TITLE: The Effect of Alfalfa Perimeter Plantings on Strawberry Pollen Consumption by Syrphidae (Diptera) in Wisconsin

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ABSTRACT

The Effect of Alfalfa Perimeter Plantings on Strawberry Pollen Consumption by Syrphidae (Diptera) in Wisconsin

Syrphid flies visit flowers to feed on nectar and pollen and provide an important pollination service in many cropping systems. We previously observed that perimeter plantings of alfalfa concentrate syrphid populations in trap-cropped June-bearing strawberry fields, but whether this trap cropping strategy impacts syrphid visitation to strawberry flowers remains unknown. This study examined whether trap cropping influences syrphid visitation to strawberry flowers by comparing the proportion of strawberry pollen in the syrphid digestive tract. We expect that the gut content of syrphids collected from strawberry fields with an alfalfa border will have a higher proportion of strawberry pollen than those from fields with the weedy border. Although syrphids are abundant in alfalfa strips, alfalfa and strawberry flower at different times, whereas weeds present in the field border compete with strawberry. We conducted gut content analysis on syrphids by dissecting syrphids and pulverizing their abdomen. Calberla's staining solution was added to visualize pollen under the microscope, allowing individual grains to be identified and counted. We found no evidence that alfalfa perimeter plantings influence the average proportion of strawberry pollen found in the digestive tract of syrphids. However, strawberry pollen represented a significantly higher proportion of gut contents of syrphids collected from the perimeter compared to those collected from strawberry fields, and there was a lower proportion of strawberry pollen in syrphids collected in week two compared to week one and three. Differences in strawberry pollen consumption and floral visitation may reflect patterns of syrphid activity over time. Future research including direct observations of syrphid movement in strawberry fields with an alfalfa border, studying the efficiency of syrphids as strawberry pollinators, and analyzing week two samples to determine whether there was a particular type of pollen other than strawberry that was high in proportion to strawberry pollen will provide useful information about this vital, but understudied group of pollinators.

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INTRODUCTION

Insects are known to be important for facilitating major services such as pollination as well as biological control in agricultural ecosystems. Flies in the family Syrphidae (Hover Flies) are dual service providers as they visit generalist flowers and actively forage on nectar and pollen as adults, while their larvae display predatory feeding behavior (Dunn et al. 2020). According to Correa (2019) the Syrphidae family has 3 subfamilies and 15 tribes with over 6,000 species and are the most recognized group of fly pollinators. They visit a range of flowering plants and agricultural crops in various ecosystems worldwide. In this study, we investigate the non-target effects of a trap-cropping experiment targeting tarnished plant bugs (*Lygus lineolaris*) in strawberry fields (Hetherington et al., unpublished data) by observing the effect of alfalfa perimeter plantings on the proportion of strawberry pollen found in the gut content of syrphids versus a standard weedy border which is held as our control.

Although strawberries are self-fertile, insect pollination significantly improves yield (de Oliveira et al. 1991). Poor pollination, caused by inadequate pollen transfer (Ernest, 2019), leads to misshapen fruits, reduced crop yield, and reduced fruit quality. Hecht (2019) observed that syprhids represented 33% and 74% of visitors to strawberry flowers in 2017 and 2018, respectively, suggesting that syrphids play a critical role in strawberry pollination in the great lakes region.

Typically, female syrphids contain more pollen than males because they feed on more pollen for the purpose of the maturation of their reproductive system, however, males need nectar to provide energy for mate seeking. A study conducted by Wratten et al. (1995) indicated a higher proportion of female syrphids containing pollen than males in the *Melangyna* *novaezelandiae* species. Furthermore, according to Primack (1983), the same group of syrphids (*Melangyna novaezelandiae*) visited more plant species than all other flower visitors in the study. This aligns with the idea that syrphids are widely accepted as highly generalized feeders. Another study by Wratten et al. (2003) indicated that field boundaries can provide food sources for hover flies but they can also create issues for dispersal. The study determined that the proportion of Hover flies with pollen was significantly affected by both boundary type and side. As it relates to field boundaries in agroecosystems, it is important that beneficial arthropods such as syrphids are considered and this is why we have decided to study the behavior of syrphids in the presence of field boundaries intended to target plant pests.

The study assesses the effect alfalfa perimeter plantings near strawberry fields have on syrphid visitation in strawberries by measuring the proportion of strawberry pollen found in the guts of syrphid flies. This study came about as a result of a previous study undertaken regarding the effects of alfalfa perimeter plantings on syrphid abundance in Southern Wisconsin strawberry fields. We chose to study syrphids because of their frequent visitation in upper Midwest strawberries, and because they provide dual ecosystem services unlike the single service providing honeybees. The alfalfa perimeter plantings are intended to curtail the frequency of *Lygus* spp. in the strawberry fields in the broader experiment. However, we hypothesize that the gut of hover flies collected from strawberry fields with an alfalfa border (treatment) will have a higher proportion of strawberry pollen than those from fields with weedy borders (control) because we expect less competition between alfalfa and strawberries given that they flower at different times, whereas the weedy border has a variety of flowers possibly flowering earlier than strawberry thereby increasing competition.

METHODS

Strawberry fields were divided into quarter acre paired treatment and control plots. Alfalfa was planted along either side of the treatment plots running parallel to the strawberry row. Whereas control plots were surrounded by a standard turf border rather than alfalfa (Figure 1). Both treatment and control plots were separated by an eighth-acre buffer zone. Syrphids were collected with sweep nets between 2020 and 2022 from strawberry fields with and without alfalfa perimeter strips, but we only analyzed syrphid data from 2021 for the experiment. Syrphids were killed by freezing and stored dry at –20°C before being transferred to vials with 70% ethanol with other insects.

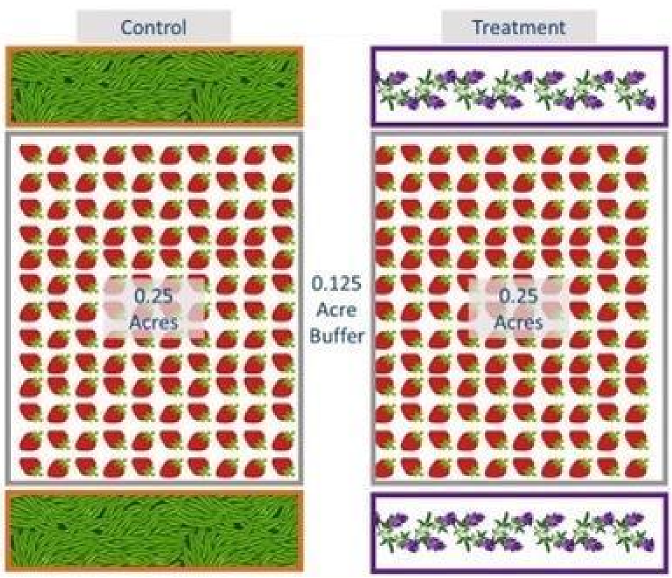

Figure 1: Control plot paired with treatment plot at each of the strawberry farms

In order to do gut content analysis, flies were separated from ethanol and allowed to dry. The wings and head of the fly were removed before the abdomen was gently pulled away from the thorax. The abdomen was placed into a microcentrifuge tube and pulverized with a plastic pestle. Calberla's Pollen Stain, which stains pollen to a light pink color, was made using 5 mL glycerol, 10 mL 95% ethanol, 15 mL distilled water, and 2 drops of saturated aqueous solution of basic fuchsin. The pestle was rinsed in 100 ul of staining solution (Calberla's Pollen Stain) to remove any remaining pollen grains into the microcentrifuge tube. 10 ul of the solution was added to each well of a glass hemocytometer and covered with a coverslip (Figure 2). The pollen grains in each of the 4 corner squares were counted. The counts were averaged across samples and mean abundance of each pollen type compared. Pollen references were developed prior to this and training was undergone to differentiate strawberry pollen from other types of pollen using pollen from a strawberry field as well as online sources.

Figure 2: Overview of Hemocytometer. (A) View of Hemocytometer with glass coverslip. Black arrow denotes notch used for loading of cell samples. (B) Hemocytometer counting area. Boxes 1-4 (in red) are used to count cells.

STATISTICAL ANALYSIS

We used R, version 4.2.3 for the statistical analysis of our data. A generalized linear fixed effects model was applied to examine the influence of alfalfa perimeter plantings, area from which insects were collected, and week of collection on the proportion of strawberry pollen. Site was included as a random effect to account for the non-independence of data based on the location of sampling. The distribution of the raw data was not normal as the data was very left skewed with a value of -1.830. Transforming that data failed to improve the distribution of model residuals, so a generalized linear modeling approach was applied. The data most closely followed a beta distribution, but the presence of 0s and 1s in the dataset prevented its use. Thus, the data were slightly compressed to meet the requirements, with 1s replaced by 0.9999 and 0s replaced by 0.0001. The glmmTMB function (Brooks et al., 2017) was applied to fit the model. The beta model was used to measure the effect of treatment, week, and row (predictors) on changes in the proportion of strawberry pollen (response variable). Wald's test was used to assess the effect of each predictor. Geometric means were then compared using emmeans.

RESULTS

The aim of the experiment was to determine whether the proportion of strawberry pollen in syrphids would change based on whether they were found in control or treatment plots to determine the effect of a trap cropping experiment on syrphid visitation in strawberries. Overall, the average proportion of strawberry pollen found in the digestive tract of syrphids was 83.18%. The predictor variables treatment, week, and row, were used to measure changes in the outcome variable of the proportion of strawberry pollen in Southern Wisconsin strawberry fields. Using Wald's test to determine which of the groups deferred, there appeared to be a significant effect of week on the average proportion of strawberry pollen ($\chi2$ = 8.70, DF = 2, P = 0.013) but no effect was observed by Treatment ($\chi2$ = 0.046, DF = 1, P = 0.831) nor Row ($\chi2$ = 3.508, DF = 1, P = 0.060). Using the beta model we were able to make comparisons between the independent variables to see which had the most influence on the dependent variable. When the estimated marginal means of the treatment and control plots were compared, it was determined that the proportion of strawberry pollen in treatment and control plots was not significantly different (β = -0.0105, z ratio = –.213, P = 0.8314) which is demonstrated in figure 3. Figure 4 shows the proportion of strawberry pollen found in the digestive tract of syrphids collected in week 2 was significantly lower than the proportion of strawberry pollen in syrphids collected in weeks 1 and 3 (Week 1 - Week 2: β = 0.217, z ratio = 2.664, P = 0.0211; Week 2 - Week 3: β = -0.208, z ratio = -2.663, P = 0.0211). When the estimated marginal means were compared by row, the perimeter plantings had around 14.44% more strawberry pollen in treatments and controls compared to the proportion of strawberry pollen found in the guts of syrphids in the strawberry fields (β = 0.122, z ratio = 2.120, P = 0.034) as depicted in figure 5.

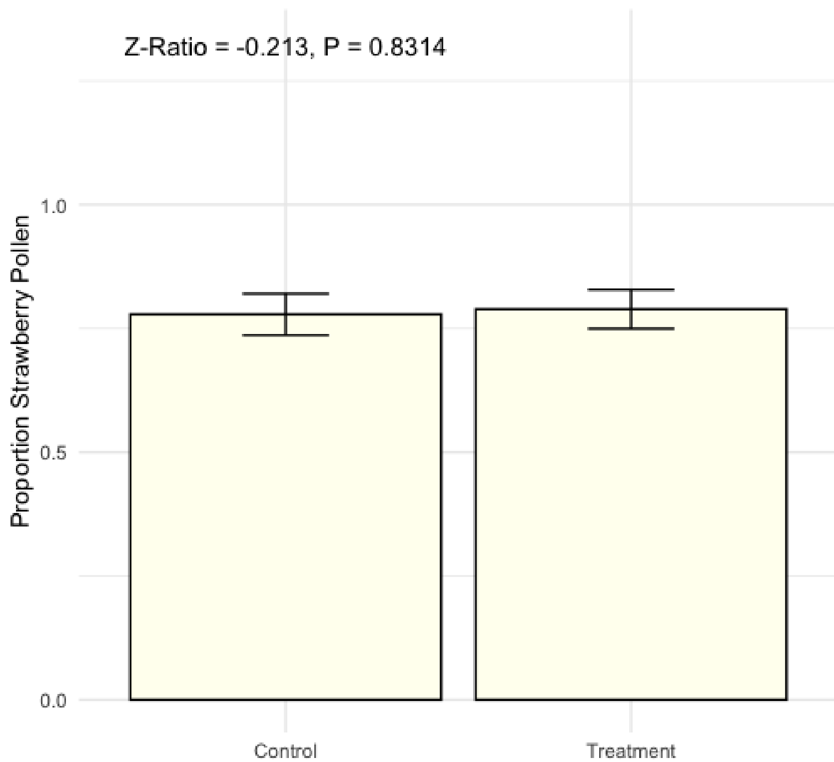

Figure 3: A Comparison of the Estimated Marginal Means for the Proportion of Strawberry Pollen for Treatment and Control plots with error bars to represent standard error predicted by the model

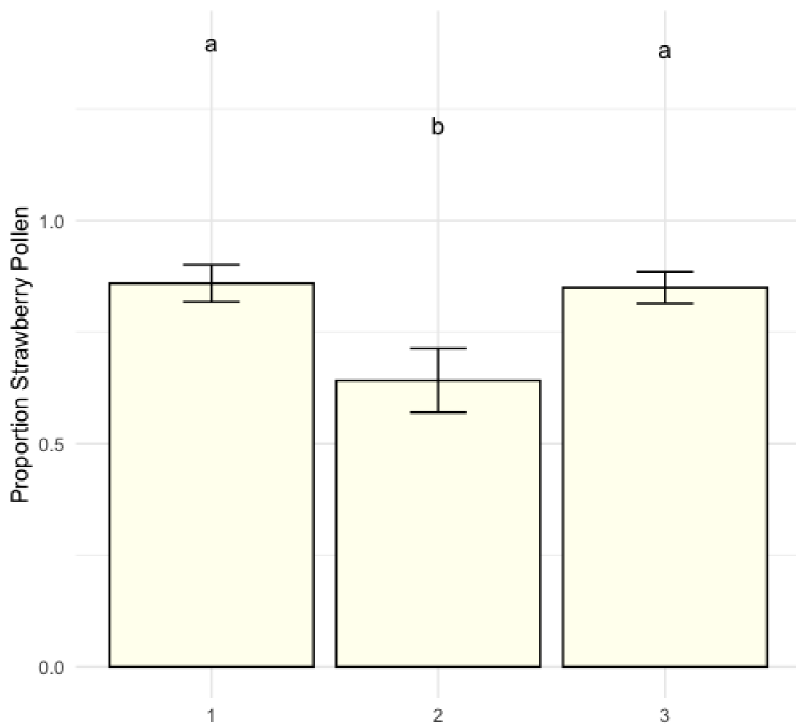

Figure 4: Proportion of Strawberry Pollen found in Syrphids collected by week (1-3) with error bars included to represent standard error predicted by the model

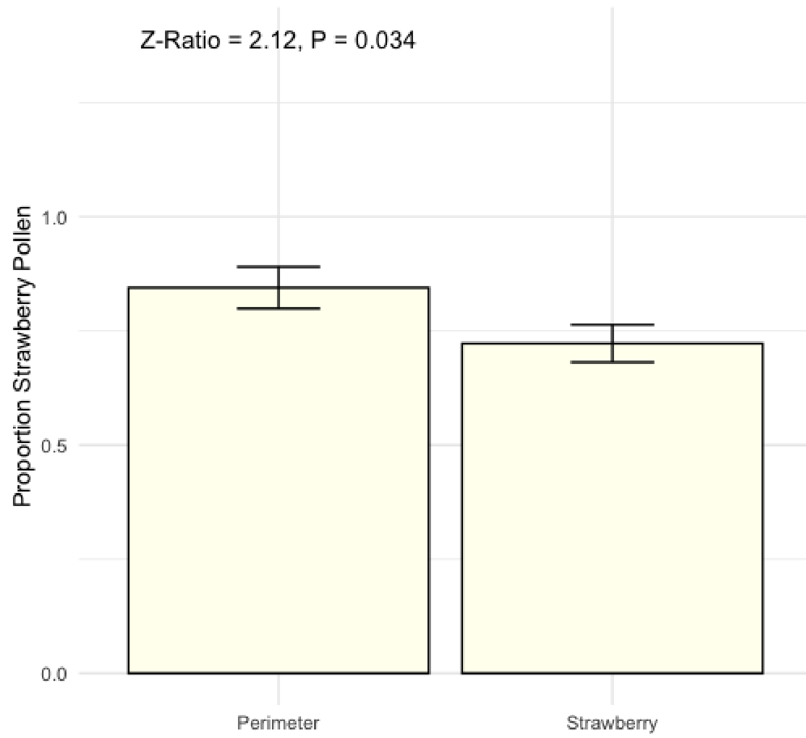

Figure 5: Proportion of Strawberry pollen found in Syrphids collected in the Perimeter plantings versus the strawberry field for both treatment and control plots with error bars included to represent standard error predicted by the model

DISCUSSION

The study examined the effect of alfalfa perimeter plantings on the proportion of strawberry pollen in syrphid digestive tracts. After a thorough analysis of the literature relating to syrphids based on gut content analysis, morphology, behaviors, and life cycles, to determine whether there foraging patterns in strawberry fields would change in the presence of alternative floral resources, we anticipated that there would be more strawberry pollen found in the gut of syrphids in the treatment because we expected less competition between the alfalfa and strawberry floral resources due to timing of flowering. The results of the generalized linear model indicated an insignificant relationship between the proportion of strawberry pollen by week, treatment, and row. However, using a beta model to measure the response in the outcome variable, the results were significant by week and row.

Our experiment provides evidence that syrphid foraging in strawberry fields is unaffected by alfalfa perimeter plantings as a method to manage tarnished plant bugs as a pest affecting strawberry fields in Southern Wisconsin. As figure 3 shows, there was no difference in the proportion of pollen found in the gut of syrphids found in treatment and syrphids collected from control plots. Interestingly, Syrphids had a higher proportion of strawberry pollen in perimeter plantings than those that were collected from the strawberry fields in figure 5. Some adult syrphids begin to emerge with an undeveloped reproductive system (Irshad, 2014) in the months of April and May which coincides with the timing that aphid populations increase (Gilbert, 1980). Female syrphids require food in the form of nectar, pollen, water, and honeydew in order for them to produce viable eggs and most of these resources can be found in strawberries so it is likely that they first visit strawberry resources in the field. After completing these activities in week one, it is likely that syrphids migrated into the alfalfa plantings for oviposition because of the presence of aphids in the alfalfa that functions as an insectary plant grown in an effort to attract and provide shelter for insect parasites and for the enhancement of biological control. Syrphid larvae feed on soft-bodied insects such as aphids, and tarnished plant bugs that are both common in alfalfa. Both honeydew and aphid pheromones are important for signaling adult hoverflies to locate aphid colonies according to Jiang et al. (2020) and based on research by Sadgehi and Gilbert (2000), adult syrphids lay their eggs in areas that provide good larval food sources. This could potentially provide justification for why syprhids in the perimeter had a higher proportion of strawberry pollen than syrphids collected from strawberry fields because syrphids were migrating into the alfalfa planting after foraging on strawberry pollen (figure 5).

As for the decrease in the proportion of strawberry pollen collected in week two, it is possible that in week two, a competitive short-lived weed appeared in the perimeter that was more attractive for syrphids during this time, so that the proportion of other pollen consumed increased compared to weeks one and three. This is likely because syrphids are visual creatures that respond to visual signals. Once the resources declined, syrphids would have resumed foraging on strawberries for pollen and nectar the following week.

Insect pollinators play a big role in global food security because of the value of pollination in agriculture which is primarily provided by bees. In fact, a massive 35% of the global food supply is dependent on animal pollinators (Klein et al., 2006). Thus, it can be concluded that if insect pollinator populations decline, food production will also be decreased. With the recent declines in wild pollinators, this presents a significant threat to the provision of pollination services in strawberries. Insect pollinators alone are responsible for an estimated 45% strawberry crop yields (Hodgkiss et al., 2018). This highlights the important role of syrphids in strawberries, as according to Dunn et al. they are generalist feeders that actively forage on pollen and nectar as adults. As previously mentioned, hoverflies are frequent floral visitors to strawberry flowers in Minnesota (Hecht, 2019). This not only emphasizes the important role of syrphids in strawberries generally, but also their critical importance in the Great Lakes region. In a previous trap-cropping experiment where alfalfa perimeter plantings were grown on either side of strawberry fields to remove tarnished plant bugs from the strawberry and compared to control plots with a standard weedy border, we saw syrphids were more abundant in the alfalfa. So, it was important to determine whether this would have detrimental effects on strawberry pollination given the important role of syrphids in this process. However, the results show that there is no difference in the proportion of strawberry pollen in syrphids collected in treatment and control plots.

This study addresses the possible impacts and unintended consequences to beneficial insects such as syrphids resulting from trap cropping. Consequently, based on the results of the study, it is safe to say that this form of management strategy can be safely implemented by growers in strawberry in Southern Wisconsin agroecosystems without having negative impacts on beneficial insects within the system. However, to confirm the accuracy of the study, future directions include looking at strawberry flower visitation through direct observation and identifying the non-strawberry pollen in the guts of syrphids to see if there were weeds that possibly drove the decline in strawberry pollen in week two, as well as assessing the efficiency of syrphids as strawberry pollinators. The dual roles hover flies provide as pollinators and biological control agents which is seen in studies by Dunn et al. (2020) compared to their single service-providing counterparts (honeybees) may further provide justification and incentive for syrphid introduction in certain crops or possibly alongside honeybees.

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REFERENCES

Brooks, M., E., Kristensen, K., Benthem, K., J. ,van, Magnusson, A., Berg, C., W., Nielsen, A., Skaug, H., J., Mächler, M., & Bolker, B., M. (2017). GLMMTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378. https://doi.org/10.32614/rj-2017-066

Correa, J. (2019). Syrphidae: A Guide to Natural History and Identification of Common Genera in Santa Cruz County. *Syrphid Guide*. Retrieved November 18, 2021, from https://randallmorganinitiative.ucsc.edu/data-and-research/images/correa-syrphidguide.pdf

de Oliveira, D., Savoie, L., & Vincent, C. (1991). Pollinators of cultivated strawberry in Québec. *Acta Horticulturae*, (288), 420–424. https://doi.org/10.17660/actahortic.1991.288.71

Dunn, L., Lequerica, M., Reid, C. R., & Latty, T. (2020). Dual ecosystem services of syrphid flies (Diptera: Syrphidae): Pollinators and biological control agents. *Pest Management Science*, 76(6), 1973–1979. https://doi.org/10.1002/ps.580

Ernest, E. G. (2019, May 16). Misshapen Strawberry Fruits. Weekly Crop Update. Retrieved November 18, 2021, from https://sites.udel.edu/weeklycropupdate/?p=13340.

Gilbert, F. S. (1980). Flower visiting by hoverflies (Syrphidae). *Journal of Biological Education*, *14*(1), 70–74. https://doi.org/10.1080/00219266.1980.9654289

Hecht, Nathan. (2019). Influence Of Flower Strips On Insect Pollinator Recruitment And Crop Yield In Day-Neutral Strawberry Production. Retrieved from the University of Minnesota Digital Conservancy, https://hdl.handle.net/11299/206175.

Hodgkiss, D., Brown, M. J. F., & Fountain, M. T. (2018). Syrphine hoverflies are effective pollinators of commercial strawberry. *Journal of Pollination Ecology*, *22*, 55–66. https://doi.org/10.26786/1920-7603

Irshad, M. (2014). Review: Role of Syrphids (Diptera: Syrphidae) asbiotic agents and pollinators in Pakis. *Journal of Bioresource Management*, *1*(2). https://doi.org/10.35691/jbm.4102.0007

Jiang, H., Kong, J. J., Chen, H. C., Xiang, Z. Y., Zhang, W. P., Han, Z. D., Liao, P. C., & Lee, Y. I. (2020). Cypripedium subtropicum (Orchidaceae) employs aphid colony mimicry to attract hoverfly (Syrphidae) pollinators. *New Phytologist*, 227(4), 1213–1221. https://doi.org/10.1111/nph.16623

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2006). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Primack, R. B. (1983). Insect pollination in the new zealand mountain flora. *New Zealand Journal of Botany*, *21*(3), 317–333. https://doi.org/10.1080/0028825x.1983.10428561

Sadeghi, H., & Gilbert, F. (2000). Oviposition preferences of aphidophagous hoverflies. *Ecological Entomology*, 25(1), 91–100. https://doi.org/10.1046/j.1365-2311.2000.00234.x

Wratten, S. D., Bowie, M. H., Hickman, J. M., Evans, A. M., Sedcole, J. R., & Tylianakis, J. M. (2003). Field boundaries as barriers to movement of hover flies (Diptera: Syrphidae) in cultivatedland. *Oecologia*, *134*(4), 605–611. https://doi.org/10.1007/s00442-002-1128-9

Wratten, S. D., White, A. J., Bowie, M. H., Berry, N. A., & Weigmann, U. (1995). Phenology and ecology of hoverflies (Diptera: Syrphidae) in New Zealand. *Environmental Entomology*, *24*(3), 595–600. https://doi.org/10.1093/ee/24.3.595

SUPPLEMENTAL MATERIALS

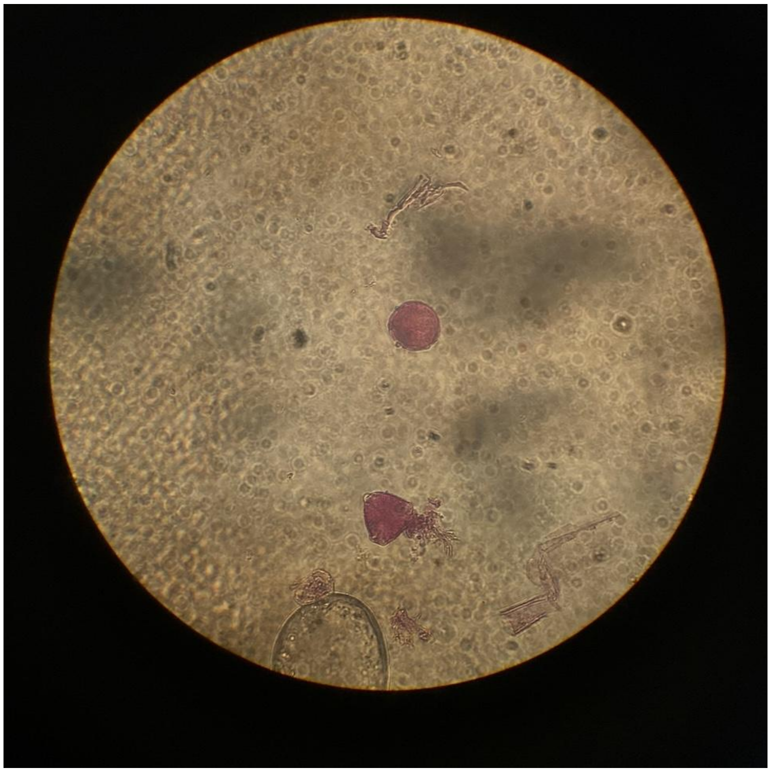

Figure 6: Strawberry Pollen Reference Slide