Journal of Economic Entomology, XX(XX), 2020, 1–7
doi: 10.1093/jee/toaa144
Research

Mortality of Potato Psyllid (Hemiptera: Triozidae) on Host Clippings Inoculated With Ergot Alkaloids

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Subject Editor: Blake Bextine

Received 22 March 2020; Editorial decision 10 June 2020

Abstract

Our previous study provided correlative evidence that morning glory species harboring endophytic fungi (*Periglandula*) are resistant to potato psyllid [*Bactericera cockerelli* (Šulc)], whereas species free of fungi often allowed psyllid development. In this study, we manipulated levels of ergot alkaloids in host tissues by inoculating clippings from potato plants with extracts from morning glories that harbor *Periglandula* [*Ipomoea leptophylla* Torrey, *Ipomoea imperati* (Vahl) Grisebach, *Ipomoea tricolor* Cavanilles, *Ipomoea pandurata* (L.) G. F. Meyer, and *Turbina corymbosa* (L.)] and one species (*Ipomoea alba* L.) that does not harbor the endophyte. Ergot alkaloids (clavines, lysergic acid amides, and ergopeptines) were detected in potato clippings, thus confirming that leaves had taken up compounds from solutions of crude extracts. Psyllid mortality rates on inoculated clippings ranged between 53 and 93% in treatments producing biochemically detectable levels of alkaloids, when compared with 15% mortality in water controls or the alkaloid-free *I. alba*. We then tested synthetic analogs from each of the three alkaloid classes that had been detected in the crude extracts. Each compound was assayed by inoculating clippings of two host species (potato and tomato) at increasing concentrations (0, 1, 10, and 100 µg/ml in solution). Psyllids exhibited a large and significant increase in mortality rate beginning at the lowest two concentrations, indicating that even very small quantities of these chemicals led to mortality. Feeding by nymphs on artificial diets containing synthetic compounds resulted in 100% mortality within 48 h, irrespective of compound. Further testing of ergot alkaloids to characterize the mode of action that leads to psyllid mortality is warranted.

Key words: Convolvulaceae, *Periglandula*, Clavicipitaceae, mutualism, potato psyllid

Several fungi in the Clavicipitaceae (Ascomycota) produce a class of bioactive compounds known as ergot alkaloids (Eich 2008, Steiner and Leistner 2018). The alkaloids are produced by fungi in association with plants either as pathogens or as mutualistic symbionts. Ergot alkaloids are generally categorized into one of the three groups (clavines, lysergic acid amides, and ergopeptines) based on structural characteristics of the tetracyclic ergoline ring common to this class of compounds (Panaccione et al. 2014, Young et al. 2015). Monocotyledonous plants (Poaceae, Cyperaceae, and Juncaceae) are particularly known for their symbiotic association with clavicipitaceous fungi, where production of ergot alkaloids by the fungi benefits the plants by protecting them against herbivores or by enhancing their ability to withstand certain abiotic stresses (Clay and Cheplick 1989, Malinowski and Belesky 2000, Brem and Leuchtmann 2002, Cheplick and Faeth 2009).

Plants in the morning glory family (Convolvulaceae) are unusual among the dicots in exhibiting a symbiotic association with Clavicipitaceae. These fungi, from the genus *Periglandula*, were described from two species of morning glories, *Ipomoea asarifolia* (Desr.) and *Turbina corymbosa* (L.) (Steiner et al. 2011). *Periglandula* has not been detected outside of the Convolvulaceae. The symbiosis leads to the production of several ergot alkaloids, with representatives from the same three classes that are found in the monocots. Chemical profiles differ extensively among morning glory taxa (Eich 2008) and may comprise different mixtures of clavines (e.g., chanoclavine, agroclavine, and lysergol), lysergic acid amides (ergonovine, lysergic acid α-hydroxyethylamide, and ergine), and ergopeptines (ergobalansine; Steiner et al. 2006, Markert et al. 2008, Beaulieu et al. 2013, Kaur et al. 2018). The *Periglandula* symbiosis appears to be most common in *Ipomoea* and closely related genera,

possibly encompassing more than 450 plant species worldwide (Eich 2008, Eserman et al. 2014).

Research showing that ergot alkaloids protect grasses from herbivory has led us to hypothesize that this same class of alkaloids produced by *Periglandula* fungi protect Convolvulaceae in a similar manner. Kaur et al. (2018) recently studied development of a psyllid, *Bactericera cockerelli* (Šulc), on 15 species of Convolvulaceae across four genera, and showed that psyllid nymphs developed successfully on some species but died rapidly on others. Although this psyllid is best known as a pest of Solanaceae (Martin 2008), it will develop on certain species of Convolvulaceae (Knowlton and Thomas 1934, Pletsch 1947, Puketapu and Roskruge 2011, Kaur et al. 2018). It is however not known why some Convolvulaceae allow psyllid survival, whereas others do not. Kaur et al. (2018) detected *Periglandula* in 11 of the 15 species assayed in their study, including in two genera (*Calystegia*, *Convolvulus*) not known to harbor the fungi. Developmental failure was found to correlate with presence of *Periglandula* and ergot alkaloids (Kaur et al. 2018). Successful development was observed only on species found to be free of fungi and alkaloids. These findings led Kaur et al. (2018) to hypothesize that ergot alkaloids have strong anti-psyllid properties.

Assays in which concentrations of specific compounds are experimentally manipulated would be useful to directly show effects of ergot alkaloids beyond the strictly correlative evidence found in Kaur et al. (2018). The objectives of this study were to examine the effects of ergot alkaloids on survival of *B. cockerelli* via inoculation of alkaloid-free leaf material or artificial diets containing these toxins. We first tested whether inoculation of alkaloid-free host (potato) foliage with crude extracts from psyllicidal Convolvulaceae prevents development of *B. cockerelli*. This trial was followed by assays using synthetic analogs of individual alkaloids to determine which specific compounds within the ergot alkaloid complex affected psyllid development. Two types of assays were conducted in which concentrations of individual compounds were manipulated. We first inoculated leaf clippings of two host plants (potato and tomato) with ergot alkaloids at a range of concentrations and determined the effects of concentration on survival of psyllids. These leaf assays were followed by a second set of assays in which compounds were incorporated into an artificial diet, and psyllid feeding, and mortality were measured.

Materials and Methods

Source of Insects

A colony of potato psyllids was started from insects collected in the summer and autumn of 2016 from potato or weedy Solanaceae near Wapato and Prosser, WA. The colony was maintained on potato (Russet Burbank cultivar) at 22°C and a long-day photoperiod (16:8 [L:D] h cycle). Insects were of the Northwestern haplotype, as confirmed using high-resolution melting analysis (Swisher et al. 2012). The colony was checked periodically for the presence of *Candidatus* Liberibacter solanacearum (causal agent of zebra chip disease in potato) using PCR (Jagoueix et al. 1996). Pathogen-free psyllids were used in all assays.

Inoculation of Foliage with Crude Extracts

We first assayed crude extracts from known alkaloid-rich Convolvulaceae to confirm that clippings of the potato host would absorb psyllicidal compounds from alkaloid-rich solutions. This proof-of-concept trial evaluated six species of Convolvulaceae that differed in both alkaloid profile (Eich 2008) and in whether or not they supported psyllid development (Kaur et al. 2018): *Ipomoea imperati* (Vahl) Grisebach (South Padre Island, TX), *Ipomoea leptophylla* Torrey (Georgia Vines, Claxton, GA), *Ipomoea pandurata* (L.) G. F. Meyer (Georgia Vines, Claxton, GA), *Ipomoea tricolor* Cavanilles (J.L. Hudson, Seedsman, LaHonda, CA), *Ipomoea alba* L. (The Sample Seed Shop, Buffalo, NY), and *T. corymbosa* (L.) Rafinesque (J.L. Hudson, Seedsman, LaHonda, CA). *Ipomoea imperati*, *I. leptophylla*, *I. pandurata*, *I. tricolor*, and *T. corymbosa* harbor *Periglandula* and ergot alkaloids and do not allow the development of potato psyllid (Kaur et al. 2018). Our control species, *I. alba*, is free of both *Periglandula* and ergot alkaloids and is a highly suitable host for the potato psyllid (Kaur et al. 2018). Morning glory seeds were scarified with sandpaper, then soaked for 24 h in gibberellic acid (1,000 µg/ml in water) before planting. Seedlings were grown in 10-cm pots containing four parts commercial potting soil (Miracle-Gro Moisture Control Potting Mix, Scotts Co., Marysville, OH), one-part clean sand, and one-part perlite (Miracle-Gro Perlite, Scotts Co.). Plants were grown in a greenhouse under ambient light supplemented with grow lights following a light:dark cycle of 16:8 h. A fully expanded leaf was collected from each plant and dried at room temperature (22–25°C) for 3–5 d in preparation for extraction. Approximately 1-g dried plant sample was ground in 95% HPLC grade methanol (Thermo Fisher Scientific, Waltham, MA) using a mortar and pestle. Homogenate was then filtered through a 0.2 µm HT Tuffyrn Membrane (Pall Corporation, Port Washington, NY). The methanol was evaporated off and the filtrate was reconstituted using 1 ml of water. The crude extracts were stored in amber vials at 4°C until insect bioassays were performed.

Clippings from potato ('Russet Burbank', Skone and Conner, Eltopia, WA) were inoculated with ergot alkaloids by immersing cut ends in solutions of crude extract. The clippings were taken from plants grown under the same conditions used in rearing the five morning glory species. Each clipping was enclosed in an arena constructed of a plastic solo-cup (1 fl oz; 29.5 ml) covered with a vented lid (Fig. 1). A small hole was made at the bottom of each cup to accommodate the stem end of the clipping. A hole of similar size was

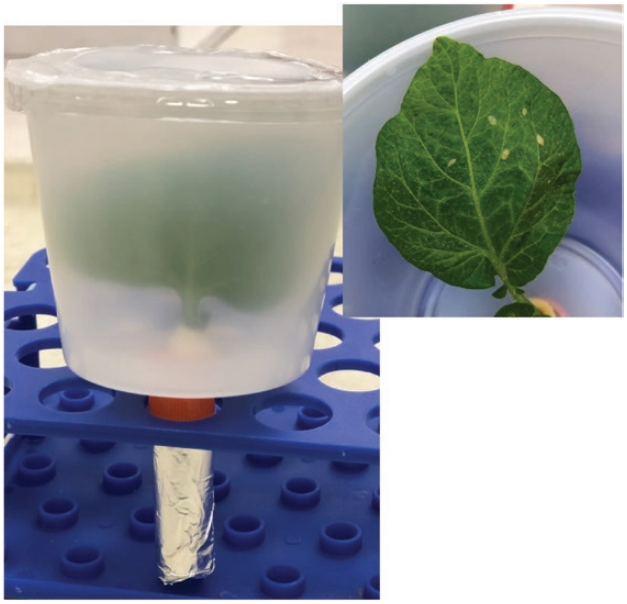

Fig. 1. Ventilated cup arena modified from Shymanovich et al. (2015) used in testing extract- or alkaloid-inoculated potato clippings for effects on mortality of potato psyllid.

made in the screw top from a 1.5-ml plastic vial. The screw top was glued to the bottom of the plastic cup. A 1.5-ml vial containing 1 ml of extract or 1 ml of water (control) was attached to the screw top vial. The cut end of each clipping was fully immersed in liquid within each vial. A small piece of sponge was wrapped around the stem of each clipping at the cup–vial junction to minimize physical damage to stem and to prevent insect escape.

Assays were conducted in controlled environment rooms with conditions set to 22°C and a light:dark cycle of 16:8 h. Once respective treatments were administered to leaf clippings, nymphs were added immediately. Psyllid nymphs (third- to fourth-instar stage) were collected from colonies and placed on the leaflets using a small paint brush. We assayed five nymphs per arena. Each treatment (six morning glory species plus water control) was replicated three times. Numbers of live and dead nymphs were recorded every 24 h for 3 d. Dead nymphs appeared desiccated and were often found off the leaf surface at the bottom of the cup. At the end of the 3-d assay, the clippings were collected and air-dried at room temperature for 1–2 d. The dried material was evaluated for ergot alkaloid content (see below) to confirm that the clippings had taken up the ergot alkaloid solutions.

Inoculation with Pure Compounds

Synthesized compounds from each of the three classes of ergot alkaloids previously detected in crude extracts were evaluated for their effect on mortality of *B. cockerelli*. The five compounds included two clavines (agroclavine and lysergol), one lysergic acid amide (ergonovine maleate), and two ergopeptines (ergocornine and ergosine). Agroclavine, ergocornine, and ergosine were obtained from Romer Labs (Newark, DE). Lysergol was obtained from Toronto Research Chemicals (North York, ON, Canada) and ergonovine maleate was procured from Sigma–Aldrich (St. Louis, MO). Each compound was assayed at four concentrations: 100, 10, 1, and 0 µg/ml (control) in 1 ml of distilled water. Two assays were conducted: 1) inoculation of host clippings and 2) inoculation of artificial diet.

Inoculation of Potato and Tomato Clippings

We inoculated foliage with individual ergot alkaloid compounds by immersing cut ends of host clippings into solutions using the methods developed in examining crude extracts. Leaf clippings from both potato ('Russet Burbank') and tomato ('Beefsteak Tomato' Botanical Interests, Inc. Broomfield, CO) plants were assayed. Three replicates were examined for each combination of host plant (potato vs tomato), type of alkaloid, and alkaloid concentration. Five nymphs (third- to fourth-instar stage) were placed on each clipping using the methods described above. Insect mortality was recorded every 24 h for 3 d.

Inoculation of Artificial Diet

A second test examined the effects of synthetic analogues by incorporating the compounds into a sucrose-based artificial diet enclosed inside feeding plates placed upside down (Jimenez et al. 2017). For each feeding arena, a parafilm sheet was stretched over the bottom of a 10-cm-diameter petri dish. Edges of the parafilm were secured by pulling the corners of film over the sides of the dish. Three milliliters of a 30% sucrose diet (in water) inoculated with alkaloids at each of the four rates used in the leaf clipping assay was dispensed under the parafilm. Four nymphs (third- to fourth-instar stage) were confined to each arena beneath the petri dish lid. A screen spline with a diameter of 4.8 mm was used to create a space between the petri dish lid and the parafilm. At 24 h, nymphs were examined to determine what percentage were feeding (insects with stylets penetrating the parafilm to reach the diet) and to monitor mortality (individuals were dried-up, had distorted molting, and were usually on their backs). Control plates contained 30% sucrose only. Three replications were evaluated per alkaloid and concentration.

Biochemical Analysis

To verify that host clippings had taken up ergot alkaloids from solutions composed of crude extracts or of commercially obtained pure compounds, samples of clippings from the inoculation trials were evaluated for ergot alkaloid content. Subsamples of plant material from the leaf inoculation assays were collected following each assay and air-dried for 3–5 d. The dried tissues were then ground using a mortar and pestle and extracted according to details as described in Kaur et al. (2018). Samples were analyzed for ergoline and ergopeptide compounds via LC–MS/MS and multiple-reaction monitoring following parameters as specified in Sulyok et al. (2007) and Kaur et al. (2018) using an ABI/SCIEX 3200 QTRAP LC–MS/MS system (Applied Biosystems, Foster City, CA) coupled with a Perkin Elmer (Waltham, MA) Series 200 HPLC for compound separation.

The limits of detection and quantification were maintained as in Kaur et al. (2018), with the addition of ergovaline (1, 2 ng/ml); elymoclavine (1, 5 ng/ml); fumigaclavine (5, 5 ng/ml); and oxidized luol (50, 100 ng/ml). No commercial standards were available for chanoclavine, festuclavine, ergine, dihydrolysergol, dihydroergosine, and dihydroergotamine. These ergot alkaloids were compared using a scale of '++' indicating a large peak area (≥5,000 counts per second [cps]) and '+' (present but <5,000 cps) among the plant species extracted based on relative peak area for all samples.

Sources of Chemicals for LC–MS/MS

Solvents (acetonitrile, methanol [LC–MS grade]; acetic acid [OmniTrace Ultra]) were procured from EMD Millipore (Darmstadt, Germany), while ammonium acetate (>99.0%, HPLC grade) was purchased from Sigma–Aldrich. Ergot alkaloid standards were obtained from Romer Labs (Tulln, Austria; biopure mix 6-ergocornine, ergocristine, α-ergocryptine, ergometrine, ergosine, and ergotamine), Sigma–Aldrich (ergonovine, agroclavine, lysergic acid, and lysergol), and Bioviotica (Dransfeld, Germany; fumigaclavine). Elymoclavine and oxidized luol were procured from Dr. Miroslav Flieger (Academy of Sciences of the Czech Republic, Prague). Ergovaline tartrate (>98% purity) was synthesized by Dr. Forrest Smith of Auburn University. Ultrapure 18 mΩ/cm water was obtained from an Elga (Marlow, Buckinghamshire, UK) PURELAB Ultra Genetic system.

Statistical Analyses

The proportion of nymphs that had died by the end of each 3-d assay was compared among treatments by logistic regression (PROC GLIMMIX; SAS Institute 2013). We specified a binomial response and logit link and modeled the response variable using the 'events/trials' syntax (numbers dead/numbers assayed) in each MODEL statement. Means were back-transformed into proportions using the ILINK option in the LSMEANS statement. The treatment factor for the crude extract assay was plant source having seven levels (six species plus water control). We extracted single degrees of freedom (df) contrasts to compare mortality rates between plant species, using results of the biochemistry analyses to define comparisons of interest (see Results). Comparisons were specified using CONTRAST statements (SAS Institute 2013). Assays with pure compounds were analyzed as a 2 × 4 factorial design (2 plant species [potato vs tomato]

× 4 concentrations [0, 1, 10, and 100 µg of compound per ml of water]). A statistical comparison of compounds was not possible in this analysis because the different compounds were assayed on different dates due to logistic limitation (i.e., availability of space and insects). To examine concentration effects, we estimated linear and quadratic effects of solution concentration by use of polynomial contrasts. Because concentration levels (0, 1, 10, 100) were not equally spaced, we used the ORPOL function in PROC IML (SAS 2013) to obtain the coefficients necessary for the CONTRAST statement.

Results

Inoculation with Crude Plant Extracts

No phytotoxic effects (visual signs of leaf discoloration or distortion) of extracts were observed in clippings from any of the assays. Biochemical analyses detected three classes of ergot alkaloids in the inoculated potato clippings, confirming that potato clippings did take up alkaloids from solutions of crude extracts (Table 1): Clavines (chanoclavine, agroclavine, lysergol, elymoclavine), a lysergic acid amide (ergonovine), and ergopeptines (ergotamine, α-ergocryptine). None of the other compounds screened for in clippings were detected (see full list of targeted compounds in Materials and Methods). No alkaloids were detected in clippings that had been inoculated with water or with crude extracts from *I. alba* or *I. tricolor* (Table 1). Chemical profiles differed among plant species used as the source of crude extracts (Table 1). Detectable levels of all three classes were found in clippings inoculated with extracts from *T. corymbosa*. Clippings inoculated with *I. imperati* or *T. corymbosa* also had detectable levels of a lysergic acid amide (ergonovine in trace amounts). Ergopeptines were detected in clippings inoculated with extracts from *I. leptophylla* and *T. corymbosa*. Only clavines were detected from the clippings inoculated with *I. pandurata* extracts.

Mean proportion of psyllid nymphs that were dead at 3 d was significantly affected by source of extract (Fig. 2; $F = 4.17$; df = 6, 14; $P = 0.013$). Live nymphs were actively feeding as shown by the presence of honeydew near the feeding nymph and at the bottom of the assay chambers. Dead nymphs were desiccated and had a distorted body appearance. The colored boxes above the bar chart in Fig. 2 show which alkaloid classes were present in leaf clippings (summarized from data in Table 1). The dark red boxes indicate the presence of compounds corresponding to peak areas ≥5,000 cps, whereas the lighter boxes indicate presence of compounds that exhibited peak areas ≤5,000 cps or that were quantified at a level below 1 µg/ml. Mortality rates appear to have been the highest when high amounts of clavines were present in the clippings (Fig. 2: colored boxes). We used the biochemical results to guide additional statistical tests of psyllid mortality; tests were done by extraction of single-degree of freedom contrasts. Mean psyllid mortality was similar between the water control and the two morning glory species (*I. alba*, *I. tricolor*) that failed to produce detectable levels of alkaloids in clippings (Fig. 2; $F = 0.62$; df = 1, 14; $P = 0.44$). Mortality rates were significantly higher in the four treatments in which alkaloids were detected (*I. leptophylla*, *I. pandurata*, *I. imperati*, and *T. corymbosa*) than in the two plant treatments (*I. alba*, *I. tricolor*) that did not produce detectable levels of alkaloids ($F = 15.92$; df = 1, 14; $P = 0.001$). The presence of clavines (*I. pandurata*, *I. imperati*, *T. corymbosa*) caused significant increases in mortality compared with treatments (*I. alba*, *I. tricolor*, and *I. leptophylla*) that did not produce detectable levels of clavines ($F = 15.90$; df = 1, 14; $P = 0.001$).

Inoculation with Pure Compounds

Leaf Clippings

To verify that leaf clippings placed in solutions of pure synthetic compounds would take up the compounds, samples of potato clippings inoculated with 100 µg of compound per ml of water were analyzed biochemically. In addition, we wanted to be certain that the targeted alkaloids were the only compounds present in treated clippings. Ergot alkaloids may rapidly break down into other metabolites during the biosynthesis process (Florea et al. 2017), may be modified *in planta*, or may epimerize based upon the solvent they are dissolved in (Smith and Shappell 2002). To ensure that we optimized our chances of detecting measurable quantities of treatment compounds—and thus could demonstrate unequivocally that clippings in solution did indeed absorb the targeted compound—we analyzed clippings from the high concentration (100 µg/ml) treatment. Clippings that had been inoculated with agroclavine, lysergol, or ergonovine maleate harbored detectable levels only of agroclavine (3.1 µg of compound per g of dried host tissue), lysergol (20.4 µg/g), or ergonovine (19.8 µg/g), respectively. Clippings that had been inoculated with ergocornine harbored ergocornine (10.9 µg/g) plus a trace amount (0.2 µg/g) of ergonovine, whereas clippings inoculated with ergosine harbored ergosine (0.9 µg/g) and a trace amount of ergonovine (0.09 µg/g). Ergonovine was present in the latter two samples most likely as a breakdown product from the targeted compounds (Florea et al. 2017). No ergot alkaloids were detected in clippings inoculated with water.

The main effect of host plant (potato vs tomato) and the interaction of host plant and concentration were nonsignificant in all statistical analyses; thus, the main effect means for the concentration treatment are presented (Fig. 3). The mean proportion of psyllid nymphs that were dead at the end of the 3-d assay increased significantly with solution concentration for four of the five compounds (Fig. 3 and associated *F*-tests). Agroclavine alone failed to produce a statistically significant increase in mortality. We extracted single

Table 1. Detection of ergot alkaloids (ng/g) in host (potato) clipping inoculated with crude extracts of morning glory plants (symbol ++ = large peaks ≥5,000 cps, symbol + = present but below the level of quantification ≤5,000 cps) and (symbol – = not detected)

	Clavines				Lysergic acid amide	Ergopeptine	
Source of crude extracts	Chanoclavine	Agroclavine	Lysergol	Elymoclavine	Ergonovine	Ergotamine	α-Ergocryptine
Water (control)	–	–	–	–	–	–	–
Ipomoea alba	–	–	–	–	–	–	–
Ipomoea tricolor	–	–	–	–	–	–	–
Ipomoea leptophylla	–	–	–	–	141	11	–
Ipomoea pandurata	–	130	26	++	–	–	–
Ipomoea imperati	–	128	45	++	80	–	–
Turbina corymbosa	++	–	34	++	40	30	12

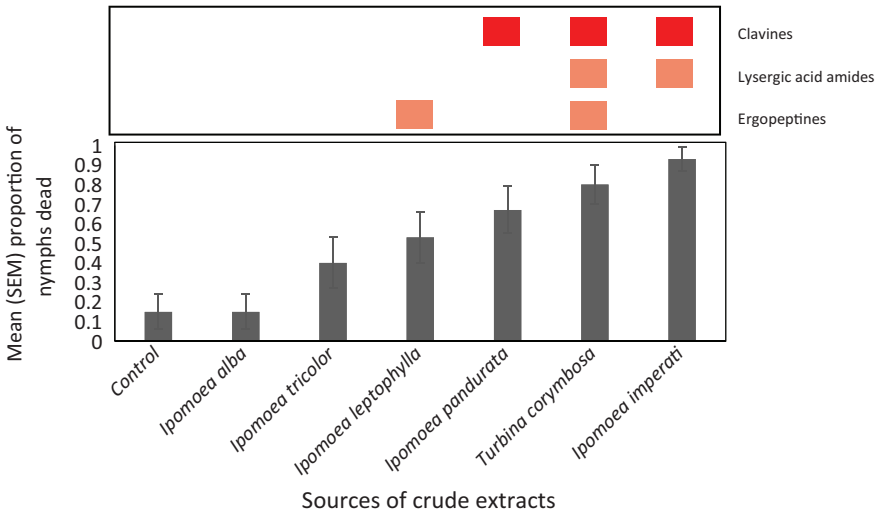

Fig. 2. Mean proportion (±SEM) of potato psyllid nymphs dead after 3 d on potato clippings inoculated with crude extracts from Convolvulaceae. Colored boxes above histograms show which classes of ergot alkaloids were in clippings (from data in Table 1; the dark red boxes indicate the presence of compounds corresponding to peak areas ≥5,000 cps, whereas the lighter boxes indicate presence of compounds that exhibited peak areas ≤5,000 cps or that were quantified at a level below 1 µg/ml [for color figure refer online version]).

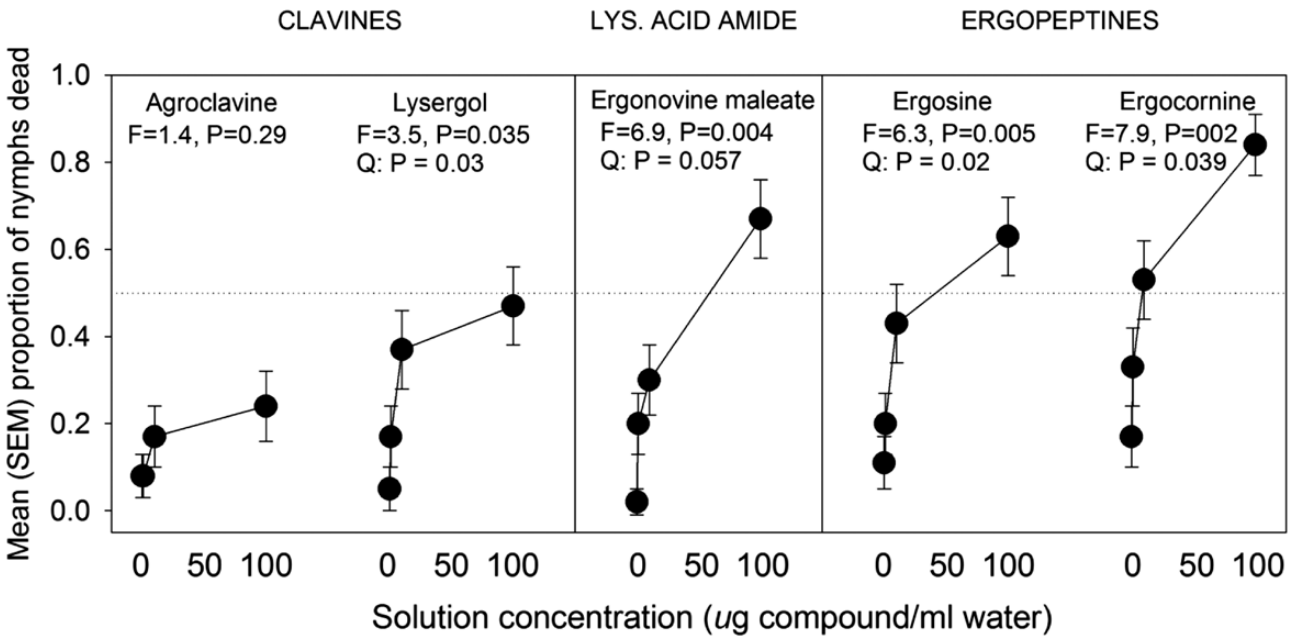

Fig. 3. Mean proportion (±SEM) of potato psyllid nymphs dead after 3 d on host clippings inoculated with synthesized ergot alkaloids. Four solution concentrations were assayed for each compound: 0, 1, 10, and 100 µg of compound per ml of water. *F*- and *P*-statistics are from analysis of variance comparing mean mortality rates across four concentrations. *Q*: significance of quadratic effect extracted as single df contrast.

df contrasts to examine quadratic effects of concentration (Fig. 3). Mortality increased as a quadratic function of solution concentration for all four of the compounds that had been found to exhibit significant concentration effects in the full model (*P*-statistics for quadratic effects shown below ANOVA statistics in Fig. 3). The line graphs show that mortality rates exhibited a large increase between the 0 and 10 µg/ml concentrations followed by a smaller uptick between the two highest concentrations (10 and 100 µg/ml solutions). This trend is consistent with the presence of statistically significant quadratic effects.

Artificial Diet

We observed 100% mortality of nymphs within 48 h for all five compounds at all concentrations (100, 10, and 1 µg/ml); therefore, no statistical tests of concentration effects were conducted. Nymphs were typically desiccated and deformed; several of the dead insects appeared to have died during the molting process. In control dishes (no alkaloids), nymphs were active and healthy and often were observed to have their stylets inserted in the artificial diet.

Discussion

A recent meta-analysis showed that entomopathogenic and nonentomopathogenic fungal endophytes in plants may act as plant bodyguards by producing secondary metabolites that protect the hosts from herbivory (Gange et al. 2019). The adverse effects of these metabolites are particularly noticeable against phloem-feeding insects, presumably because these insects feed in the plant tissues in which these metabolites concentrate (Gange et al. 2019). In a previous study, we examined survival of a phloem-feeding insect (the potato psyllid, *B. cockerelli*) on multiple species of Convolvulaceae and found that egg-to-adult development occurred only on plant species not harboring detectable levels of ergot alkaloids (Kaur et al. 2018). These alkaloids in the Convolvulaceae are produced by the fungal endophyte *Periglandula*; the plant species that lack the fungus also lack ergot alkaloids (Beaulieu et al. 2013, Kaur et al. 2018). Kaur et al. (2018) provided correlative evidence that the presence of the fungal endophyte produces ergot alkaloids that protects plants in the Convolvulaceae against insect herbivory.

In this study, we manipulated levels of ergot alkaloids in host plant tissues to determine whether we could directly confirm the correlative results of Kaur et al. (2018). We showed that host (potato) clippings inoculated with extracts from endophyte-infected Convolvulaceae led to significant increases in mortality rates of potato psyllid (Fig. 2). Biochemical analyses showed that clippings inoculated with crude extracts from *I. leptophylla*, *I. pandurata*, *T. corymbosa*, and *I. imperati* harbored detectable levels of ergot alkaloids in three biochemical classes. Nymphal mortality rates were significantly higher in these treatments than on the alkaloid-free clippings produced from extracts of *I. alba* and *I. tricolor* or water controls (Fig. 2). Only one other study has examined the effects of Convolvulaceae and ergot alkaloids on herbivorous insects by experimentally manipulating insect diet. Amor-Prats and Harborne (1993) showed that crude extracts of *Ipomoea parasitica* (HBK) G Don added to an artificial diet led to reduced feeding and digestive efficiency of the *Heliothis virescens* (Fabr.) larvae compared with those on an extract-free diet. As in the present study, the primary compounds detected in extracts were clavines (lysergol and elymoclavine; Table 1).

Results of the crude extract study (Fig. 2) suggested that trace amounts of lysergic acid amides or ergopeptines in clippings that also harbored clavines did not lead to noticeable increases in psyllid mortality beyond that produced by the clavines alone (Fig. 2). Thus, there was no statistical difference in mortality rates of psyllids reared on *T. corymbosa* or *I. imperati* (both plant species harboring a mixture of alkaloid classes; Fig. 2) versus psyllids reared on a species (*I. pandurata*) harboring clavines only (Fig. 2). It is difficult to know from these results whether nonclavine compounds did not affect potato psyllid or whether concentrations were too low in clippings to produce an effect beyond that produced by the more abundant clavines. Clay and Cheplick (1989) showed that fall armyworm [*Spodoptera frugiperda* (Smith)] exhibited reduced fitness on artificial diets inoculated with ergopeptines as compared to diets containing clavines or lysergic acid amides; thus, there is indeed evidence that compounds other than clavines can have biologically significant insecticidal effects. To evaluate this question for the potato psyllid, we assayed pure compounds individually from all three alkaloid classes in a series of dose–mortality assays. Synthetic analogs of lysergol, ergonovine maleate, ergosine, and ergocornine all caused psyllid mortality in a dose-dependent manner (Fig. 3). Biochemical analysis on clippings used during bioassays confirmed uptake of compounds used to inoculate these clippings. Clippings were found to harbor only the targeted compounds and minimal concentrations of their breakdown products. The relationship between mortality rates and concentration was quadratic in appearance due to a large increase in mortality at the lowest two concentrations, followed by a flattening out of the dose–response curve at the highest concentration (Fig 3). Shymanovich et al. (2015) supplemented clippings of oats with ergonovine and produced oat leaves harboring concentrations of this compound at much lower levels than what occur naturally in endophyte-infected plants (inoculated leaves had levels of ergonovine approximately 0.1% of what was found in naturally growing oat plants). Despite these low concentrations, inoculated oat leaves exhibited insecticidal properties against the bird-cherry oat aphid, *Rhopalosiphum padi* (L.). These results, combined with our dose–mortality study, suggest that individual ergot alkaloids from multiple classes can have insecticidal properties against phloem-feeding insects even at very low concentrations.

Ergot alkaloids are known to cause vasoconstriction, uncontrolled muscle contraction, and disturbance in the central nervous and reproductive systems in mammals by acting as agonists or antagonists of the neurotransmitters serotonin, dopamine, adrenaline, and noradrenaline (Panaccione et al. 2014, Steiner and Leistner 2018). We consistently observed abnormalities in molting of psyllid nymphs in our tests that should be investigated to determine physiological or behavioral modes of action of these compounds against psyllids and other insect herbivores. Some fungal alkaloids are known to induce insect juvenile hormone biosynthesis (Hoffmann and Lorenz 1998), causing disruption during the molting process due to their effects on the biosynthesis and function of insect growth hormones. It is possible that a similar mode of action is activated after ingestion of ergot alkaloids by insect herbivores. An understanding of how these compounds affect psyllids and other herbivores would be a first step toward developing uses for these products in pest control programs.

Acknowledgments

We thank Sally Longoria, Jen Stout, Heather Headrick, and Katie Wentz for technical assistance. We also thank Todd Wixson, IR4 USDA-ARS Wapato, WA, for guidance on the purchase of ergot alkaloids for research use at our facility. We are grateful to Andy Jensen and Karol Krey for reviews of the manuscript. This work was supported by the ARS Federal-State Partnership Potato Research Grants program, the Northwest Potato Research Consortium, and USDA-NIFA-SCRI (#2015-51181-24292).

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