populations feeding on *A. thaliana*?

Watering the *A. thaliana* plants with salinized water was predicted to cause decreased plant growth and a reduction in aphid population size for several reasons. Continual high salinity in plants results in the cessation of plant leaf surface expansion, a decrease in the productivity of photosynthesis, and plant death (Parida and Das, 2005). This causes aphid populations, which rely on the phloem sap of plants for nutrition (Giordanengo, et al., 2010), to be left without a food source. Additionally, high salinity in plants increases plant mesophyll and epidermal thickness (Boughalleb, Denden and Tiba, 2009). This may make it more difficult for the aphids to pierce the leaves for food (Giordanengo, et al., 2010).

By observing the effect of saline soil on *A. thaliana* and *M. persicae*, the present study aims to better understand the effects of road de-icing on primary producers and the organisms that feed on them. The results of the study may have important implications for the future of ecosystems both around Cootes Paradise and around the world.

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN

For the experiment, 24 healthy wild type *A. thaliana* rosettes were chosen out of a selection of plants grown in the McMaster University Greenhouse in Hamilton. These plants were deemed healthy based on their lack of discoloration or torn leaves. All chosen plants either had not begun the bolting stage or had bolts that had been previously removed. The study used 96 viviparous female *M. persicae* (green peach aphid) that had been reared on either *A. thaliana* or tobacco leaves. Randomization of the treatments was done using a random number table (Petrie and Sabin, 2009). To make the randomization process more efficient, a four-number range was chosen for each plant. For example, numbers one to four corresponded to plant A, numbers five to eight corresponded to plant B, etc. (Table 1). The random number table was then used to assign treatments to plants in the systematic pattern of “no salt”, “low salt”, and “high salt”, resulting in eight plants per treatment.

**Table 1:** The *Arabidopsis thaliana* plants and their corresponding random table number ranges and treatments. Although only plants A-G are shown in the table, the remainder of the plants were assigned a letter, a four-number range, and a treatment in the same manner.

Plant	Range of Numbers	Salt Level
A	1-4	High
B	5-8	None
C	9-12	None
D	13-16	Low
E	17-20	Low
F	21-24	None
G	25-28	High

This random number table was used again to determine the placement of the plants in the two trays. The sequence of two-digit numbers in the table dictated the order in which plants were placed in the tray. Plants were placed one at a time into Tray 1, first filling up a row of three and then going on to the next until there were four rows of three plants each. Tray 2 was filled in the same manner (Figure 1). Plants were arranged in a staggered fashion to minimize the contact between plants and to reduce the chance of aphids migrating between plants.

### SALINE TREATMENT SET-UP

Micropipette tips were used to facilitate the watering of the plants. 96 tips were retrieved and cut at the second line from the bottom so that each tip was 4.5 cm long. This allowed water to travel through at a fast-

Tray 1		
G	W	K
Q	O	B
S	I	H
U	R	M

Tray 2		
A	X	D
L	E	V
T	J	C
F	N	P

**Figure 1:** Plant treatments (red - no salt, green - low salt, blue - high salt) and tray placement.

er rate. Four of the modified micropipette tips were placed near the edge of the container in the middle of each side. They were then pushed in at an angle approximately  $65^\circ$  from the horizontal into the soil until their tops aligned with the surface (Figure 2).

The plants were watered with a 20 mM salt solution, a 60 mM salt solution, or regular tap water depending on their treatment group. Two 500 mL solutions were created for each concentration so that plants could be watered with the same solution each time. Tap water was used instead of distilled water to ensure that the plants still received some of the minerals that are found in tap water.

The plants were watered one tray at a time. A micropipette was used to fill each of the four modified micropipette tips with tap water in all the plants in the “no salt” treatment group (i.e. the control plants) on the first tray. Each of the 4 micropipette tips were first filled with 1 mL of water, adding up to a total of 4 mL per plant. Immediately after, the same plants were watered with another 4 mL of tap water, resulting in a final volume of 8 mL of tap water in each control plant. The water was allowed to sink into the soil through the micropipette tips while the plants in Tray 2 were being watered in the same manner. This process was repeated one more time, so that in the end, each control plant had received a total of 16 mL of tap water. The process was repeated in its entirety using the 20 mM and the 60 mM salt solutions for the plants in the “low salt” and “high salt” treatments, respectively.

### INOCULATION OF *M. PERSICAE*

Every plant was first inoculated with two aphids using a metallic probe and a magnifying glass. This number was chosen to ensure that there was a sufficient number

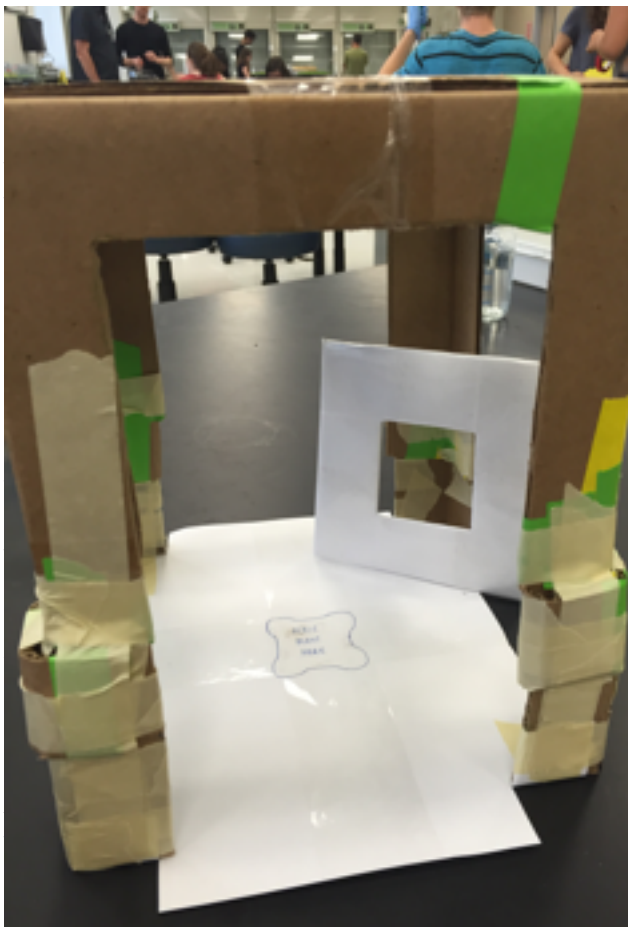


**Figure 2:** A top-view image of a plant that was taken using the quadripod. The four circles at the north, east, south, and west sides of the plant pot are the micropipette tips that were inserted at an angle of approximately  $65^\circ$  and used to water the plant.

of aphids for all 24 plants. Each plant was put on the lab bench after inoculation to avoid mixing up inoculated and uninoculated plants. After every plant had received two aphids, a second round of inoculation occurred, whereby each plant was populated in the same way with two more aphids. In the end, each plant had a total of four aphids.

### DATA COLLECTION

Front-view and top-view photos were taken of each plant for comparison throughout the study. A quadripod was built out of a cardboard box in order to ensure that the top-view photo of every plant was taken from the exact same position, height and angle each time (Figure 3). Several holes were cut out of the box to allow the maximum amount of light to reach the plant. An outline was drawn on the top of the box to ensure that the photography device (an iPhone 6) was placed in the same location for each photo. A sheet of blank paper with an outline of the bottom of the plant pot was attached to the legs of the quadripod to aid with the placement of the plant. A small piece of cardboard was used to make a frame with a white background to give sufficient contrast for the pictures. A ruler was taped to the cardboard to provide a scale for pictures. The photos of all the plants were taken in the exact same



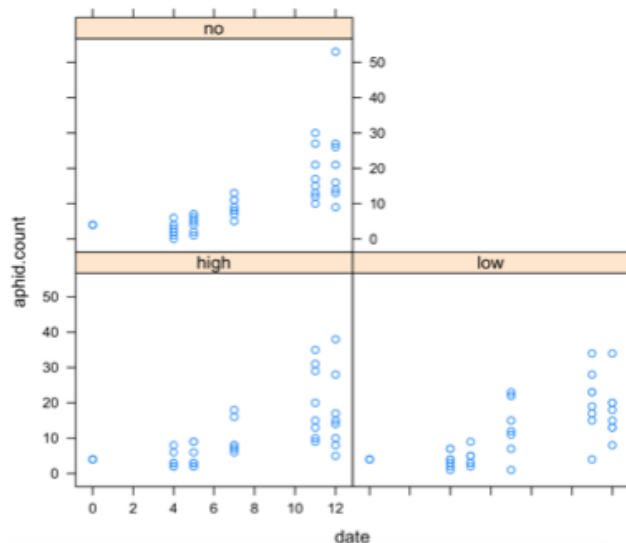
**Figure 3:** The cardboard quadripod for taking top-view images of the plants (the ruler is not present in the photo). The square frame was used to provide a white background to enable better visualization of the rosette leaves. The plant pot was placed in the outlined area on the sheet of paper and the iPhone 6 was positioned using the outline on the top of the quadripod.

visualization and analysis of the data as the effect of treatment was separated from date. A two-way analysis of variance (ANOVA) test was conducted to see whether treatment and date had a significant effect on aphid population growth and whether there was a significant interaction effect from the two variables. This interaction effect would indicate that variance in aphid populations between treatments depended on the number of days elapsed.

The aerial photos of the plants were analyzed using an image processing software, ImageJ. Using the software, the surface area of the exposed rosette leaves (in  $\text{cm}^2$ ) of each plant was calculated for every observation day. This value was used as an estimate of total plant leaf surface area. The scale of the program was calibrated using the ruler in the plant image. A boundary was drawn by hand around the plant leaves to accurately select the area for measurement. This data was analyzed using the same statistical method (a two-way ANOVA) as the aphid population data to see if treatments had an effect

on the *A. thaliana*.

Any significant results ( $p < 0.05$ ) from the two-way ANOVA analysis were further explored using a post-hoc Tukey's Honest Significant Difference (HSD) test to compare the means of the different treatment groups.

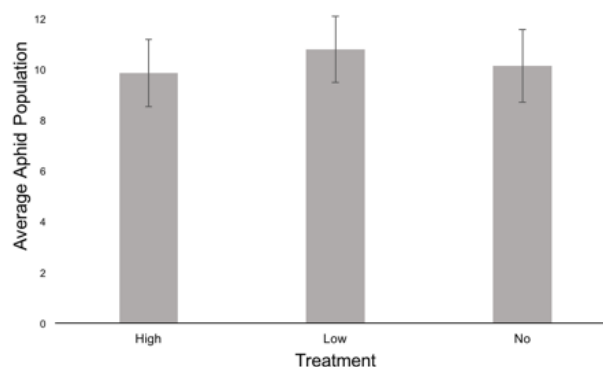


**Figure 4:** Aphid population change throughout time on plants watered with normal water, with a low-concentration salt solution, and with a high-concentration salt solution.

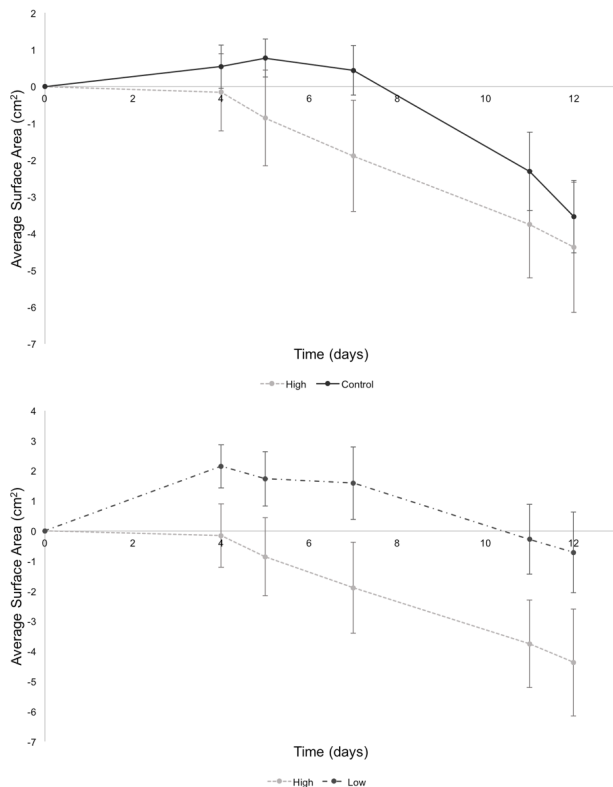
## RESULTS

### APHID POPULATION CHANGE

Within the different treatments, there were no noticeable trends with respect to aphid population growth. Both of the treatments and the control saw the aphid population increase with time, and there was no noticeable difference regarding the manner in which the populations increased (Figure 4). This suggests that the salt treatments did not have an impact on the aphid population growth. This is also evident when the average aphid population count is compared within each of



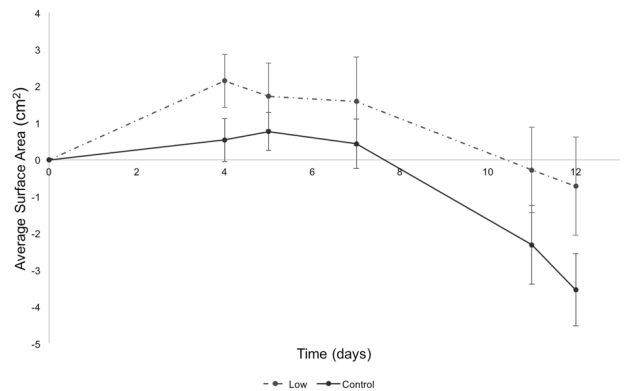
**Figure 5:** The average population of aphids on plants watered with tap water, a low-concentration salt solution and a high-concentration salt solution. The error bars represent  $\pm$ SE.



the three treatments (Figure 5). A two-way ANOVA test was conducted on the relationship between the aphid population growth and both the treatment and the date. The relationship between aphid population growth and the treatment was deemed insignificant ( $p = 0.76$ ). The only variable that was significant in relation to aphid population growth was the date ( $p < 0.001$ ). In other words, the different salt solution used to water the plants did not have any impact on the growth of aphid populations.

### PLANT SURFACE AREA CHANGE

Using ImageJ, the surface area of the exposed rosette leaves of each plant was measured for each of the days that they were surveyed. Each plant began with a unique and distinct surface area. In order to account for this difference, the surface area change of each plant through time was compared. Initially, it was difficult to notice any trends in the data collected. As well, each plant's growth and shrinkage pattern was unique, making visual analysis of the data impossible. Due to the three distinct variables (high salt, low salt, and no salt), a two-way ANOVA was used. The relationship between surface area change and treatment was



**Figure 6:** Average plant surface area change throughout time of plants watered with a high-concentration saline solution (60 mM), a low-concentration saline solution (20 mM), and tap water. (A) shows the relationship between the “high salt” treatment and the control; (B) shows the relationship between the “low salt” treatment and the control. (C) shows the relationship between the “high salt” treatment and the “low salt” treatment. The Tukey HSD test indicated that there was a significant difference between the high and the low treatments ( $p = 5.276 \times 10^{-4}$ ). All the error bars were calculated by measuring the standard error of the data points.

significant ( $p = 3.27 \times 10^{-4}$ ). It should be noted that time also had a significant effect on the plant surface area ( $p = 2.45 \times 10^{-5}$ ). However, time and treatment affected the plants independently of each other ( $p = 0.856$ ). Thus, the manner in which the date affected the surface area change did not impact the way in which the treatment affected the surface area change.

To determine which treatment had an effect on the plant surface area change, a Tukey HSD test was run (Table 2). The test revealed that there was a significant difference between the high and the low treatments ( $p = 5.276 \times 10^{-4}$ ). Interestingly, it also revealed that there was no significant difference between the control and the high salt treatment or the control and the low salt treatment ( $p = 0.1884$  and  $p = 0.09536$ , respectively).

To determine how the distinct treatments affected the plant surface area growth with respect to time, the change in surface area of all plants for each treatment was averaged for each day that data was collected. The averages of the variable interactions in Table 2 were graphed against each other (Figure 6). The plant surface area of the plants in the high salt treatment group decreased the most throughout time; however, all of the treatments had a net negative impact on plant growth

**Table 2:** Result of a Tukey Honest Significant Difference test with a 95% family-wise confidence level analyzing the differences between the impact of the salt treatments on *Arabidopsis thaliana* plants.

Variable Interactions	Difference	Lower bound	Upper Bound	Adjusted p-value
Low - High	2.497	0.9595	4.035	0.0005276
No - High	1.140	-0.3980	2.678	0.1884
No - Low	-1.358	-2.895	0.1804	0.09536

over time. There was no significant difference between the control plants and the low salt treatment group or between the control and the high salt treatment (Figure 6A and 6B). The significant difference between the low and high treatments is visible since the error bars of the graph do not overlap (Figure 6C). In other words, the three graphs support the results obtained using the Tukey HSD test.

## DISCUSSION

These results give some insight into the impact of winter road salting on both plant and aphid populations. First, this study suggests that varying saline concentrations in the soil have an impact on *A. thaliana* growth. The results of this experiment did not entirely confirm the initial hypothesis because the control group did not differ from either treatment groups. Had the hypothesis been entirely accurate, the decrease in plant surface area would have been significantly greater in the high salinity plants than in the control, which was not the case. However, the results of this study did indicate that increasing the salt concentration from 20 mM to 60 mM negatively impacted plant leaf area. Salted soils are known to impede the growth of many plant species (Qados, 2011). Increasing salinity has been shown to decrease water uptake by the roots, alter leaf osmotic potential, modify protein content, increase sodium and chloride ions in the chloroplasts, reduce the quantity of photosynthetic pigments present in the leaves, and inhibit photosynthesis (Ueda, Tsutsumi and Fujimoto, 2016; Heidari, et al., 2011; Qados, 2011; Gama, et al., 2009). This, in turn, can have a negative effect on leaf number, plant dry mass, and leaf area (Qados, 2011). Additionally, exposure to salt stiffens the epidermal cell wall of the plant (Zörb, et al., 2015). The present study indicates that *A. thaliana* may be susceptible to salt stress and that exposure to salted soils might negatively impact its fitness.

Contrary to *A. thaliana*, however, the *M. persicae* population was in no way affected by increased salinity. This rejects the hypothesis that was initially postulated. *M. persicae* feeds by sucking the phloem of the plant (Dutton, et al., 2002). If the salinity of the soil had caused a change in phloem chemical composition or availability, it should have negatively impacted the population growth of *M. persicae*. Additionally, since salt causes plants to thicken and stiffen their epidermis (Zörb, et al., 2015), it could have been possible that the aphids were unable to pierce it with their stylets and therefore died from lack of food. However, no such effect was observed.

There are a few potential reasons why the aphid population was not affected by the increase in salinity. *A. thaliana* defends itself from aphids in multiple differ-

ent ways. Its constitutive defences include trichomes and toxic antibiotics in its phloem (Louis and Shah, 2013). The plant also makes use of inductive defences such as increasing starch and glucosinolate concentration when attacked, or inducing premature death of leaves on which *M. persicae* colonies are established (Louis and Shah, 2013). While effective, these inductive mechanisms of defence are costly to the plant. It is possible that, when under stress from increased salt concentration, the *A. thaliana* would preferentially allocate resources to survival rather than defence. If this were the case, *M. persicae* would not be impeded in its feeding and the colonies would thrive. Another possibility is that although the leaf surface area decreased in the high-salinity treatment, this did not have a population-limiting effect on the aphids. During the study, the aphids seemed to aggregate preferentially on the stems and bolts, so perhaps a slight reduction in leaf area would not have caused a measurable impact on aphid habitat.

These findings carry some heavy implications with regards to the use of salts for road de-icing. Naturally high levels of soil salinity already impede plant growth in several locations around the world, and further salt addition through road de-icing only exacerbates the problem (Qados, 2011). By some estimates, salinization of arable land will have caused a 50% loss of agricultural space by 2050 (Heidari, et al., 2011). Furthermore, studies have shown that some plants are even more vulnerable to high salt concentrations when under drought conditions (Ha, et al., 2008). Given the water shortages currently devastating several regions of the world, the safekeeping of our agricultural lands is crucial. Additionally, if herbivores such as *M. persicae* are unaffected by the increase in salinity, plants will have to face the double burden of salinization and predation. This could have dire consequences on food production as well as on fragile ecosystems such as Hamilton's Cootes Paradise.

In light of these potential consequences, it seems vital to continue research in this direction. The present study had some limitations that future research could address. In this experiment, data was collected by manually counting the live aphids, which may have allowed for some errors during the count. In future studies, a more accurate way of assessing aphid population growth should be employed. This could include the use of microscopes as well as a greater number of independent counts of each plant. As well, ImageJ, the program used to calculate rosette leaf surface area, has limitations as it can only analyze images two-dimensionally. This did not account for the fact that some leaves naturally lie more flat while others grow in an upward direction. In future studies, a more accurate technique should be em-

ployed. Furthermore, the potted plants were kept very close to each other over the course of this experiment, which may have allowed for migration of the aphids from one plant to another. It is also worth noting that the plants did not all start off at the same stage of growth. Some were already bolting, while others were still in the rosette stage. In an effort to normalize the growth stage, the bolts were cut off of the more mature plants. However, this may have further impacted their growth. Additionally, the saline solutions used to water the plants were of relatively low salt concentrations. *A. thaliana* has been known to survive at saline concentrations of 100 mM (Ueda, Tsutsumi and Fujimoto, 2016). It is possible that repeating this experiment with higher salt levels would have a greater impact on the plants, thus yielding clearer results. Finally, and perhaps most importantly, the experiment only lasted for a duration of 13 days. While this allows us to gain some information on the effect of salinization on aphid populations, the salt levels that accumulated in the soil over 13 days are not entirely comparable to a season of road salt build-up. Future studies could investigate this system over a greater time period.

## CONCLUSION

This experiment demonstrated the negative effects salt has on plant growth and health. The aphid population sizes were not significantly correlated with increased salinity levels, but the leaf surface area of the *A. thaliana* decreased with increased saline treatments.

It should be noted that, although no effect on herbivores was observed, future research should be done to determine whether this is true for other herbivores and plant types. Overall, the negative impact that salt had on *A. thaliana* in this experiment provides further justification as to why an alternative method for de-icing should be researched, as protection from salinization is crucial for ecosystem preservation.

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## AUTHOR CONTRIBUTIONS

All authors contributed equally towards the gathering of background research, designing of the experiment, collection of data, and editing of the final manuscript. In terms of composing the manuscript, M.T. and A.T. wrote the abstract and A.F. focused on the introduction. M.G-A. and A.T. wrote the methods, while M.T. performed the statistical analyses, wrote the results, and produced the figures. P.B. focused on the discussion and A.F. wrote the conclusion.

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# Testing the effects of nitrogen on the interaction of *M. persicae* and *A. thaliana*

LIZ BALINT, LEENA ALSHENAIBER, NOEL KIM, ISOBEL SHARPE, MEGAN SWING

Integrated Science Program, Class of 2019, McMaster University

## SUMMARY

Natural elements serve as the building blocks of ecosystems, and cycle through the biosphere. Nitrogen is one of the most important elements for plant growth. To further increase plant growth, nitrogen is artificially added to ecosystems as fertilizer, though it may put nearby organisms at risk. The impact of fertilizer runoff affects many environments and the organisms that inhabit them. For these reasons, it is important to understand the effects of increased amounts of nitrogen on plant-animal interactions. To do so, we studied the effect of varying ammonium nitrate (AN) concentrations, a compound commonly found in fertilizer, on the interaction between *Arabidopsis thaliana* and *Myzus persicae*. The control group *A. thaliana* plants were treated with water, while low and high dose groups were treated with varying concentrations of AN. We counted the number of *M. persicae* present on each *A. thaliana* plant throughout the study period. The low dose group begins to plateau after the sixth day, while the control and high dose groups grew. These results suggest that soil nitrogen content affects trophic interactions between plants and herbivores. The optimal treatment was a low dose of AN, as population growth of *M. persicae* plateaued, limiting herbivory and potentially benefiting *A. thaliana*.

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**Keywords:** *A. thaliana*, *M. persicae*, aphid, nitrogen, soil, fertilizer, resource allocation, plant-animal interactions

## INTRODUCTION

### NITROGEN AND PLANTS

Plants are the basis of ecosystems, and changes in plant growth have a substantial effect on said ecosystems (Maron and Crone, 2006). The rapidly increasing human population has led to an increased demand for plants used for food, oil, and energy (PRB, 2016). In order to fuel this demand, agriculturalists rely heavily on fertilizers such as inorganic nitrogen, or ammonium nitrate (AN). AN is readily absorbed by plants, as it has a positive charge that can be held by the abundant negatively charged ions in soil (Liu et al., 2014; UCAR, 2016). Nitrogen is an essential nutrient for plants due to its use in metabolic processes and plant structure. By extension, increased levels of nitrogen give plants

stronger defenses against herbivory (Sohlenkamp et al., 2002). Thus, it is commonly used in fertilizers. Despite its benefits, nitrogen fertilizer can also cause environmental damage through runoff from crops to surrounding biota (Liu et al., 2014). This affects plant growth, and can eventually alter plant-animal dynamics.

### STUDY SYSTEM

*Arabidopsis thaliana*, known as thale cress, is a weed commonly used as a model organism due to its small genome and relatively short life span (Koornneef, Alonso-Blanco and Vreugdenhil, 2004). *Myzus persicae*, another model organism commonly known as the green peach aphid, is a generalist that feeds on the phloem of a wide variety of plants, including *A. thaliana* (Louis, Singh, and Shah, 2012). In general, plants employ a number of chemical and mechanical defenses to deter

this sort of herbivory. In our study system, *A. thaliana* has several defenses against the phloem-sucking herbivory of *M. persicae* (Kuśnierczyk et al., 2008).

## PLANT DEFENSE

*A. thaliana* has a variety of characteristics used to defend itself against the herbivory of *M. persicae*. Defensive responses in the plant are elicited by the saliva secreted by *M. persicae* when sucking on the phloem of *A. thaliana*, as well as the penetration of the phloem cell wall itself (Miles, 1999; Kuśnierczyk et al., 2008). Components of *M. persicae* saliva lead to the accumulation of jasmonic acid in the plant, a compound known to transduce signals of attack throughout the plant (Kuśnierczyk et al., 2008). Salicylic acid signalling pathways are also induced during herbivory, although jasmonic acid pathways are more efficient. Due to antagonistic crosstalk between the two signalling pathways, *M. persicae* can induce salicylic acid signalling responses, thereby suppressing jasmonic acid signalling (Wees et al., 2000; De Vos, Kim and Jander, 2007). During herbivore feeding,  $\text{Ca}^{2+}$  membrane channels also open, allowing areas of the sieve element membrane that were broken by *M. persicae* to be plugged by proteins (Will and van Bell, 2006; Kuśnierczyk et al., 2008). *M. persicae* then attempt to prevent these defensive responses by injecting  $\text{Ca}^{2+}$  binding proteins into the sieve element membranes, which stops *A. thaliana*'s proteins from repairing membrane damage.

## OUR STUDY

The interaction between nitrogen fertilizers and various plants has been studied, demonstrating that nitrogen addition to the soil affects some aspects of plant growth and development, but not others (Chechin and Fumis, 2004; Houle and Moore, 2008). Additionally, the interactions between *M. persicae* and *A. thaliana* have been studied extensively (De Vos and Jander, 2009; Kim and Jander, 2007). Beyond this, the impact of *M. persicae* on nitrogen metabolism in plants such as alfalfa has been explored by Girousse et al., 2005, who found that *M. persicae* infestation led to reduced nitrogen content and associated negative nitrogen deposition rates. Altered nitrogen availability influences the magnitude of response to herbivory by altering the levels of compounds such as jasmonic acid, which alert plants to biotic and abiotic stressors (Schmelz et al., 2003). However, the effects of nitrogen fertilizer on the interactions between *M. persicae* and *A. thaliana* have not been demonstrated. Subsequently, in this study we propose to investigate how varying amounts of nitrogenous compounds in the soil of *A. thaliana* affect the per capita growth rate of *M. persicae*. We used the pop-

ulation of *M. persicae* on *A. thaliana* to infer the potential effects of runoff water contaminated with nitrogen fertilizer on plant growth. We hypothesize that the *M. persicae* population will initially increase with higher concentrations of nitrogenous compounds available in the soil. Over time, we predict that *A. thaliana* will use the added nitrogen in the soil to strengthen its defenses against the increasing rates of herbivory.

## MATERIALS AND METHODS

### EXPERIMENTAL SETUP

This experiment was conducted over a 13-day period in a laboratory at McMaster University in Hamilton, Ontario during early autumn. 24 wild type (Col) *A. thaliana* plants were obtained in the rosette stage of growth. The plants received sunlight from a north-facing window and were kept at room temperature.

### TREATMENT AND INOCULATION

The *A. thaliana* plants were randomly divided into three groups of eight: control, low dose, and high dose. Two concentrations of AN were created by dissolving solid crystal AN in water, with a concentration of 60 ppm for the low dose group and 300 ppm for the high dose group, which are realistic fertilizer runoff concentrations (Kim et al., 1986). Next, 12 mL of distilled water was added to the control plants, and 12 mL of the low and high dose AN solutions were added to the respective plants, using a pie pump. The AN was dissolved in water to allow for a faster uptake of nitrogen from soil to plant. The solution was added to the control group first, then to the low group, and lastly to the high group in order to avoid contamination of solutions by the pie pump. Using a teaser needle, each *A. thaliana* was inoculated with four *M. persicae*. This is a suitable number of *M. persicae*; even if one or two insects do not survive, there is still opportunity for population growth. The plants were labelled and placed into three large plastic trays which were covered with clear plastic lids to avoid external contamination and the transfer of *M. persicae*.

### DATA COLLECTION

Data was collected on days 4, 5, 6, 7, 11, and 12 after inoculation. The number of *M. persicae* was counted for each plant by removing one pot at a time from the lidded containers to prevent any alates from escaping. To reduce human error, each plant was counted by two researchers, and the mean of the two counts was used for analysis. On each of these days, we also noted whether the plant was bolting and the number of leaves beginning to yellow per plant. Photographs of each of the



plants were also taken.

## STATISTICAL ANALYSIS

All analyses were done using the statistical software, R (R, 2015). An analysis of covariance (ANCOVA) was performed on the mean *M. persicae* count, the treatment group (control, low dose, high dose), with a continuous predictor of time (measured in days). An interaction term between treatment and day on the mean *M. persicae* count was also analyzed in the ANCOVA. Plant H6 from day 12 was an outlier and removed from analysis.

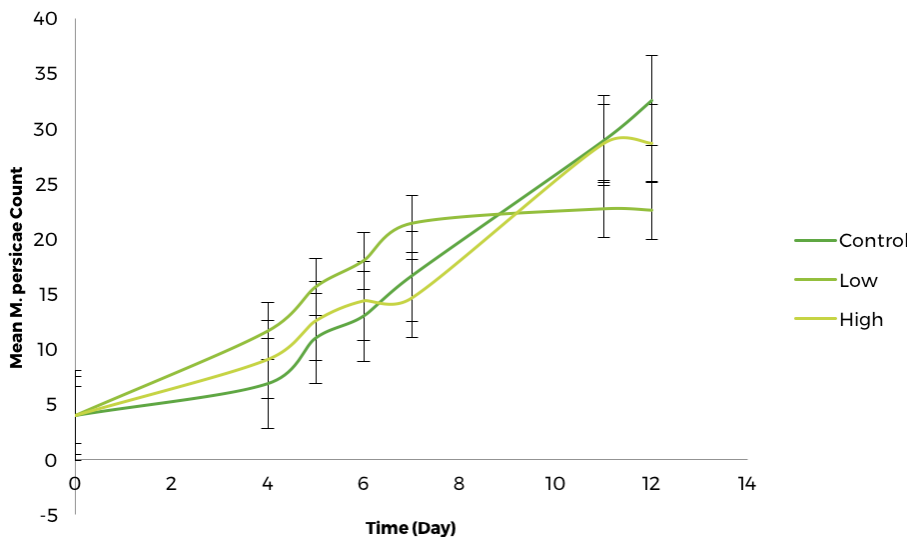
**Table 1: Summary of ANCOVA for the effect of treatment, day, and the interaction between treatment and day on mean *M. persicae* count over a 13-day period.**

Mean <i>M. persicae</i> count				
	Df	Mean Sq	F value	Pr(>F)
Treatment	2	9.5	0.1160	0.89060
Day	1	6653.2	84.4438	1.454e-15
Treatment:Day	2	430.8	5.2731	0.006214
Residuals		11191.6	81.7	

## RESULTS

The effect of time on mean *M. persicae* count was statistically significant ( $p < 0.05$ ; Table 1). Furthermore, the interaction term between treatment and day was statistically significant (Table 1). The treatment as the sole factor did not have a statistically significant influence on the mean *M. persicae* count (Table 1).

For the first four days of the experiment, the number of *M. persicae* increased over time (Figure 1). The low dose increased at the highest rate, then the high dose, then the control. At 5 days, there was a small peak in the mean *M. persicae* count for all treatment groups. After 6 days, the mean *M. persicae* count plateaued for the low dose treatment group, while the control and high dose group continued to increase.



**Figure 1: Mean *M. persicae* count over the course of 12 days for plants with no nitrogen added to the soil (Control), plants with 60 ppm ammonium nitrate (AN) added to the soil (Low), and plants with 300 ppm AN added to the soil (High). Error bars represent standard error.**

## DISCUSSION

Our results suggest that the effectiveness of plant defenses may change based on the concentration of AN added to the soil. Based on our findings, the addition of low doses of AN to the soil of *A. thaliana* can be beneficial when limiting herbivore populations. However, adding higher doses of AN can increase herbivore populations. During the first four days of the study period, the control, low dose, and high dose groups all experienced an increase in mean *M. persicae* count over time (Figure 1). These trends suggest the beginning of an exponential population growth due to back-and-forth competition between the herbivore and plant (Ayala et al., 1973).

On the sixth day, the mean count for the low dose group began to plateau, which demonstrates the potential benefit of a low dose of AN (Figure 1). Uptake of nitrates occurs in the roots of the plant. Here, various transporters move the nitrates from the apoplast of root epidermal and cortical cells, located outside of the plasma membrane of plant cells, into the symplast, located inside the plasma membrane (Sohlenkamp et al., 2002). Nitrogen is an essential nutrient to plants, as it is an important structural component and is essential for metabolic processes, including photosynthesis. By extension, increased levels of nitrogen allow *A. thaliana* to have stronger defenses against herbivory. Our results suggest that the low dose of AN gave *A. thaliana* the extra resources needed to strengthen its defenses, preventing the population of *M. persicae* from growing larger (Kuśnierczyk et al., 2008). Stout, Brovont and Duffey (1998) found a similar result. They reported that low levels of AN addition to the soil produced larger increases in phenolic levels in tomatoes compared to plants with high levels of AN addition. Phenolics are a secondary metabolite that plants release in response to stress. Overall, the plateau in our low dose group rep-

resents the ability of *A. thaliana* to strengthen its defenses against herbivores using AN from the soil.

The benefits of a low dose of AN to the plant also aligns with the theory of resource allocation. All plants must use their resources, including nitrogen, for three main functions: reproduction, defense, and growth, which all interact and compete for resources (Bazzaz et al., 1987). These three functions interact and compete for resources. Allocation to these functions varies by individual and over time, as well as among populations and species (Bazzaz et al., 1987). In the case of our study system, we may see the plateau for the low dose group after the sixth day because the plant has had the chance to react to the herbivory of *M. persicae* and allocate more resources to defense. To confirm this theory, future studies should measure plant biomass and reproductive rates. As demonstrated by Pratt et al. (2005), plants damaged by herbivory will have lower rates of reproduction than undamaged plants. In the case of *M. quinquenervia*, the paperbark tea tree, the undamaged plants were 36 times more likely to reproduce than the plants damaged by herbivory. This points towards resource allocation for defenses, taking away energy from reproduction and growth.

The control group population continued to increase for the duration of the experiment, which suggests the beginning of exponential population growth over our short experimental time period (Figure 1). The high dose group also followed this trend, suggesting that high amounts of AN are not beneficial in promoting the defenses of *A. thaliana* against herbivores. In accordance with this result, Minkenberg and Fredrix (1989) show that leafminers, namely *Liriomyza trifolii*, prefer to feed on plants that were fertilized with higher levels of nitrogen, suggesting that the plant is more attractive to herbivores like *M. persicae*. Other studies have found similar results (Chen, Olson and Ruberson, 2010). In the context of our study system, the increase in attractiveness along with nitrogen concentrations may account for our observed results in the high dose group. Although the *A. thaliana* in the high dose group may have been more attractive to the herbivores, the plateau in the population of *M. persicae* suggests that *A. thaliana* may have exhibited increased defenses. These counteracting forces provide an explanation for the trends observed in our results for the high dose group, which were similar to the control group. Additionally, high levels of nitrogen may have allowed *A. thaliana* to grow larger and thus be able to support more *M. persicae*. However, in our study we did not directly measure plant growth. Further studies should measure growth rate and foliar biomass of *A. thaliana* to determine if increased attractiveness is directly due to increased nitrogen concentrations, or indirectly due to increased

foliar biomass resulting from an increase in nitrogen concentrations.

Despite our promising results, there are several factors that may have altered our data. The *A. thaliana* plants available for this experiment were not of consistent size, age or health, representing a confounding variable. For example, some of the plants entered the bolting stage earlier than others, and based on our observations, there were higher numbers of *M. persicae* on bolting plants than on rosettes. Additionally, since all plants in one treatment group were kept in the same lidded container, there was a possibility of winged alates traveling between plants. Alates may have developed due to lack of space and changes in quality of the host plant (Dixon, 1977). To reduce this error, all plants could have been kept in separate containers. Another source of error is the fact that for the low and high dose groups, we added AN to the soil but did not measure concentrations of nitrogen in the soil itself. These values can be different from one another, as Mulvaney, Khan and Ellsworth (2008) found that addition of AN fertilizer to the soil decreases amounts of natural soil nitrogen. This is because when nitrogen is added to the soil, it can undergo changes in its availability to plants. From this study, we can apply our findings on the effect of nitrogen on aphid population growth to surrounding ecosystem. For example, trophic levels may be affected by nitrogen fertilizers through tri-trophic interactions. This involves the effects of plants, herbivores, and herbivore predators on one another. Nitrogen can affect nutrient availability to plants, which is a bottom-up force that involves the removal of a primary consumer or producer. This bottom-up force can either increase or decrease plant populations (Capinera, 2008). Additionally, this can alter animal community composition, influencing herbivores. This was observed in our study through the interaction between the nitrogen fertilizer treatment and the change in the population of herbivores. Herbivores are also influenced by a top-down force by their predators, carnivores (Capinera, 2008). Carnivores are affected by the populations of their prey, which we know from our study are influenced by the interaction between nitrogen availability for plants, and time (Capinera, 2008). Therefore, all three trophic levels are affected by the addition of nitrogen fertilizer in plants showing the ecological impact of our study and further studies involving nitrogen fertilizers in plants.

Overall, this study provides greater insight into the interactions between plants and animals. Through a controlled laboratory experiment, we found that the addition of ammonium nitrate to the soil of *A. thaliana* has a significant effect on *M. persicae* population growth over time. These findings can be used to better understand

the potential impacts of nitrogen fertilizer runoff on surrounding ecosystems. In addition, our research can be used by future studies as an analogous experiment to apply over a longer time period to analyze the long-term effects of nitrogen on interactions between plants and animals. Further studies must be done to understand all effects of nitrogen fertilizer on the interaction of *A. thaliana* and *M. persicae*, as well as interactions between other plants and herbivores in susceptible environments.

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## AUTHOR CONTRIBUTIONS

L.A. contributed to the introduction and discussion. N.K. contributed to the methods and results and performed the statistical analysis. L.B. helped write the introduction, methods, and results and performed the statistical analysis. I.S. wrote the discussion section and the methods for treatment and inoculation. M.S. contributed to the introduction, abstract, and methods sections. All authors contributed equally to experimental design, data collection, and editing.sdfdf

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# Effects of anthropogenic acetylsalicylic acid contamination on ecological interactions

ANGELA DITTRICH, JOSEPHINE AGUECI, TAYLOR LUU, MEGH RATHOD, NADIA AL HASHEMI

Integrated Science Program, Class of 2020, McMaster University

## SUMMARY

Pharmaceutical soil contamination has become a rising concern based on its potential impacts on ecological interactions. Acetylsalicylic acid (ASA), also known as Aspirin, is a commonly used medication that has been found to have an impact on plant defence by enhancing salicylic acid immunity pathways. This study aimed to determine the impact of varying ASA soil concentrations on the population growth of *Myzus persicae* on genotypically different strains of *Arabidopsis thaliana*. Additionally, this study analyzed how varying ASA soil concentrations impact the plant growth of *A. thaliana*. Two ascension lines of *A. thaliana* were used in this experiment: the wild-type Col line and the mutant salicylic acid induction-deficient *sid2*. Each plant was treated with one of four aqueous ASA concentrations (0M, 1.1nM, 11nM, 0.2mM). Overall, *sid2* plants experienced a negative mean aphid population growth rate relative to the Col plants. It was concluded that neither genotype nor ASA concentration had a significant impact on the population growth of *M. persicae*. Interactions between genotype and ASA concentrations were found to be significant for both aphid population and bolt height responses. A greater reduction in aphid population was observed as ASA concentration was increased for both genotypes, while an inverse relationship was observed between ASA concentration and bolt height growth. There were no observable effects of bolt height on *M. persicae* population growth. Overall, investigating the interactions between *M. persicae* and *A. thaliana* in the presence of acetylsalicylic acid provides further evidence for the ecological impact of pharmaceuticals, and the importance of minimizing this contamination.

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## INTRODUCTION

For centuries, humans have been altering their surrounding environment in detrimental, and often irreversible, ways. The anthropogenic contributions to climate change have directly and indirectly impacted ecosystems by altering pH levels, temperature, climate, humidity, salinity, and nutrient availability in soil and water. One major concern that has become prevalent in the last decade is the introduction of pharmaceuticals to the natural environment (Association of Metropolitan

Water Agencies, 2008). It is estimated that pharmaceutical use continues to rise 10 to 15 percent annually in North America (Health Canada, 2012). This increase, combined with over-prescription and improper disposal of medication, results in increasing chemical contamination of lakes and rivers via direct runoff or through human excretion (Crowe, 2014). While a wide variety of studies have observed and analyzed the effects of antidepressants (Metcalf, Miao, Koenig and Steuger, 2003; Schultz et al., 2010; Lajeunesse et al., 2012) and contraceptives on the endocrine systems

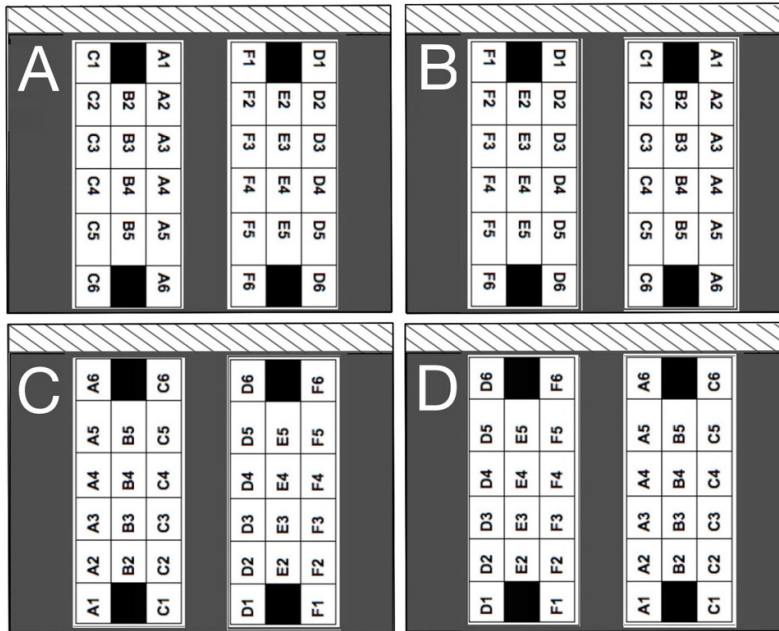
of aquatic organisms such as fish and frogs (Park and Kidd, 2005; Kloas et al., 2009; Runnalls et al., 2013; Orlando and Ellestad, 2014), the impacts of pharmaceutical absorption by plants is severely understudied. Many plants rely on inducible chemical pathways as a defence mechanism, so altering the presence or concentration of various compounds even slightly could have major consequences (War et al., 2012). Thus, it is crucial that the effects of common medications on ecological interactions is examined to quantify the magnitude of these consequences. An investigation was conducted on the impacts of soil contaminated with acetylsalicylic acid on plant-animal interactions. Commonly known by the brand name Aspirin, this nonsteroidal anti-inflammatory drug is a derivative of salicylic acid (SA), a signalling molecule that plays an important role in the plant immune system via SA-inducible defence pathways (Clissold, 1996). Salicylic acid acts as a stress messenger and has been linked to resistance against biotrophic pathogens and promotion of Systemic Acquired Resistance (SAR), which 'primes' plants following an attack, ensuring faster and more effective defence responses against a second attack. (Conrath, 2006, Morkunas; Mai and Gabrys, 2011). Another major plant defence is the jasmonic acid (JA)-inducible pathways, which also involve the use of JA molecules as messengers that activate certain genes to address predation. However, unlike the SA pathways, JA pathways have been observed to be an effective response to stressors such as necrotrophic pathogens and herbivorous insects (Conrath, 2006). The presence and activation of both pathways within an individual plant results in crosstalk in an attempt to optimize resource allocation and employ the most effective defence response respective to the predator. However, it is evident in the following studies that it has been observed that SA and JA pathways share an antagonistic relationship when confronted by certain biotic stresses. Although JA-induced mechanisms are more effective against predators such as generalist aphids, the SA signalling pathway has been observed to suppress the JA pathway by means that are still unclear. Leon-Reyes et al. (2010) concluded that SA-mediated suppression of the JA signalling pathway targeted regions downstream of the JA biosynthesis pathway. Observations have also been provided towards SA-mediated suppression of JA-inducible genes (Kourneef et al., 2008). Nevertheless, suppression of the JA signalling pathway has provided evidence for the secretion of SA-inducible compounds by green peach aphids such as *Myzus persicae*, in order to upregulate the less effective plant defence mechanism (Louis and Shah, 2013). Interest in crosstalk between the SA and JA signaling pathways has led to the experimental use of ascension lines deficient in one of the mechanisms (Nishimu-

ra and Dangl, 2011). In the present investigation, the Columbia (wild-type) ascension possesses functional SA and JA pathways, while the *sid2* ascension possesses a mutation to the SA-induction deficient 2 gene in *Arabidopsis thaliana*. This gene codes for isochorismate synthase, an enzyme which plays a crucial role in SA biosynthesis (Ghazijahani et al., 2014). Mutation of this gene results in plants with a reduced ability to accumulate SA, and thus enhanced susceptibility to pathogens and environmental stresses. Salicylic acid (SA) is one of the most readily available plant growth regulating materials, and is also effective in other forms such as methyl salicylate (Ghazijahani et al., 2014).

The objective of this study was to address the following two questions: (1) How do varying ASA soil concentrations impact the population growth of *M. persicae* on *A. thaliana* across different plant genotypes? (2) How do varying ASA soil concentrations impact the growth and overall quality of *A. thaliana*? The corresponding null hypotheses were the following: (1) ASA concentration and genotype do not affect aphid population. (2) ASA concentration does not impact the growth and overall quality of *A. thaliana*.

## MATERIALS AND METHODS

This experiment was conducted over a span of 12 days in an indoor facility at McMaster University in Hamilton, Ontario, from September 21st to October 3rd, 2017. All 32 *A. thaliana* plants (16 *Col* and 16 *sid2*), were in rosette or early bolting stage. The average lifespan of *A. thaliana* is 60 days (Serino and Gusmaroli, 2011), so selecting plants in these stages minimized flowering and plant death prior to the completion of the experiment. Plants were divided into two separate groups based on ascension line (*Col* or *sid2*), with four different ASA treatments within each ascension line (0M, 1.1mM, 11mM, 0.2mM). The justification for the four concentrations were: 0M was the control to observe response variables without exposure to ASA, 1.1mM and 11mM were the lower and upper bounds of ASA concentrations that have been recorded in the environment (Cleuvers, 2004), and 0.2mM was a physiologically active concentration observed to induce plant immune responses (Senaratna et al., 2000). A double tray apparatus was used to minimize cross contamination of different water treatments between neighbouring plants. The trays were structured with a solid plastic tray on the bottom, and a permeable tray layered overtop to allow for drainage of water and the solute it may contain. A gap between the trays was created by folding paper towels and wedging them along the perimeter of the two trays creating separation. Each tray corner was labelled, creating a standardized grid system to define the location of each plant and the varying orientations of the trays



**Figure 1:** Initial experimental setup. The *Col* tray contained plants A1- C6, while the *sid2* tray contained plants D1- F6. The initial setup on Day 0 (A) had the *Col* tray to the west of the *sid2* tray, with the “1” side of the tray against the north window ledge as illustrated by the striped area. The trays were switched on Day 5 (B) with the “1” side of the tray against the north window ledge but the *sid2* tray to the west of the *Col* tray. The trays were switched on Day 5 (B) with the “1” side of the tray against the north window ledge but the *sid2* tray was placed to the west of the *Col* tray. On day 8 (C), the “6” side of the tray was placed against the north window ledge with the *Col* tray to the west of the *sid2* tray. On day 11 (D), the “6” side of the tray was placed against the north window ledge with the *sid2* tray to the west of the *Col* tray.

throughout the experiment. Trays were re-oriented on days 5, 8, and 11, to eliminate any environmental bias (see Figure 1). A number generating software (Haahr, 1998) was implemented to randomly assign a location and ASA concentration to each plant.

## PREPARING TREATMENT SOLUTIONS AND INOCULATION WITH APHIDS

The ASA solutions were prepared from 100 g of 99% powdered acetylsalicylic acid and distilled water on a VMR stirrer. 10 drops of MeOH were added to ensure solubility in water. 15 millilitres of the corresponding SA solutions were exogenously delivered to the plant via soil drenching. The plants were first watered on Day 0 with respective solutions and twice more throughout the duration of the experiment (Day 4 and Day 11). On Day 4, three aphids were inoculated from pre-existing *A. thaliana* plants onto the rosette leaves of each of the 32 test plants. The aphids were all in an intermediate development instar stage and were reared on a wild type (*Col*) *Arabidopsis thaliana*. Rearing them on *A. thaliana* ensures the aphids were not starved prior to inoculation, avoiding any malnourishment. As well, during the investigation, the aphids were subjected to the same conditions by maintaining spatial and temporal proximity of the population. Following the inoculation, aphid numbers were counted for the duration of the experiment by inspection with a probe and magnifying glass. Bolt height was measured on days 0, 4, 8, 11, and 12, as a quantitative factor coupled with qualitative observations, serving as a proxy for plant quality.

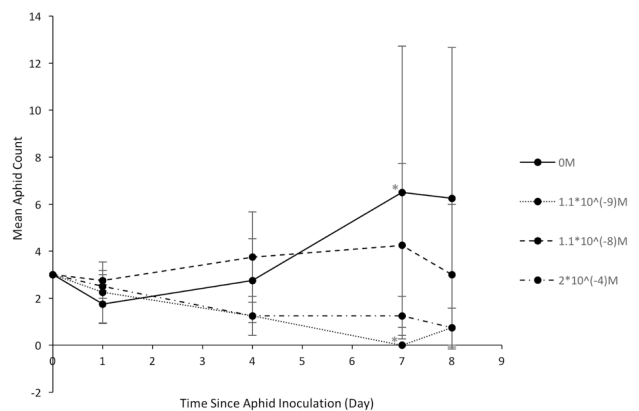
## STATISTICAL ANALYSIS

The effects of genotype and ASA concentration on aphid population growth were assessed with an ANCOVA, followed by a post hoc Holm’s test. Time,

measured in days, was interpreted as a continuous independent variable. The effects of genotype and ASA concentration on bolt height growth were also assessed with an ANCOVA followed by Holm’s test. Possible block effects arising from growth of *Col* and *sid2* plants in different trays were included in the above analyses. The potential impacts of bolt height on aphid population growth were assessed using a linear regression model. All analyses were carried out using R version 3.3.2 (R Development Core Team, 2016).

## RESULTS

### APHID POPULATION GROWTH



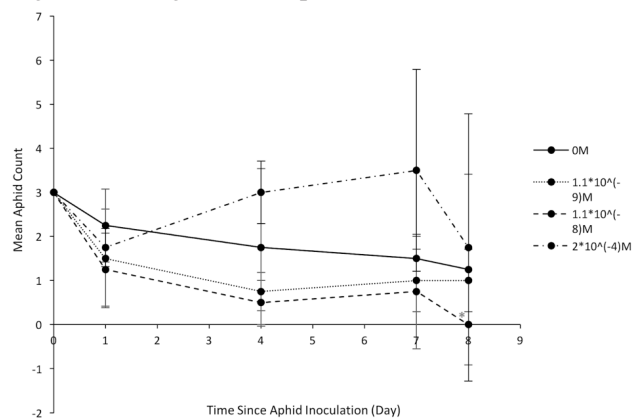
**Figure 2:** Mean Aphid Count of *Col* for Four Concentrations over Eight Day Period. Control = 0M, Low = 1.1nM, Intermediate = 11nM, High = 0.2mM. Error bars show standard deviation (<0.95 interval).

Each *A. thaliana* plant used in this experiment was exposed to one of four ASA concentrations: 0M (control), 1.1nM (low), 11nM (intermediate) or 0.2mM (high). Among all eight combinations of genotype and treat-

**Table 1: Three-Way Analysis of Covariance of the Effect of Genotype and ASA Concentration Difference on *Myzus persicae* Population Growth Across Eight Day Test Period. \* indicates a significant p-value.**

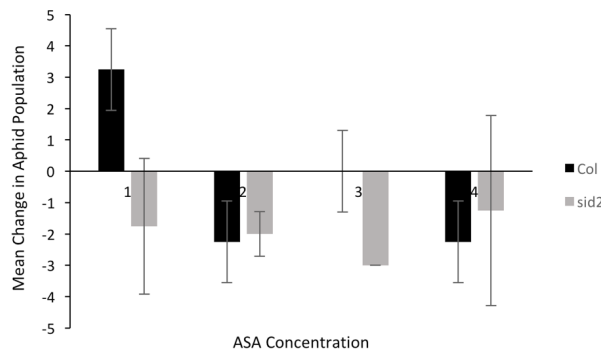
Source	d.f.	Mean Square	F Value	Pr(>F)
Plant x Treatment x Day	3	20.7156	4.7946	0.003255*
Treatment x Day	3	13.2290	3.0618	0.030168*
Plant x Day	1	12.4256	2.8759	0.092078
Plant x Treatment	3	23.7750	5.5026	0.001318*
Day	1	8.2656	1.9130	0.168766
Treatment	3	16.0417	3.7128	0.013064*
Plant	1	30.6250	7.0880	0.008643*
Residuals	144	4.3207		

ment, the control *Col* plants experienced the only net increase in mean aphid count over the test period, peaking at a mean of  $6.50 \pm 6.22$  aphids on Day 11 (Figure 2). This peak is significantly different ( $P < 0.05$ ) from only two other treatment combinations (low *Col* on Day 11, d.f. = 3,  $p = 0.039$ ; and intermediate *sid2* on Day 12, d.f. = 3,  $p = 0.039$ ) (Table 1). These were the only significant differences found with interaction between genotype, treatment, and day. The high *sid2* plants also had a similar trend, exhibiting the largest increase among all *sid2* treatments and peaking at  $3.50 \pm 2.29$  aphids on Day 11 (Figure 3). The high and low *Col* plants exhibited a gradual decrease in mean aphid count, while the intermediate *Col* plants gradually increased over the test period, but had no overall change. However, for *sid2*, mean aphid count decreased from Day 4 to Day 12 for all concentrations without a significant difference between one another, suggesting that they were similarly negative during this time period.



**Figure 3: Mean aphid count of *sid2* for four ASA concentrations of eight day period. Error bars show standard deviation (<0.95 interval).**

Additionally, observations were made regarding overall change in aphid count from Day 4 to Day 12 (Figure 4). The control *Col* experienced the only increase observed among all treatments and genotypes with a mean increase of  $3.25 \pm 6.42$  aphids, approximately double that of the *sid2* decrease. The low concentration group ex-



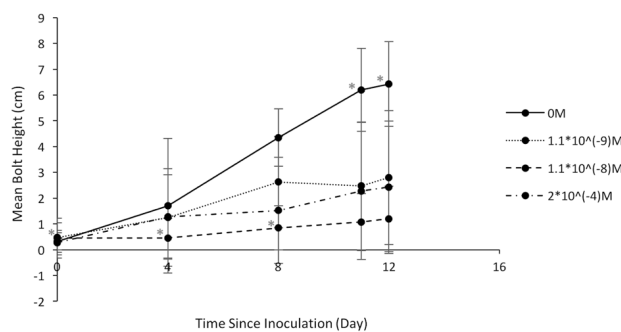
**Figure 4: Mean Change in Aphid Count of *Col* and *sid2* Plants at Various ASA Levels over Eight Day Test Period. '1', '2', '3', and '4' represent OM, 1.1nM, 1.1nM and 0.2mM. Error bars show standard deviation (<0.95 interval).**

pressed a similar overall decrease in mean aphid count in both the *Col* and *sid2* plants of  $-2.25 \pm 0.83$  and  $-2 \pm 0.71$ , respectively. The intermediate concentration group exhibited  $0 \pm 3.00$  in mean aphid population for *Col* plants and a  $-3 \pm 0.00$  change in aphid population count in the *sid2* plants, the largest decrease observed among the genotypes and treatments. The high *Col* and high *sid2* plants expressed a change in aphid count of  $-2.25 \pm 0.83$  and  $-1.25 \pm 3.03$ , respectively. No significant differences were found in the overall change in mean aphid count between genotypes for any treatment (d.f. = 3,  $p = 1.000$  for almost all of differences in mean) (Table 2).

**Table 2: Significant interactions ( $P < 0.05$ ) between genotype and ASA concentration on aphid population count across eight day period since ASA inoculation**

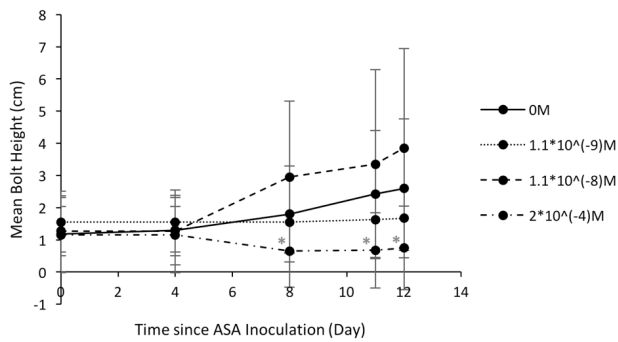
Genotype. Conc.Day	Sid. high.8	Col. control.11	Sid. control.11	Col. low.11	Sid. low.11	Col. int.11
Col.low.11	1.000	0.039	1.000	-	-	-
Sid.int.12	1.000	0.039	1.000	1.000	1.000	1.000

## BOLT HEIGHT

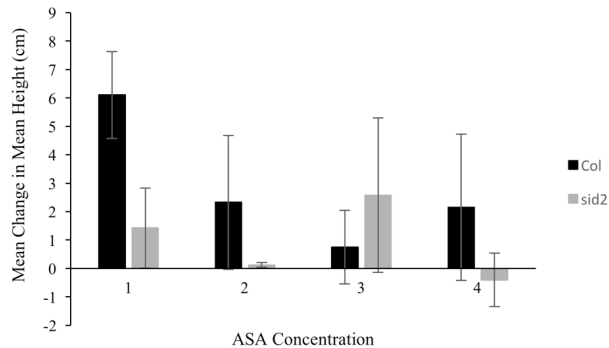


**Figure 5: Mean bolt height of *Col* plants for four treatments for 12 days since initial inoculation with ASA. T0 = 0M; T1 =  $1.1 \times 10^{-9}$ M; T2 =  $1.1 \times 10^{-8}$ M; T3 =  $2.0 \times 10^{-4}$ M. Error bars show standard deviation (<0.95 interval).**

Interactive effects between genotype and concentration on mean bolt heights of *Col* (Figure 5) and *sid2* (Figure 6) plants were observed on specific days over the 12-day test period (Figure 7). All *Col* ASA concentra-



**Figure 6:** Mean bolt height of *sid2* plants for 4 treatments over 12 days from initial ASA inoculation. Error bars show standard deviation (<0.95 interval).



**Figure 7:** Mean change in plant bolt height of *col* and *sid2* plants at various ASA levels over 12-day test period. '1', '2', '3', and '4' represent 0M, 1.1nm, 11nm and 2.0µm. Error bars show standard deviation (<0.95 interval).

tions exhibited positive bolt height growth, while *sid2* exhibited varying trends across different ASA concentrations. Control *Col* plants demonstrated the greatest mean bolt height growth compared to the three other ASA concentrations, with a steady increase ending at 6.43±1.64cm. Bolt height means on Day 11 and Day 12 for control *Col* were significantly different from Day 0 means for all treatment groups (Table 3; d.f. = 3, p = 0.0109, p = 0.0094, p = 0.0159, and p = 0.0170, from control to high respectively). Control *Col* on Day 12 was also significantly different compared to high *sid2* on Day 8 (P<0.05, d.f. = 3, p = 0.0283) and Day 11 (d.f. = 3, p = 0.0304). Bolt height growth for low and high *Col* were similar in both progression and final heights of 2.8±2.60cm and 2.43±2.56cm, respectively, while intermediate *Col* demonstrated the smallest bolt height growth (1.20±1.27cm). In addition, *Col* exhibits greater bolt growth at all ASA concentrations compared to *sid2*, except at intermediate concentration, where *sid2*

**Table 3:** Significant interactions (p < 0.05) between genotype and ASA concentration on bolt height across eight-day period

Genotype. Conc.Day	Col. cont.0	Col. high.0	Col. mod.0	Col. low.0	Col. mod.4	Sid. high.8	Col. mod.8	Sid. high.11	Sid. high.12
Col.control.11	0.0212	0.0183	0.0304	0.0325	0.0304	-	-	-	-
Col.control.12	0.0109	0.0094	0.0159	0.0170	0.0159	0.0283	0.0498	0.0304	0.0375

mean growth is approximately three times greater. At high ASA concentration, the only overall decrease in bolt height is observed in *sid2* plants, while *Col* bolt growth is similar to low ASA concentration.

The control and intermediate *sid2* plants demonstrated increasing bolt height growth rates, with that of the intermediate plants being at a steeper incline. Almost no change in mean bolt height was observed at low *sid2*. The notably small standard deviation (0.125±0.083cm) suggests the presence of a threshold of effect of ASA concentration on bolt height. Interestingly, all *sid2* ASA concentrations had very similar growth rates from Day 0 and Day 4, after which the different treatments diverged with rates that were not significantly different (d.f. = 3, p = 1.0000). Most importantly, bolt height growth appeared to share an inverse relationship with ASA concentration among both *Col* and *sid2* plants.

### HEIGHT ON APHID POPULATION GROWTH

The intercept of the linear regression plot of bolt height against aphid population was significant (Table 4; p = 5.82e-15) while the slope was not (d.f. = 1, p = 0.828) (Figure 8).

**Table 4:** Linear regression model of the effect of height on aphid population for all genotypes and treatments. \* indicates a significant p-value.

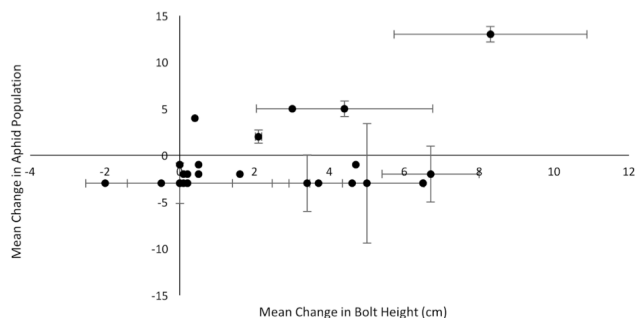
Source	d.f.	Estimate	Pr(> t )	F	Adj. R <sup>2</sup>
Intercept		2.07448	5.82e-15*	0.04741	-0.007679
Height	1	-0.01722	0.828		
Residuals	124				

### DISCUSSION

#### APHID POPULATION

Control *Col* exhibited the greatest overall increase in *M. persicae* populations, peaking on Day 11 (Figure 2 and Figure 3). This was contrary to the expectation that control *Col* would experience the greatest decrease in aphid population compared to all other *Col* treatments, as the plants had the smallest degree of crosstalk between SA and JA, due to the absence of upregulation of SA by ASA. Evidence from other studies suggests that SA-inducible pathways do not play an important role in addressing aphid feeding stress. For example, mutant *A. thaliana* such as *sid2* and *npr1*, which have a loss of key SA regulator and receptor NPR1 (non-expressor of PR genes 1), have unchanged aphid population growth compared to wild-type (Moran and Thompson, 2001;



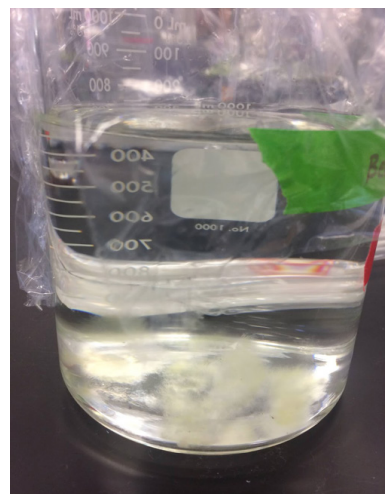


**Figure 8:** Relationship between mean change in bolt height and mean change in aphid population across all genotypes and ASA concentrations

ticeable increase in aphid population numbers on control *Col* conflict with the above evidence. The absence of ASA in control *Col* should favour SA pathways to the smallest degree among all *Col* treatments and thus result in reduced aphid populations. Although significant differences in aphid population count were only found between control *Col* on Day 11, and low *Col* and intermediate *sid2* on Day 11 and Day 12 (d.f. = 3,  $p = 0.039$ ; d.f. = 3,  $p = 0.039$ ), evidence for interaction between genotype and ASA concentration demonstrated by the trend of control *Col* may suggest a greater degree of effectiveness of SA-inducible pathways against aphid feeding than previously believed. Conflicting observations have been made on the exogenous application of SA derivatives on aphid settling and growth; application of synthetic analogs of SA has no impact (Moran and Thompson, 2001), while the SA analog benzothiadiazole has resulted in decreases in aphid reproduction (Morkunas, Mai, and Gabriš, 2011). Furthermore, several SA-related pathways have been identified as effective defences against aphid feeding. For example, the regulatory gene *PAD4* (phytoalexin deficient4) is one of the genes responsible for inducing SA synthesis, as well as activating a defence pathway that adversely impacts aphid settling and fertility (Wiermer, Feys, and Barker, 2005). In turn, SA molecules enhance *PAD4* expression, resulting in a positive feedback loop. Thus, it is suggested that SA-inducible pathways, while possibly less effective than counterpart JA pathways, may still play an important role in reducing aphid population numbers. This is supported by the low, intermediate and high treatments of both genotypes - excluding high *sid2*, which exhibited only decreases in aphid population - which suggest a more effective plant defence response to aphid feeding with greater exposure to ASA (Figure 3). The lack of significant differences between *Col* and *sid2* suggests that they were similarly effective in plant defence, and that exogenous exposure versus no exposure to ASA impacts plant response more greatly than degrees of ASA concentration. This may also suggest

that addition of ASA via soil drenching may be an effective substitute for SA in the absence of endogenous SA.

Support for a greater role for SA-inducible pathways is contradicted by observations made with high *sid2*. Exposure to the highest ASA concentration in *sid2* was expected to yield the highest aphid population numbers among all *sid2* plants due to greatest suppression of JA. However, the small mean aphid population on high *sid2* might be explainable by error. From Day 11 to Day 12, all genotype and treatment combinations experienced a decrease in aphid population, excluding a small increase observed among low *Col* that is likely a consequence of aphid migration. This shared decrease may be due to contamination of the ASA solutions produced on Day 0 and used throughout the experiment for soil drenching. A contaminant must have been introduced, as the development of an unidentified biological contaminant was observed in the high ASA concentration beaker on Day 11, thus contamination of the other solutions may have been unnoticeable to the naked eye. The contaminant was observed to be cloudy yellow-white in colour, irregular in shape, and filamentous (refer to Figure 9). This common decrease may have also arisen from the introduction of predators into the environment. Aphid predators were first noticed on Day 8, and could have potentially been responsible for any drops in aphid count observed throughout the experiment. Thus, had the experiment ended on Day 11 rather than 12 due to significant impact by error, the trend of mean aphid count on high *sid2* would have become an overall increase, suggesting that upregulation of SA-inducible pathways adversely impacts plant defence. However, this appears to be an outlier trend and may suggest the presence of a threshold ASA concentration at which ASA exposure changes from positively to negatively impacting plant defence.



**Figure 9:** Unknown contamination observed in the high ASA concentration beaker on Day 11.

Consideration of more ASA concentrations within the experimental setup of a future study may make these trends more discernable, and provide more accurate observations regarding the importance of the SA signalling pathway. This would be aided by the inclusion of an ascension line possessing a non-functioning JA signalling pathway, in order to further investigate the impacts of JA-SA crosstalk.

Interestingly, mean aphid population decreased on all plants between the first and second day since aphid inoculation, irrespective of genotype or ASA concentration. This was believed to arise from stress placed on the aphids as they were introduced to a new environment and each of the plants were watered with the appropriate ASA solution.

### BOLT HEIGHT

There is an apparent inverse relationship between ASA concentration and change in bolt height for both genotypes, supported by all treatment combinations except intermediate *Col* and *sid2*. At higher ASA concentrations, reduced bolt height growth was observed. While significant differences were only found between control *Col* on Day 11 and Day 12, and the other three ASA concentrations of *Col* and high *sid2* (Table 4), this trend was expected. SA plays an integral role in plant senescence and cell death, and exogenous application of SA causes programmed cell death (PCD) and early senescence in association with SAR (Vogelmann et al., 2012) (Brodersen, 2005). Furthermore, SA is involved in regulation of flowering time, and in response to both biotic and abiotic stresses, high SA endogenous levels are observed in plants that transition earlier from bolting to flowering to increase chances of population survival (Martinez et al., 2003) (Lyons et al., 2015). This furthers the notion that SA promotes plant resource reallocation, diverting resources from increasing biomass to processes involved in reproduction, thus increasing the overall survival of the population rather than the individual. The outlier bolt height trend of intermediate *sid2* can be explained by the large standard deviation of  $2.575 \pm 2.712$ cm. The sole significant differences between control *Col* and four other treatment groups, ASA concentration combinations may suggest that exposure or non-exposure to ASA has a greater impact on *Col* bolt height growth than different degrees of ASA exposure. Furthermore, all four ASA concentrations of *Col* experienced a net increase in mean bolt height and *sid2* exhibited various trends, suggesting a possible influence of genotype upon bolt height. However, standard deviation for the low, intermediate, and high *Col* were very large, and shared large amounts of overlap with the means and standard deviations of the *sid2*. This observation, and the lack of significant differences among low,

intermediate, and high treatments further supports the possibility that changes in bolt height are most greatly influenced by the exposure or lack of exposure to ASA. Interestingly, trends for *M. persicae* populations and bolt growth for low and high *Col* plants appeared to be quite similar, both demonstrating gradual decreases in both response variables. However, the aforementioned relationship is only speculated based off of the minimal data in this study and should be further investigated with more specific future experiments.

### BOLT HEIGHT ON APHID POPULATION

The intercept of the linear regression plot investigating possible effects of bolt height upon aphid population indicated that at changes in mean bolt height of ocm, there was an expected significant mean aphid population increase of 2.07. Interpreting change in bolt height as a proxy for plant quality, this may suggest that *A. thaliana* in poor health experience an increase in aphid population. However, the absence of a significant slope suggests that there does not exist a relationship between the two variables, and it must be acknowledged that bolt height is an unreliable indicator of plant quality that can be affected by factors such as ascension line and significant variation in the development rate of *A. thaliana* (Gnan, Marsh, and Kover, 2017) (Figure 7). Nevertheless, the significant intercept warrants further investigation, perhaps through consideration of the effects of interaction between genotype and height on aphid population.

### CONCLUSION

Overall, interactions were found between both ASA concentration, genotype and time. These results led to the following conclusions: the null hypothesis that ASA concentration and genotype do not affect aphid population was rejected. Additionally, as a significant relationship was found between ASA concentration and bolt height, the null hypothesis that ASA concentration does not affect bolt height was rejected. These findings present further evidence for the impact of anthropogenic contaminants on not only the quality of *A. thaliana* and its associated immune response, but on the population growth of insects such as *M. persicae*. The irresponsible disposal of pharmaceuticals is having a significant impact on the environment. Thus, efforts should be made to implement new policies and procedures to limit pharmaceuticals such as ASA from entering the soil and water, and decrease anthropogenic ecological impacts.

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## AUTHOR CONTRIBUTIONS

**Rathod:** Research contributions included: researched the study system of *A. thaliana* and *M. persicae* and experimental methods on exogenous delivery of substances to plants, and experimental design. Conducted research on SA and JA defense pathways. Authorship contributions included: wrote materials and methods section, and co-authored study system and results. Contributed to general editing.

**Luu:** Research contributions included: researched the SA and JA pathways, including mechanisms, genes induced, SAR, and local responses. Also researched crosstalk between SA and JA, and the impact on plant health and aphids. Conducted statistical analyses (ANOVA, ANCOVA, post hoc) for all data, created graphs alongside another group member to display trends of data. Authorship contributions included: contributed to results and discussion sections of the manuscript. Contributed to general edits and formatting for final man-

uscript.

**Al Hashemi:** Research contributions included: researched proper care and conditions for *A. thaliana*. Authorship contributions included: wrote the study system portion of the report, general editing and contributed to the discussion and conclusion.

**Agueci:** Research contributions included: Researched background information on *M. persicae* and *A. thaliana* (general traits, aphid and plant relationships with jasmonic/salicylic acid pathways, plant defence mechanisms against aphid feeding, the effects of acetylsalicylic acid on plants and aphids), experimental design. Authorship contributions included: formulation of appropriate research questions and hypotheses, created Excel graphs and analyzed their trends for the Results and Discussion sections. Contributed to edits and formulation of Abstract and Conclusion.

**Dittrich:** Research contributions included: investigated various anthropogenic ecological impacts to shape the research topic, followed by researching pharmaceutical and ASA impacts on water, soil, and plants once the topic was narrowed down, researched JA and SA pathways to determine which strains were appropriate for the experiment, and background research for the Introduction section of the manuscript. Authorship contributions included: compiled most of the abstract, wrote the Introduction section and part of the Materials/Methods section, wrote the references, and edited the rest of the manuscript.

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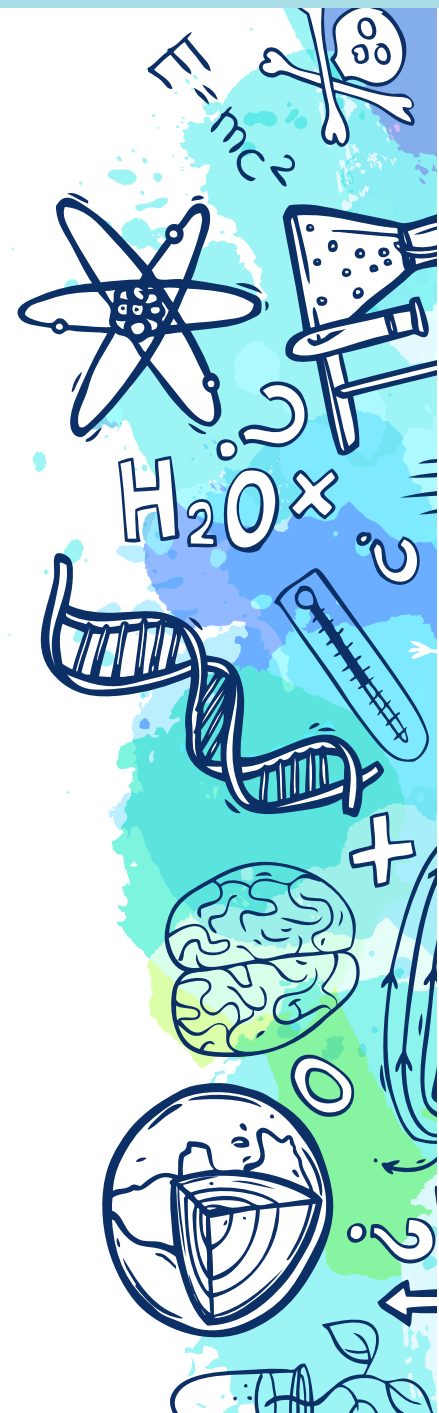
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An aerial view of the coast of Hawai'i (the Big Island) with mountains in the distance.

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