EFFECTS OF RESVERATROL ON DEVELOPMENT OF THREE LEPIDOPTERAN SPECIES VARYING IN DIET BREADTH

BY

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THESIS

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ABSTRACT

The polyphenolic stilbene resveratrol is a plant secondary compound associated with antioxidant and anti-pathogen functions. Knowledge of these functions prompted longevity and development studies on many different model organisms, including several insect species. Results from these studies have been inconsistent across species; these inconsistencies may reflect the fact that many of these model organisms do not encounter resveratrol in their natural diets. In this study, I examined the effects of resveratrol on growth and development of three species of lepidopterans: the navel orangeworm *Amyelois transitella* (Pyralidae), a nut-feeding generalist with several hostplants that produce resveratrol, the cabbage looper *Trichoplusia ni* (Noctuidae), a polyphagous folivore that rarely encounters the compound in its host plants, and the tobacco hornworm *Manduca sexta* (Sphingidae), an oligophagous folivore that feeds almost exclusively on species in the Solanaceae, some of which produce resveratrol in fruits but not in leaves. Two strains of *A. transitella* that differ in their recent ecological exposure to resveratrol were compared—a wild-caught strain from fig orchards and a laboratory strain originally collected in almond orchards and maintained for multiple generations on a semi-defined artificial diet. Growth and development of these species and strains were assayed with artificial diets containing concentrations of resveratrol based on ecologically relevant levels. Although the diet containing the highest concentration of resveratrol tested (70μg/g) led to lower pupal weights of *M. sexta* compared to all other diets, no other adverse or beneficial impacts of resveratrol were detected in any other assays. In contrast with previous studies documenting beneficial effects of resveratrol in two model insect species, neither larval survival nor adult lifespan was enhanced in any of the species in this study.

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INTRODUCTION

Trans-resveratrol (*trans*-3,5,4'-trihydroxystilbene), referred to here as resveratrol, is a polyphenolic stilbene found in a variety of plant species, including several that are frequently consumed by humans (Pallauf *et al.* 2016). As a dietary component, particularly in red wine, resveratrol attracted attention as a powerful antioxidant with health-promoting potential (Teguo *et al*. 1998; Siemann and Creasy 1992). Many studies have documented a diversity of beneficial properties *in vitro*, including anti-inflammatory, anticarcinogenic, cardioprotective, and neuroprotective activities (Salehi *et al.* 2018; Wu *et al*. 2001). After Howitz *et al.* (2003) showed that resveratrol could extend the lifespan of *Saccharomyces cerevisiae* (bread yeast) by 70%, research into resveratrol as a longevity-enhancing molecule proliferated (Reinisalo *et al.* 2015; Valenzano *et al*. 2006; Baur *et al*. 2006). Despite intense efforts, definitive evidence that resveratrol alone affects the lifespan of humans is lacking due to the many variables present in field studies of resveratrol and human subjects. In an eleven-year study (1998-2009) conducted by Semba *et al.* (2014), resveratrol did not have a substantial influence on health status or mortality risk in men and women 65 years or older. This result is in part due to the limited bioavailability and rapid metabolism of resveratrol *in vivo* and also likely due to the variability of the study group's microbiota, genetic background, and diet (Pallauf *et al.*, 2016; Reinisalo *et al.*, 2015).

In terms of its ecological functions in the plants that produce it, resveratrol serves as a phytoalexin, an antimicrobial agent induced upon infection (Sobolev *et al.* 2007). In wine grapes (*Vitis vinifera*), e.g., production of resveratrol and other stilbenes is induced in response to infection by *Botrytis cinerea*, gray mold, and inhibits its mycelial growth (Adrian *et al.* 1997; Langcake and Pryce 1976). Similarly, varieties of peanuts (*Arachis hypogaea*) synthesize

resveratrol and other stilbene phytoalexins in response to infection by *Aspergillus* species (Sobolev et al. 2007) as well by several yeast and bacteria species (Sobolev 2013). Resveratrol also inhibits the growth of toxigenic *Aspergillus* species (*Aspergillus flavus, A. parasiticus*) in grapes (Selma *et al.*, 2008) and in high concentrations prevents the capacity of the causative agent of downy mildew, *Plasmopara viticola*, to move across a plant surface (Calabrese *et al.*, 2010).

In addition to its antimicrobial activities, the possibility exists that, like many other phytochemicals, resveratrol and related stilbenes may also function as a defense against insect herbivores. Liu *et al.* (2013) discovered through phytochemical screening that resveratrol was an effective antifeedant against the coconut hispine leaf beetle *Brontispa longissima*. Its insecticidal properties were also assessed by Gabaston *et al.* (2018) against the Colorado potato beetle *Leptinotarsa decemlineata*; an extract of grapevines containing resveratrol caused chronic toxicity and inhibited larval growth in this species. In other screening studies, resveratrol, identified in extracts of the bark of *Yucca periculosa*, impaired growth of larvae of the noctuid *Spodoptera frugiperda* (Torres *et al.* 2003) and combretastatin A-4, a *cis*-stilbene related to resveratrol, inhibited growth of *Spodoptera litura* (Lv *et al.* 2014). Only three studies to date have examined the effects of resveratrol on an herbivore that routinely encounters the compound in its host plants. Sobolev *et al.* (2007) showed that, when a resistant strain of peanut was attacked by lesser cornstalk borer (*Elasmopalpus lignosellus* Zeller), the plants produced higher concentrations of stilbenes than did counterparts more susceptible to this insect pest. Sambangi and Rani (2016) demonstrated that resveratrol inhibited feeding by the larvae of the noctuids *S. litura* and *Amsacta albistriga*, both major pests of peanuts, and caused concentration-dependent weight reduction in leaf disk bioassays. Finally, Faccoli and Schlyter (2007) evaluated the effect

of resveratrol and other phenolics occurring in bark of spruce host plants on diet acceptance and tunneling activity in the bark beetle *Ips typographus* and found concentration-dependent antifeedant impacts on male beetles and reduced tunneling activity overall.

In contrast with its insecticidal effects, in a review of the extensive literature on resveratrol and lifespan in model organisms, Pallauf *et al.* (2016), identified several studies documenting longevity enhancement by resveratrol in some insect species. In the fruit fly, *Drosophila melanogaster*, resveratrol extended adult longevity but its effect varied with strain, diet, concentration and sex; in all studies of larvae, resveratrol reduced mortality. As well, in studies of *Apis mellifera*, the western honey bee, adults consuming resveratrol experienced an increase in lifespan and greater resistance to infection by the gut parasite *Nosema ceranae*.

Overall, effects of resveratrol on insect performance are thus inconsistent and its adaptive value in the life of plants with respect to interactions with herbivorous insects is unclear. In this study, I examined the effects of resveratrol on development of larvae of three species of lepidopterans with different life histories. The navel orangeworm *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) is a North American native found in the southwestern United States and through Mexico to northern South America. It is highly polyphagous, feeding preferentially on mature fruits and nuts, and is a serious economic pest in California non-native crops such as pistachios, almond, figs, and pomegranates (Bagchi *et al.* 2016; Bentley *et al*. 2016). Fruits of both pistachio (*Pistacia vera*) (Bolling *et al.* 2010) and almond (*Prunus dulcis*) (Xie and Bolling, 2014) have high levels of resveratrol and accordingly navel orangeworm populations in the Central Valley routinely encounter resveratrol in their diet, particularly when feeding on nut crops (Wade 1961). To capture the variation in likelihood of exposure to resveratrol experienced by this polyphagous species, I compared responses to resveratrol in two strains maintained in the

laboratory: a strain derived recently from individuals infesting a fig orchard (designated FIG) and a laboratory strain originally collected in almond orchards and raised in the laboratory for many years (designated CPQ).

I also examined responses to resveratrol in the cabbage looper *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), a widely distributed noctuid moth found in temperate North America. It is polyphagous in its larval stages on a diversity of herbaceous plants, including multiple crop species in the Brassicaceae (Eichlin and Cunningham 1978). Although some of its recorded host plants belong to families reported to contain stilbenes, resveratrol has not been found to occur at high concentrations in leaves of plants other than peanuts (Chung et al. 2003), grapevines (Langcake and Pryce 1976), and a few other species (Chong et al. 2009; Schouten *et al*. 2002); thus, T. ni, as a folivore, likely does not encounter resveratrol frequently in its diet.

The third species examined was the tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae), an oligophagous folivore that feeds almost exclusively on host plants in the family Solanaceae (Mechaber and Hildebrand 2000). Although resveratrol is reported in the fruit of the solanaceous plant *Lycopersicon esculentum* (tomato) (Ragab *et al.* 2006), foliage of this and other solanaceous species is not noted for resveratrol production.

In view of the ecological habits and evolutionary history of these three lepidopterans, I predicted that resveratrol would have adverse effects on *T. ni* and, to a greater extent, *M. sexta* and no adverse effect on *A. transitella* at ecologically relevant concentrations. With respect to *A. transitella*, I predicted that, after many generations in the laboratory without exposure to dietary phytochemicals, the CPQ strain would display greater sensitivity to resveratrol than the fig strain. Finally, I predicted that, with little or no ecological exposure to high concentrations of

resveratrol or any other stilbenes, the oligophagous folivore *M. sexta* would display the greatest sensitivity to adverse effects of resveratrol.

In addition to testing for toxicity, I also examined whether resistance to adverse effects of resveratrol is due to cytochrome P450-mediated detoxification. Although in humans resveratrol inhibits the activity of a number of xenobiotic-detoxifying cytochrome P450 enzymes (Guthrie *et al.* 2016), in all three lepidopterans this enzyme superfamily has been shown to play important roles in detoxifying a diversity of phenolic phytochemicals (Li *et al*. 2007; Niu *et al.* 2011, 2012). Accordingly, using piperonyl butoxide (PBO), a relatively selective cytochrome P450 inhibitor (Niu *et al*. 2011; Demkovich *et al*. 2015a, b), I provided all three species with resveratrol-containing diets with and without PBO to determine if PBO synergizes resveratrol toxicity by inhibiting its metabolism

MATERIALS AND METHODS

Chemical Source and Preparation. PBO was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and Sigma-Aldrich (MO, USA) at 90% PBO. Resveratrol (*trans*-3,5,4'-trihydroxystilbene) was purchased from Sigma-Aldrich (St. Louis, MO). Ethanol (100%) was used to dissolve all solutions. All solutions were kept at -20℃ until needed.

Insects. Two strains of *A. transitella*, CPQ (CPQ-2005: Siegel *et al.* 2010) (derived from almond and maintained in the laboratory for many generations on a wheat bran diet) and FIG (derived from individuals collected in fig (*Ficus carica*) orchards in Kern County, California in 2017 and maintained for four generations in the laboratory on wheat bran diet), were kept in an incubator at 27±4℃ with a photoperiod of 16:8 h (L:D). Larvae were kept on a wheat bran diet modified from Finney and Brinkman (1967) and were reared until pupation according to Demkovich *et al.* (2015b).

Trichoplusia ni eggs were obtained from Benzon Research Inc. (Carlisle, PA). Colony care was modified from Levy (2018). Larvae were raised on a semi-defined artificial diet developed by Waldbauer *et al.* (1984) (Table 1, Table 2) and were raised in 30 mL soufflé cups until pupation. Pupae were removed from their silken cocoons, weighed, and then rinsed *en masse* with a 10% chlorine bleach (Clorox, Oakland, CA, dissolved in deionized water) solution for about 10 seconds, after which they were rinsed with tap water for 10 seconds or until the bleach odor was imperceptible. During resveratrol assays, pupae and adults were kept in a 38-cm^3 flight chamber box at 23±4°C under laboratory conditions with adjacent windows providing natural light. The chamber was provided with wax paper as an oviposition substrate and lightly moistened paper towels as a floor to promote higher internal humidity. A 50-mL plastic screw-cap tube was used to provide the moths with food (10% sucrose in deionized water); a hole was

created in the side of the tube with a soldering iron and a moistened cotton ball was inserted as a wick for the sugar solution. During resveratrol and PBO assays, pupae and adults were placed in a 26 x 19 x 11 cm lidded plastic cage in an incubator at 28±4°C with a photoperiod of 16:8 h (L:D) (Percival Incubator, Percival Scientific, IA, USA). The cage was ventilated with a 12 x 6.5 cm mesh on the lid; the bottom of the cage was lined with paper towels and the sides were lined with wax paper as an oviposition substrate. A 50-mL plastic screw cap tube of 10% sucrose in DI water was placed inside the cage as described to provide food for the moths. For both assays, wax paper from the chamber was removed after 3 to 4 days or after a substantial number of eggs were present on the wax paper. Then the wax paper with eggs was gently rinsed in 10% chlorine bleach solution (Clorox, Oakland, CA, dissolved in deionized water) for sterilization, followed by a gentle rinse in deionized water. The wax paper with eggs was then placed into plastic reclosable bags with paper towels.

Manduca sexta neonate larvae were obtained from a stock colony at UIUC. Larvae were fed a semi-defined artificial diet (Table 3, Table 4) in an incubator (Hotpack Corporation, PA, USA) at 26±4°C with a photoperiod of 16:8 h (L:D).

Experimental diets. During *A. transitella* and *T. ni* assays, solutions of each resveratrol treatment were prepared at different concentrations as micrograms or nanograms of active ingredient per gram of diet (Resveratrol: 80 ng/g, 7 µg/g, and 70 µg/g). The lowest concentrations are based on concentrations of resveratrol reported in pistachios and almond (Grippi *et al*., 2008; Xie and Bolling, 2014) and the highest concentration (70 µg/g) is ten times higher than the concentration reported in almonds (7 µg/g). The minimum amount of solvent used to dissolve resveratrol was 175 µL of ethanol, which was also used as the control for the resveratrol treatments, combining for a total of four treatments. For synergism studies, all

concentrations of resveratrol and the control were used in the assay in combination with PBO; with a PBO control, this experiment comprised nine treatments. The fixed concentration of PBO was 200 µg/g, previously reported by Niu *et al.* (2012) to be nonlethal for first instar larvae from a colony of *A. transitella* SPIRL-1966 strain (Siegel *et al.*, 2010). Resveratrol and/or PBO were mixed into the diet when the temperature of the diet fell below 60°C.

Bioassays of *A. transitella* performance with and without resveratrol in the diet.

For this bioassay six variables were measured: days to pupation, days to eclosion, total lifespan, adult longevity, pupal weight (mg), and eggs laid. Larvae were placed individually in soufflé cups containing approximately 5 g of the treatment diet. Pupae were removed from silken cocoons using a pair of iridectomy scissors and featherweight forceps, sexed, and placed in a new soufflé cup. Adult individuals of the opposite sex within the same treatment were partnered as a mating pair, each of which was placed in a soufflé cup with an applicator stick as a perching substrate; the top was lined with a paper towel as an oviposition substrate. The lid was punctured to allow DI water to be added to wet the paper towel every 24 hours for three days. For longevity assays, individuals were placed in similarly prepared soufflé cups; DI water was added to wet the paper towel every 24 hours until the individual died.

Bioassays of performance of *T. ni* with and without resveratrol in the diet.

For this bioassay six variables were measured: days to pupation, days to eclosion, total lifespan, adult longevity, pupal weight (mg), and eggs laid. Larvae were placed individually in soufflé cups containing ~5 g of the treatment diet. Pupae were rinsed individually in a 10% bleach (Clorox) solution for at least 10 seconds and then a 60 mL deionized water bath for at least 10 seconds. Cleaned pupae were sexed and placed in new soufflé cups as already described. Adults of each sex within each treatment were set up as mating pairs in a 266 mL plastic lidded

container with a wooden applicator stick, a small cotton ball soaked in a 10% sucrose in DI water as a food source, and wax paper that lined the inside of the container. Oviposition assays lasted three days and pairs were checked every 24 hours. For longevity assays, adults were kept in soufflé cups individually and water was added to the paper towel fixed by the lid every 24 hours.

Bioassays of performance of *M. sexta* with and without resveratrol in the diet.

For this bioassay two variables were measured: days to pupation and pupal weight (mg). For tobacco hornworm assays, 204.8 mg of resveratrol was dissolved in 5 mL of 100% ethanol and added to make 2926.3 g of diet containing resveratrol at a concentration of 70 µg/g. The control diet contained an equivalent amount of ethanol. Larvae and pupae were kept in an incubator at 28±4°C with a photoperiod of 16:8 h (L:D). Larvae were raised in 30 mL plastic cups and were transferred to 266 mL plastic cups once they reached third instar. New diet was added and frass was removed from the cups as needed. Because older larvae often have difficulties molting, on occasion I removed exuviae that were not completely shed. Once larvae reached the prepupal or wandering stage they were removed from the cup, rinsed clean of their diet and frass with tap water and placed in holes drilled in blocks of pine wood 7.5 cm deep with a diameter of 2.3 cm to accommodate the pupae. The holes were plugged with rubber or soft plastic stoppers. Once they pupated, pupae were sexed and stored in a separate incubator kept at 31°C with no light (GCA Corporation Precision Gravity Convection Incubator, Precision Scientific Group). Pupae were in 266 mL lidded cups lined with paper towels.

Bioassays of potential synergism between PBO and resveratrol.

For resveratrol assays that included PBO, seven variables (days to pupation, eclosion, and death, adult lifespan, pupal weight (mg), eggs laid (log), percent eggs fertilized) were recorded for two species (*A. transitella, T. ni*).

Statistical Analyses. The Quartile function in Microsoft Excel was used to remove outliers in *A. transitella* resveratrol assays. Excel files were imported as csv files into the programming software R (R Core Team, 2016). R was used in primary and post hoc analysis. Linear models were created for data analyzed with a one or two-way ANOVA. For resveratrol assays, the CPQ and FIG strains of *A. transitella* were analyzed using a two-way ANOVA to compare strain and treatment, *T. ni* assay data were analyzed with a one-way ANOVA to compare the treatments, and *M. sexta* assay data were analyzed with a Student's t test because only two variables were compared. For the resveratrol and PBO assays, results from both *A. transitella* and *T. ni* assays were analyzed with a two-way ANOVA. The three controls for the PBO assay (PBO, EtOH, EtOH+PBO) were also analyzed separately from the main PBO assay using a one-way ANOVA. For two-way ANOVAs, in the event of a significant main effect or interaction effect I partitioned the variance to the significant effect by turning the two-way ANOVA into a one-way ANOVA, keeping the significant effect. Data normality was assessed via residuals with the Shapiro-Wilks normality test and homoscedasticity was assessed using Levene's test accessed from the R package lawstat (Gastwirth *et al.,* 2017) with the null hypothesis being data are normal and homoscedastic ($\alpha = 0.05$). Transformation of data was informed by the use of the Box-Cox power transformation test accessed through the R package MASS (Venables and Ripley, 2002). All transformed data were reanalyzed to determine normality and homoscedasticity. All reported values are normal and homoscedastic.

RESULTS AND DISCUSSION

Resveratrol Trial Results. *M. sexta* pupal weights differed significantly between treatments, with larvae on the control diet having a higher average weight (2934.7 mg) than larvae on the diet containing 70 µg/g resveratrol (2702.6 mg) (Table 5; t = 2.10, df = 90, p = 0.04). For eggs laid, the two strains of *A. transitella* differed on diets containing 7µg/g resveratrol (Table 5; ANOVA: p = 0.004, F = 10.4, df = 1; lsmeans contrast: p = 0.045, df = 16, t ratio = 2.17). For CPQ and FIG, both days to pupation and pupal weight differed according to strain but no contrasts between treatments were significant (Table 5; p = 0.015, F = 6.93, df = 1, and p = 0.014, F = 7.16, df = 1, respectively).

Resveratrol and PBO Trial Results. For *A. transitella*, the least squares means contrast as part of the post hoc analysis completed using a t-test showed that average adult lifespan was longer for the 7 µg/g resveratrol treatment (17.5 days) than the same treatment with PBO (13.2 days) (Table 6; p = 0.027, t-ratio = 2.44, df = 13). For days to pupation in *T. ni*, the main effect of PBO in the diet had significant ANOVA table values (Table 6; p = 0.0023, F = 11.9, df = 1) but no contrasts between treatments were significant. In other words, the significant difference detected was attributable to the effect of PBO alone. For days to eclosion in *T. ni*, the main effect of PBO in the diet had significant ANOVA table values (p = 0.004, F = 10.4, df= 1) but no contrasts between treatments, again indicating differences between treatments with and without PBO but no treatment main effects. This again indicates that the significant differences detected were attributable to the PBO treatment alone.

Bioassays for synergistic interactions depends on the use of the putative synergist at concentrations that have no adverse effects on the test organism; in my study, however, PBO had significant effects on *T. ni* development, including pupal weight, days to pupation, and adult

longevity. Accordingly, for these assays, I removed the PBO main effects and the interaction effects from the two-way ANOVAs and re-analyzed them as one-way ANOVAs with the main effect being resveratrol in the diet. Results from the reanalysis showed that the resveratrol had no effect on *T. ni* in the study (Table 7).

Discussion. The original hypothesis I set out to test in this study was that the impact of resveratrol on lepidopteran larvae would vary with the frequency with which it is encountered in the natural diet. Thus, my expectation was that *A. transitella*, having a longstanding ecological relationship with resveratrol-producing plant hosts, would not be adversely affected by resveratrol. Results of assays conducted with both strains of *A. transitella* (CPQ and FIG) confirmed this hypothesis. No significant differences were observed for resveratrol treatments of the CPQ or FIG strains, although there were significant differences between strains for days to pupation, pupal weight, and eggs laid. These findings suggest that the CPQ and FIG strains have differences in their life histories and biology irrespective of resveratrol consumption.

M. sexta was predicted to be affected to the greatest extent by resveratrol. At the highest concentration of resveratrol (70 µg/g), I detected, consistent with the hypothesis, a significant difference between the resveratrol and the control treatment for pupal weight. Because I was unable for logistical reasons to test effects of resveratrol on this species at the lower concentrations reported from almond and pistachio, I was unable to establish whether responses to resveratrol may be concentration-dependent.

T. ni was also predicted to be affected by resveratrol, although to a lesser extent than *M. sexta*. In fact, in contrast with *M. sexta*, no adverse effects of resveratrol consumption were observed. As previously indicated, *T. ni* is a highly polyphagous folivore that may on occasion encounter resveratrol in its broad diet. Generalist lepidopterans such as *T. ni* typically are broadly

tolerant of a diversity of dietary phytochemicals (e.g., Li et al. 2004); thus, the absence of toxicity observed in my study is consistent with its biology, even if relatively few of its host plants may contain significant levels of foliar resveratrol.

The purpose of carrying out assays of resveratrol effects on lepidopteran larvae in the presence and absence of PBO was to determine if resveratrol metabolism is P450-mediated. Adult lifespan was, on average, reduced in *A. transitella* (CPQ) with the addition of PBO at the intermediate concentration of resveratrol (7 µg/g). Synergism can be detected only if the PBO concentration alone has no adverse effects on the target organism, which was not the case in the *T. ni* assays. Metabolism of resveratrol may be due to action by detoxification enzymes other than P450s, such as esterases and glutathione-S-transferases; moreover, the possibility exists that tolerance in these insects is due to excretory adaptations (Schuler, 1996).

With resveratrol affecting only one life history trait in one species (*M. sexta*, pupal weight), conclusions about its functions in an ecological context based on this study are limited. A tentative conclusion is that resveratrol functions as an inhibitor of insect development for specialist folivores. With resveratrol as a monomer on which many other stilbene compounds are based (Chong *et al*. 2009), it is possible that toxic properties observed in studies with other insects based on extracts are a result of multiple compounds working in concert with each other. A single stilbene may have little or no effect, but multiple stilbenes together may have entirely different toxic properties. Whether multiple types of stilbenes combined with resveratrol can affect development of insect folivores to a greater extent than resveratrol alone remains to be determined.

Selecting for plant mutants that produce a higher level of stilbenes, including resveratrol, or searching for resveratrol-rich varieties for pathogen management has already been considered

for breeding programs in peanuts (Sobolev *et al.* 2007); substantially increasing levels of resveratrol or increasing the number of stilbenes produced in crop plants may have concomitant effects on herbivores that, given the paucity of studies and the inconclusive nature of their findings, might be difficult to predict.

TABLES

Table 1. Vanderzant Vitamin solution prepared onsite. Ingredients mixed with mortar and pestle.

Vanderzant Vitamin Recipe	
Ingredient	**Grams**
Sucrose	598
Ascorbic acid	270
Calcium panthothenate	1
Thiamine HCL	0.25
Myo-inositol	20
Niacinamide	1
Pyridoxine HCL	0.25
Vitamin B12	2
Riboflavin	0.5
Folic acid	0.25
Choline	90.62
Biotin	0.02
Vitamin E (alpha tocopherol)	16

Table 2. Standard insect diet modified from Waldbauer *et al.* (1984).

Standard Insect Diet	
Group A	
Agar (g)	13
Water (mL)	550
Group B	
Vitamin-free casein (g)	31.5
Sucrose (g)	24
Wheat germ (g)	27
Wesson's Salt mix (g)	9
Alphacel (g)	10
Water (mL)	220
4 M KOH (mL)	5
Group C	
Vanderzant Vitamins* (g)	18
Sorbic Acid (g)	1.6
Methyl Paraben (g)	1.6
Ascorbic Acid (g)	3.2
Streptomycin (g)	0.12
Wheatgerm oil (mL)	4
10% Formaldehyde (mL)	2

*see Table 1

Table 3. Recipe for artificial diet optimized for *Manduca sexta* rearing (James and Joyce Nardi, UIUC).

Manduca Diet	
Distilled Water (mL)	1100
Agar (g)	24
Wheat Germ (g)	120
Casein (g)	54
Sucrose (g)	48
Wesson's Salts (g)	18
Yeast (g)	24
Cholesterol (g)	5.25
Sorbic Acid (g)	3
Methyl-p-hydroxy-benzoate (g)	3
Ascorbic Acid (g)	7.5
Streptomycin Sulfate (g)	0.3
Kanamycin Sulfate (g)	0.08
10% Formalin (mL)	35
Hornworm vitamin mixture* (mL)	15
Linseed oil (mL)	6

* see Table 4

Table 4. Recipe for vitamin mixture optimized for *Manduca sexta* artificial diet. (James Nardi, personal communication)

***Manduca sexta* Vitamin Mixture**	
Distilled Water (mL)	150
Nicotinic Acid (mg)	150
Riboflavin (mg)	75
Thiamine (mg)	35
Pyridoxine (mg)	35
Folic Acid (mg)	35
Biotin (mg)	3

Table 5. Effects of dietary resveratrol on performance of three species of Lepidoptera. Values are means; for each species respectively, values followed by the same letter are not significantly different. For two-way ANOVA, values followed by the same letter are not significantly different *within* each strain and values followed by the same symbol (*) represent significant differences *between* strains.

Statistical Analysis	Species	Treatment	Days to Pupation	Pupal Weight (mg)	Eggs Laid
Two way ANOVA	*A. transitella* (CPQ)	EtOH 175µL	19.1 ± 0.8 a	40.05 ± 1.3 a	188.26 ± 39.7 a
		80ng/g Resv	19.0 ± 2.0 a	44.19 ± 3.3 a	225.7 ± 87.8 a
		7µg/g Resv	18.9 ± 0.7 a	43.36 ± 0.6 a	234.2 ± 60.0 a*
		70µg/g Resv	19.6 ± 1.5 a	41.09 ± 0.9 a	203.8 ± 29.0 a
	A. transitella (FIG)	EtOH 175µL	22.3 ± 3.3 a	44.64 ± 2.0 a	54.0 ± 27.2 a
		80ng/g Resv	24.1 ± 4.5 a	45.35 ± 1.2 a	156.7 ± 41.3 a
		7µg/g Resv	22.3 ± 1.9 a	48.87 ± 2.7 a	64.0 ± 20.9 a*
		70µg/g Resv	24.0 ± 2.4 a	45.78 ± 3.4 a	111.9 ± 89.1 a
One way ANOVA	*T. ni*	EtOH 175µL	10.3 ± 0.1 a	233.8 ± 5.2 a	392.6 ± 144.0 a
		80ng/g Resv	10.0 ± 0.2 a	253.4 ± 2.9 a	172.4 ± 133.8 a
		7µg/g Resv	10.3 ± 0.2 a	245.6 ± 6.8 a	244.7 ± 106.5 a
		70µg/g Resv	10.2 ± 0.2 a	233.0 ± 5.7 a	205.6 ± 171.9 a
Student's t-test	*M. sexta*	EtOH 175µL	23.2 ± 0.3 a	2934.7 ± 78.3 a	-
		70µg/g Resv	23.7 ± 0.3 a	2702.6 ± 77.7 b	-

* values followed by the same symbol represent significant difference between strain

Table 6. Effects of resveratrol and PBO on life history traits of three lepidopterans. Values are means; means followed by the same letter are not significantly different *within* each treatment type (with and without PBO), values followed by the same symbol (*, ǂ) represent significant difference *between* treatments with and without PBO. This tables highlights the significant effects of PBO on *T. ni*, with significance stemming from the addition of PBO, particularly on Days to Death.

Species	Treatment	Time to Stage (days)				Pupal Weight (mg)	Eggs Laid	% Eggs Fertilized
		Pupation	*Eclosion*	*Death*	*Adult Lifespan*			
A. transitella (CPQ)	EtOH 175µL	25.0 ± 0.6 a	31.6 ± 1.8 a	46.3 ± 1.8 a	14.7 ± 0.2 a	41.27 ± 0.3 a	173.5 ± 1.2 a	81.4 ± 6.0 a
	EtOH + PBO	27.7 ± 2.1 a	33.9 ± 0.8 a	51.2 ± 2.1 a	17.3 ± 2.1 a	38.29 ± 2.5 a	255.6 ± 112.0 a	66.18 ± 20.1 a
	80ng/g Resv	22.3 ± 1.6 a	28.9 ± 1.6 a	43.5 ± 1.5 a	14.7 ± 1.3 a	42.17 ± 2.6 a	188.9 ± 53.2 a	77.87 ± 15.0 a
	80ng/g Resv + PBO	24.0 ± 1.4 a	29.2 ± 1.2 a	45.3 ± 1.0 a	16.1 ± 0.4 a	43.21 ± 1.8 a	208.5 ± 25.3 a	80.53 ± 5.2 a
	7µg/g Resv	24.0 ± 2.3 a	31.5 ± 2.3 a	49.0 ± 4.4 a	17.5 ± 2.2 a*	40.88 ± 3.0 a	110.2 ± 48.3 a	61.47 ± 6.9 a
	7µg/g Resv + PBO	23.7 ± 1.4 a	30.2 ± 0.8 a	43.7 ± 0.9 a	13.2 ± 0.3 a*	39.06 ± 0.7 a	249.8 ± 108.0 a	70.31 ± 4.4 a
	70µg/g Resv	23.7 ± 2.4 a	29.2 ± 1.8 a	44.3 ± 2.8 a	15.1 ± 1.2 a	40.22 ± 2.3 a	191.5 ± 13.9 a	81.65 ± 14.4 a
	70µg/g Resv + PBO	24.5 ± 0.4 a	32.5 ± 0.2 a	46.6 ± 0.8 a	14.1 ± 0.6 a	40.05 ± 2.1 a	213.5 ± 78.6 a	66.99 ± 11.8 a
T. ni	EtOH 175µL	10.9 ± 0.6 a	17.5 ± 0.6 a	22.5 ± 1.6 aǂ	5.0 ± 1.0 a	246.52 ± 6.0 a	21.7 ± 8.1 a	-
	EtOH + PBO	12.7 ± 0.5 a	19.1 ± 1.0 a	26.7 ± 1.3 aǂ	7.5 ± 0.5 a	217.81 ± 8.9 a	213.8 ± 121.3 a	-
	80ng/g Resv	10.8 ± 0.7 a	17.2 ± 0.5 a	21.6 ± 0.7 a*	4.4 ± 0.3 a*	247.89 ± 14.0 a	-	-
	80ng/g Resv + PBO	12.4 ± 0.7 a	18.8 ± 0.6 a	26.3 ± 1.3 a*	7.5 ± 1.4 a*	222.34 ± 12.0 a	76.7 ± 23.7 a	-
	7µg/g Resv	11.2 ± 0.3 a	18.0 ± 0.5 a	24.2 ± 0.5 a	6.3 ± 0.1 a	245.71 ± 7.6 a*	341.0 ± 234.1 a	-
	7µg/g Resv + PBO	12.0 ± 0.7 a	18.5 ± 0.9 a	25.6 ± 1.5 a	7.1 ± 0.9 a	214.37 ± 12.1 a*	76.5 ± 43.7 a	-
	70µg/g Resv	11.0 ± 0.6 a	17.5 ± 0.5 a	23.7 ± 1.7 a	6.2 ± 1.3 a	240.15 ± 9.0 a	135.0 ± 114.0 a	-
	70µg/g Resv + PBO	12.4 ± 0.9 a	19.3 ± 0.7 a	25.3 ± 0.9 a	6.0 ± 0.5 a	228.89 ± 6.6 a	66.0 ± 35.7 a	-

Table 7. Effect of resveratrol on life history traits after the removal of PBO treatments. Values are means. Re-analysis as one-way ANOVA; means followed by the same letter are not significantly different within each treatment type. No significant differences between treatments of resveratrol and the control indicates no effect on development of resveratrol.

Species	Treatment	Time to Stage (days)				Pupal Weight (mg)	Eggs Laid (log)	% Eggs Fertilized
		Pupation	*Eclosion*	*Death*	*Adult Lifespan*			
A. transitella (CPQ)	EtOH 175µL	25.0 ± 0.6 a	31.6 ± 1.8 a	46.3 ± 1.8 a	14.7 ± 0.2 a	41.27 ± 0.3 a	173.5 ± 1.2 a	81.4 ± 6.0 a
	80ng/g Resv	22.3 ± 1.6 a	28.9 ± 1.6 a	43.5 ± 1.5 a	14.7 ± 1.3 a	42.17 ± 2.6 a	188.9 ± 53.2 a	77.87 ± 15.0 a
	7µg/g Resv	24.0 ± 2.3 a	31.5 ± 2.3 a	49.0 ± 4.4 a	17.5 ± 2.2 a	40.88 ± 3.0 a	110.2 ± 48.3 a	61.47 ± 6.9 a
	70µg/g Resv	23.7 ± 2.4 a	29.2 ± 1.8 a	44.3 ± 2.8 a	15.1 ± 1.2 a	40.22 ± 2.3 a	191.5 ± 13.9 a	81.65 ± 14.4 a
T. ni	EtOH 175µL	10.9 ± 0.6 a	17.5 ± 0.6 a	22.5 ± 1.6 a	5.0 ± 1.0 a	246.52 ± 6.0 a	21.7 ± 8.1 a	-
	80ng/g Resv	10.8 ± 0.7 a	17.2 ± 0.5 a	21.6 ± 0.7 a	4.4 ± 0.3 a	247.89 ± 14.0 a	-	-
	7µg/g Resv	11.2 ± 0.3 a	18.0 ± 0.5 a	24.2 ± 0.5 a	6.3 ± 0.1 a	245.71 ± 7.6 a	341.0 ± 234.1 a	-
	70µg/g Resv	11.0 ± 0.6 a	17.5 ± 0.5 a	23.7 ± 1.7 a	6.2 ± 1.3 a	240.15 ± 9.0 a	135.0 ± 114.0 a	-

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