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Original article

Laboratory studies on the photostability of fumagillin, the active ingredient of Fumidil B[1]

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Abstract – Fumagillin (as the dicyclohexylammonium salt) has been found to be extremely unstable when solutions in 50% ethanol are exposed to sunlight in small vials, undergoing a series of degradations with half-lives in the range of seconds to minutes. Similar results were obtained with photolyses of Fumidil B in either 50% ethanol or sugar syrup. Decomposition also occurred in fluorescent room light. Exposure of fumagillin to sunlight for three days caused almost complete disappearance of UV absorption of fumagillin and all its immediate photoproducts. The reactions involved in the photolyses are apparently reversible *Z:E* rearrangements in the unsaturated ester portion of the molecule, and do not involve the pharmacologically active moiety. Samples of fumagillin in syrup, irradiated for 0, 0.5, 5, 30, or 360 minutes were all effective in protecting caged bees from nosema disease. While long exposures to sunlight probably should be avoided, brief exposure causes no obvious loss of activity.

fumagillin / stability / photolysis / nosema disease / *Apis mellifera*

1. INTRODUCTION

Fumagillin (Fig. 1) is an antibiotic isolated from *Aspergillus fumigatus*. The production and properties were first described in 1951 (Eble and Hanson, 1951; McCowen et al., 1951), and the structure was published by Tarbell et al. (1961). It was first reported to be active against nosema disease of honey bees (*Apis mellifera* L.) in the early 1950s (Katznelson and Jamieson, 1952; Bailey, 1953a, b). Subsequent reports of work (see Goetze and Zeutzschel, 1959) indicated that fumagillin was effective in improving survival of nosema-infected colonies and increasing honey yields, but some residual infection usually remained.

Different methods of application have been used: syrup (Furgala and Gochnauer, 1969), powdered sugar (Wyborn and McCutcheon, 1987; Szabo and Heikel, 1987), and extender patties (Williams, 1973). A formulation of dicyclohexylammonium fumagillin was introduced commercially in 1954 as 'Fumidil B' for the treatment of *Nosema apis* Zander, the pathogenic agent of nosema disease (Cmejla and Otto, 1955). Syrup treatments have generally been shown to be most effective, and are the method of administration listed on the Fumidil B label.

Stability under various laboratory conditions was discussed in a series of papers in 1954 (Garrett and Eble, 1954; Eble and Garrett,

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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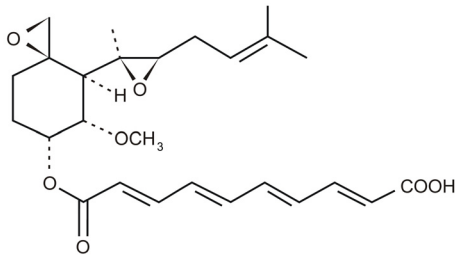

Figure 1. Fumagillin.

1954; Garrett, 1954; Assil and Sporns, 1991). Fumagillin was shown to decompose on exposure to intense (carbon arc, high in UV) light, both in solution and as the solid, with UV absorbance of the total sample at 351 nm decreasing with a half-life of about 12 hours in solution. Absorbance of the solid decreased to a constant level about 80% of the initial value over about 50 hours. Biological activity in a *Staphylococcus aureus* bacteriophage assay also decreased, although the rate was not clear from the data presented.

The Fumidil B label recommends "Store in a dark place at temperatures not to exceed 86°F" (30 °C). There is no mention of sensitivity of Fumidil solutions to light, nor does the bee literature indicate any studies of the effectiveness of the irradiated material against nosema disease. Anecdotal reports (Nasr, unpublished data) suggested that feeding bees sugar syrup medicated with fumagillin in outdoor bulk feeders failed to control nosema disease. To clarify this question, we began a study of fumagillin photostability and the effect of light on fumagillin activity against nosema disease.

2. MATERIALS AND METHODS

2.1. Materials

A laboratory standard of dicyclohexylammonium fumagillin (Abbot, Agricultural and Veterinary Products Division, North Chicago IL, code 32540, lot 53-120-CD) was in laboratory stock. The labeled fumagillin assay was 720 µg/mg; by calculation, pure dicyclohexylammonium fumagillin is 717 µg/mg free fumagillin by weight. It had been stored at ≤ 5 °C in a brown bottle and showed a single peak in the 10–15 minute range on HPLC analysis (see below). References to 'fumagillin' in any solution preparation refer to this material unless otherwise stated, and concentrations in this paper are expressed as this dicyclohexylammonium salt.

Fumidil B, lot 700721, was manufactured by Rhône Mérieux, Canada, Inc. (Victoriaville, Quebec) for Mid-Continent Agrimarketing, Inc. (Overland Park, KS). The nominal concentration of fumagillin in Fumidil B was 20 mg/g (Furgala and Boch, 1970). It consisted of a mixture of fine light tan powder and larger brown granules. HPLC assay of manually separated samples of the granules and powder under the conditions below showed that the granules contained 24.6% dicyclohexylammonium fumagillin by weight, while the powder was free from fumagillin. There were no obvious photoproducts present. The material was mixed as thoroughly as possible before sampling. Label directions require the use of 75–100 mg fumagillin activity/gallon, which corresponds to 27–36 µg/mL of dicyclohexylammonium fumagillin.

2.2. Analysis

HPLC analyses were carried out on a 4.6 mm × 250 mm column packed with C_{18} silica gel (Supelcosil LC-18-DB, 5 µm particle size, Supelco, Inc., Bellefonte, PA), using a 20 µL injector loop. The eluent was a mixture of acetonitrile/water/acetic acid in a ratio of 500:500:1.5 by volume (Brackett et al., 1988; Assil and Sporns, 1991). Flow rate was 1.5 mL/min. Fumagillin concentrations were determined with a SpectraSYSTEM UV2000 detector attached to an SP4400 integrator (Thermo Separation Products, San Jose, CA). Fumagillin and its decomposition products were determined at a wavelength of 350 nm. Concentrations of fumagillin were determined by comparison of detector peak areas with those produced by a series of known standards. It was assumed that the photoproducts had the same absorbance properties as fumagillin, which is probably true only to a limited extent.

Acetonitrile (HPLC grade) was obtained from Aldrich and 95% ethanol (USP grade) from Warner-Graham Co. (Cockeysville, MD). Water was purified with a NANOpure apparatus (Barnstead, Dubuque, IA). Sugar for syrup preparation was a commercial grade from our bee-feeding supply (Classic Granulated Cane Sugar, Sysco Corp., Houston, TX).

The light meter used was a cal-LIGHT 400 broad range lux/FC meter (Cooke Corp., Auburn Hills, MI) with nominal linear readings from 0.1 to 400 000 lux. Readings are compared below to old readings from a nearby site measured in W/m^2.

Data reduction, statistical analysis, and plotting were performed with GraphPad Prism ver. 3.02 (GraphPad Software, Inc. San Diego, CA).

2.3. Photolysis

2.3.1. Photolysis of fumagillin in 50% ethanol

Fumagillin analytical standard (20 mg) was dissolved in 50% ethanol to a total volume of 50 mL (400 μg/mL). For early runs, aliquots of this solution (100 μL) were added to 4-mL vials (foil lined caps) containing 50% ethanol (900 μL) to give the analytical solution containing 40 μg/mL in 50% ethanol. Dilutions were carried out in a room with the lights out. The only light came through the partially open door from the hall (intensity 4–4.5 lux). In order to reduce possible intersample variations during later runs, a larger volume of diluted solution was prepared and aliquoted into vials. Vials were exposed to full sunlight outdoors for varying lengths of time (see below), wrapped in aluminum foil to prevent further exposure to light, and removed to the laboratory for HPLC analysis. For irradiation times of 1 minute or less, the vials were placed in holes bored in an aluminum block, covered with aluminum foil, and placed in the shade of a cardboard box. Individual vials were removed and held by hand in sunlight for the desired time, then replaced in the block. For longer times, vials were placed on a tilted wooden rack painted white, covered with aluminum foil, carried outside, and the foil removed. At the desired times, vials were removed from the rack, wrapped in aluminum foil, and brought into the laboratory. This irradiation and the others below, with the exception of the preparative irradiation in Section 2.3.6, were done in April.

2.3.2. Photolysis of Fumidil B in 50% ethanol

Fumidil B (1.016 g, calculated equivalent to 20 mg fumagillin) was dissolved in water and a little ethanol for foam suppression and diluted with water to 50 mL. A 100 μL aliquot of this solution was diluted with 900 μL of 50% ethanol. Analysis of this sample by HPLC showed 81.2 μg/mL, so the sample was diluted at 50:950 μL instead of 100:900. This corresponded to ca. 40 μg/mL fumagillin. The variation between samples seemed larger than for the fumagillin sample and the solution was cloudy, so the stock solution was filtered through a syringe filter (0.45 μ). This solution was diluted into vials under the lighting conditions above and exposed to sunlight as above.

2.3.3. Photolysis of Fumidil B in syrup

Filtered Fumidil B stock solution from 2.3.2 (1 mL) was diluted with 50% (w/w) syrup (19 mL). The solution was stirred magnetically for five minutes and dispensed into vials for irradiation.

2.3.4. Photolysis of fumagillin in room light

A solution of fumagillin stock solution (0.5 mL) in 50% ethanol (4.5 mL) was prepared in a scintillation vial and wrapped with aluminum foil. At time zero, the aluminum foil was removed. Exposure was to normal fluorescent room light plus daylight from the windows (1.15 klux). Samples were removed at 1–2 minute intervals (timed with a stopwatch) up to 10 minutes and transferred to sample vials wrapped with aluminum foil. After these samples were taken, further injections were made in real time at 20-min intervals up to 100 minutes, then every 30 minutes up to six hours. The cap was left off the scintillation vial between injections.

2.3.5. Test for photoequilibrium during irradiation

Dicyclohexylammonium fumagillin (2 mg) was dissolved in 50% ethanol (2 mL) in room light. HPLC of a 1:24 dilution of this solution showed only 0.5% photoproduct. This stock solution was exposed to sunlight (95.4 klux) for 4 minutes with continuous agitation, giving a solution of 50% starting material and three main photoproducts. This solution (20 μL) was separated by HPLC into two fractions, the starting material (11.9–12.6 min) and a mixture of three products (12.8–15.5 min). Each of these fractions was again irradiated for 5 minutes at 102.3 klux and the products examined on HPLC.

2.3.6. Preparation of irradiated fumagillin solutions for testing against nosema disease

Syrup was prepared from sugar (1000 g) and water (1000 mL) with ca. 100 mL of the water reserved. Dicyclohexylammonium fumagillin (79 mg) was dissolved in ca. 25 mL of the reserved water and added to the prepared syrup, and the flask was rinsed with the remaining water in portions. The specific gravity of 50% sucrose syrup is published as 1.2296 (Merck Index, entry 8966); the 79 mg of dicyclohexylammonium fumagillin thus gives a concentration of 35 μg/mL of fumagillin base in the syrup. The fumagillin was weighed out in low room light (fluorescent lights off). All subsequent sample preparation steps were carried out under a photographic safelight (Wratten series OC filter, Eastman Kodak Co., Rochester, NY).

The irradiations were done by transferring ca. 275–300 mL of the treated syrup to a 190 × 100 mm

Pyrex crystallizing dish and covering with polyethylene film (0.5 mil, 12.7 μ, Glad Cling Wrap, Clorox Co., Oakland, CA; it had no significant UV absorption in the 350 nm range). These dishes were exposed to direct sunlight outdoors for various lengths of time (30 seconds, 5 minutes, 30 minutes, and 6 hours). An unirradiated sample was also kept. The 30-second and 5-minute samples were carried outside in a covered black plastic pail, removed for exposure, swirled continuously for the irradiation period, replaced in the pail and brought back indoors. The longer period samples were simply carried outdoors and exposed to sunlight. The 30-minute sample was swirled for ca. 30 seconds every 10 minutes; the 6-hour sample was swirled every hour or so. These irradiations were done in July.

All five samples were aliquoted (ca. 50 mL each) into 60-mL wide mouth amber jars (5 aliquots/sample). Samples were stored at 5 °C over the weekend, transported to New Jersey, and placed in a –20 °C freezer for storage. Small samples of all solutions were reserved in scintillation vials wrapped in aluminum foil for HPLC assay. They were stored at –20 °C until analysis.

2.3.7. Activity of irradiated fumagillin against nosema disease

The experiment was carried out by the method of Webster (1994). Bees were collected from a single colony in a bee yard in Pemberton, NJ to ensure that all bees were related genetically. Bees from brood frames were shaken into a plastic tub. Approximately 100 bees were scooped up and placed into each of 35 cages divided into 7 treatments × 5 replicates (cages). Each cage had a wire screen front and a feeder tube consisting of a 15-mL centrifuge tube with several holes in the cap to allow bees access to the syrup within. All tubes were wrapped with aluminum foil to prevent exposure to light. The cages were kept in an incubator at 33 °C.

All bees were initially starved for three hours, after which treatments were initiated. Treatments 1–6 ('inoculated') were fed 50% sugar syrup containing 6×10^5 *Nosema apis* spores/mL for 3 days, while the uninoculated control (treatment 7) received syrup without added spores. At the end of the three days, 10 bees were randomly removed from each cage for spore counts. Fumagillin treatments were started after 3 days, since in preliminary tests, feeding of *N. apis* spore suspensions of higher titer or for longer than four days caused heavy bee mortality and high spore levels under the conditions of the experiment.

The 7 treatments were as follows: (1) inoculated, fumagillin not irradiated; (2) inoculated, irradiated 30 seconds; (3) inoculated, irradiated 5 minutes; (4) inoculated, irradiated 30 minutes; (5) inoculated, irradiated 360 minutes (6 hours); (6) inoculated, no fumagillin; and (7) uninoculated, no fumagillin. These treatments were fed for five days; dead bees were removed each day. At the end of five days of treatment, all surviving bees were frozen, and ten bees were randomly removed from each cage for final spore counts. The entire guts (including midgut, rectum, and sting chamber) were removed by pulling on abdominal segment VII with forceps (Shimanuki and Knox, 2000). The guts were then ground in distilled water, and the spore count determined with a hemocytometer as described by Cantwell (1970). Pooled samples of 10 bees/cage were counted.

Spore counts were analyzed using t-test to compare between before and after treatment for each fed treatment. Due to high variations, a quadratic transformation ($y' = \ln((y+0.01)/(1.635-0.379(y+0.01)))$; Draper and Smith, 1981) was used to give normally distributed data set that could be analyzed.

The bee mortality for the seven treatments over the five days of treatment was compared. Data were transformed ($y' = \arcsin(\sqrt{(y)})$. One-way ANOVA followed by Bonferroni's multiple comparison test was used for statistical analysis of transformed mortalities.

3. RESULTS

3.1. Fumagillin irradiations

Fumagillin samples were initially irradiated for 2 minute increments to 10 minutes, then five minute increments to 30 minutes. Data fitted to a single-phase exponential decay curve with a $t_{½}$ of 1.98 minutes ($r^2 = 0.9664$) from 40 μg/mL to 11.1 μg/mL. The next run, with 10 second increments up to 60 seconds, fitted similarly to a curve with $t_{½}$ 5.49 seconds ($r^2 = 0.9986$) from 40 to 14.4 μg/mL.

A larger run (30 points) was carried out to examine these decays further. Exposure times ranged from 1 second to 6 hours. Results are shown in Figure 2. There was an initial exponential decrease from 40 to 17 μg/mL over the first 25 seconds, with a half-life of 4.6 seconds (Fig. 2a, $r^2 = 0.9895$). From 0.5 to 4 minutes, a second exponential decay to 13.3 μg/mL (Fig. 2b, $t_{½}$ = 33.4 seconds, $r^2 = 0.9792$) occurred, followed by a third, from 4 to 30 minutes to 11.75 μg/mL (Fig. 2c, $t_{½} = 4.04$ minutes, $r^2 = 0.9967$). A final decrease, from 11.5 to 5.5 μg/mL occurred over the final 5 hours of the experiment. An exponential fit had $r^2 = 0.9992$ and an estimated $t_{½}$ of 16.4 hours (Fig. 2d).

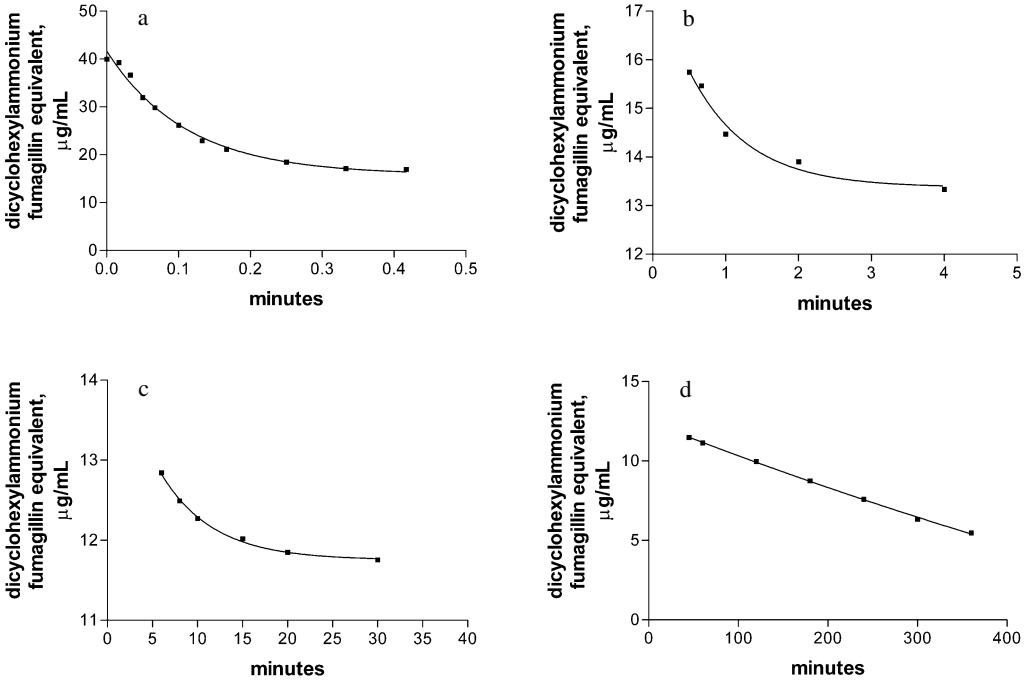

Figure 2. Fumagillin photolysis in 50% ethanol, full sunlight. a: 0–25 seconds; b: 0.5–5 minutes; c: 6–30 minutes; d: 45–360 minutes.

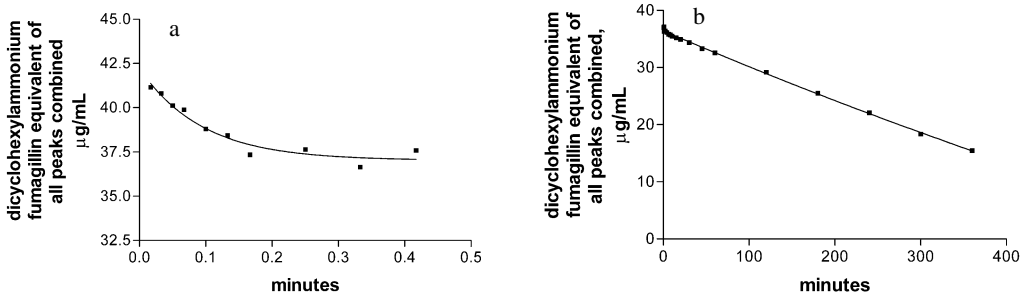

Figure 3. Fumagillin photolysis in 50% ethanol, sum of all starting materials and products. a: 0–25 seconds; b: 0.5–360 minutes.

A sample exposed to sunlight for 3 days showed almost no peaks absorbing at 350 nm (minute peaks similar to 6 hour sample, but peak intensity was too small to integrate). The sum of all the fumagillin and photoproduct peaks exhibited an initial exponential decay from 41 to 37 µg/mL, with a half-life of 3.9 seconds (r^2 = 0.9505, Fig. 3a), followed by a linear decrease to 15.5 µg/mL at 6 hours (r^2 = 0.9982) (Fig. 3b).

Photolysis of Fumidil B in 50% ethanol gave similar results. The four successive decays had half-lives of 0.82 seconds (r^2 = 0.9999), 5.1 seconds (r^2 = 0.9795), 2.91 minutes (r^2 = 0.8436), and 29.4 minutes (r^2 = 0.8324). Light caused similar changes to solutions of Fumidil B in sugar syrup over six minutes. Three decays were observed with half-lives of 2.2 seconds (r^2 = 0.9932), 6.0 seconds (r^2 = 0.9995), and

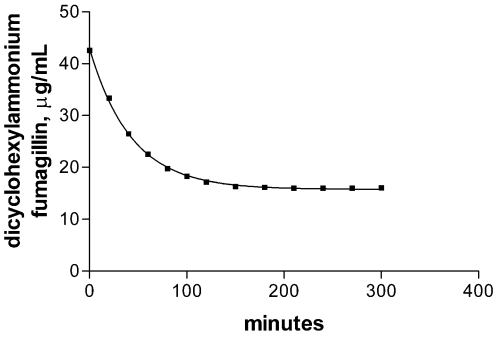

Figure 4. Fumagillin photolysis in 50% ethanol, room fluorescent lighting.

38.5 seconds ($r^2 = 0.9336$). The reaction was not followed for longer periods.

Fumagillin also decomposed in 50% ethanol under fluorescent room lighting (Fig. 4). The concentration of fumagillin decreased from 42.6 to 16 μg/mL over 5.5 hours ($t_{½} = 29.9$ minutes, $r^2 = 0.9991$). The fluorescent tubes did not indicate the type of phosphor mixture, and we had no instrumentation capable of reading w/m^2 directly. Energy distribution would vary with different types of fluorescent tubes. Nonetheless, it is clear that decomposition occurs under fluorescent lights, although much more slowly than under full sunlight.

Some of the variations in concentration values for the various half-lives for different photolyses were probably caused by differences in light intensity. Transient changes occurred when clouds obscured the sun. Photolyses were run only on clear days, but even so there were occasional clouds. Furthermore, light intensity varies with season and time of day. Observed peak intensity in late April in Beltsville (39.03° N) varied from 75 klux (8:00, all times are EDT) to 125 klux (13:00–15:00). In July, light values were 111 klux (9:00) to 130 klux (13:00–14:00). These data correspond approximately to 275 to 870 w/m^2 during April and 400 to 900 w/m^2 for clear days in July (1990 data for Baltimore, MD (39.18° N) from Renewable Resource Data Center, National Renewable Energy Laboratory, US Department of Energy, www.rredc.nrel.gov/solar/old_data/nsrdb/hourly/tab1.html).

Given the structure of fumagillin, it is likely that the photoreactions observed are *Z:E* isomerizations of the four double bonds in the acid moiety of fumagillin. To investigate this, a solution of fumagillin (initial purity 99.5%) was irradiated in full sunlight for 4 minutes. The irradiated mixture contained 50% fumagillin and three other products. This mixture was separated by HPLC into a 'starting material' fraction (97.6% fumagillin) and a 'products' fraction (6.3% fumagillin). Irradiation of the 'starting material' fraction for 5 minutes gave a mixture of six components (36.9% fumagillin); simultaneous irradiation of the 'products' fraction gave a mixture of the same components with 42.1% fumagillin.

Analysis of the syrup samples irradiated for the nosema disease inhibition study below showed the following fumagillin contents: unirradiated, 46.3 μg/mL, 30 seconds, 20.0 μg/mL; 5 minutes, 16.0 μg/mL; 30 minutes, 13.1 μg/mL; and 360 minutes, 8.0 μg/mL.

3.2. Nosema disease inhibition study

When fed to bees inoculated with *Nosema apis* spores, all treatments with unirradiated or irradiated fumagillin caused clear decreases in the spore load (Fig. 5). Statistical analysis of quadratic transformed data showed that treatments 1–5 (all treatments with fumagillin, whether irradiated or not) resulted in significant decreases in spore counts when comparing between before and after treatment (one-tailed t-test, equal variance, $P = 0.0297$, < 0.0001, 0.0085, 0.0285, and 0.0087, respectively). The two treatments not involving fumagillin were not statistically different ($P = 0.35$ and 0.47). For clarity, the data in Figure 5 have been back-transformed ($y' = (1.635e^y)/(1+0.379e^y)$). Error bars represent the 95% confidence intervals; the lower limits have been truncated at zero. The asymmetry of the confidence intervals is a result of the transformation as shown in Figure 5. Mean bee mortalities during the five days of treatment were not significantly different, with the exception of treatment 6 (inoculated, no fumagillin (Tab. I)).

4. DISCUSSION

4.1. Fumagillin photolysis

It is clear from the HPLC data that fumagillin is highly susceptible to photodegradation,

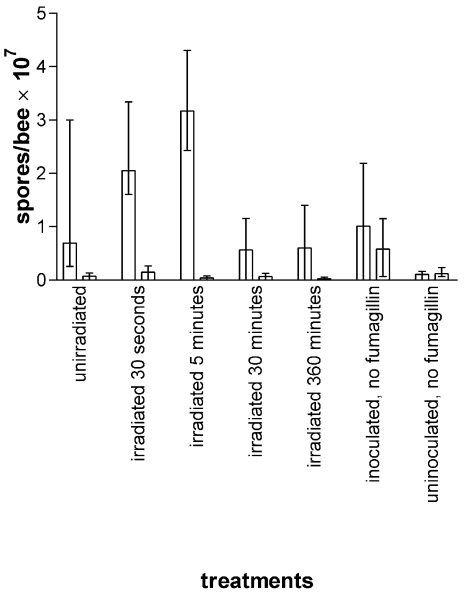

Figure 5. Effect of feeding irradiated fumagillin to bees inoculated with *Nosema apis*. Data are back-transformed (see text) for clarity. The first bar in each pair is before treatment value; the second after treatment. Error bars are 95% confidence intervals. Before treatment/after treatment differences are significant ($P < 0.05$) for all treatments involving fumagillin, but not significant for the two control treatments.

decreasing to less than half its initial concentration within 30 seconds in full sunlight. At least four successive equilibria could be observed, wherein fumagillin transforms to related compounds, which in turn are also photochemically reactive. Half-lives are in the seconds to minutes range; fumagillin is thus extremely susceptible to irradiation in either syrup or 50% ethanol, either as pure fumagillin or as Fumidil B. These rates are determined in small vials where the ultraviolet components of the sunlight can penetrate the entire sample readily. In larger vessels, the UV would be absorbed by the fumagillin in the surface layers without being able to affect the solution in the depths of the container. Photolysis would then occur as diffusion or convection caused solution to move to the surface. On the other hand, in bulk containers only the solution on the surface would be accessible to bees.

The chromophore in the molecule is decatetraenedioic acid (all *E*), which absorbs at λ 351 nm. Photolysis of straight-chain double bonds commonly leads to *Z:E* equilibria, and the initial photoproducts absorb at least to some extent in the same wavelength range. Since the starting material and product fractions from separation of a partially-photolyzed sample gave similar product mixtures on further irradiation, it is clear that at least some of the reactions occurring involve equilibria, as would be expected for double bond photoisomerizations. Decrease in UV absorbance of solutions with continued irradiation is likely to have been caused by photoisomerization to products not absorbing in the range of the UV wavelengths occurring in ambient sunlight. Garrett and Eble (1954) give possible examples of such cyclized products. Such isomerizations to the tetraene ester portion of the fumagillin molecule might not be expected to destroy activity, since the alcohol product produced by complete removal of the tetraene diacid moiety is also active against nosema disease, albeit less so by a factor of about 10 (Gochnauer and Furgala, 1962).

Table I. Mortality of bees over 5 day treatment period.

Treatment	Bee mortality (%) ± SEM
Inoculated, fumagillin not irradiated	6.8 ± 1.8b
Inoculated, irradiated 30 seconds	8.2 ± 4.6b
Inoculated, irradiated 5 minutes	8.0 ± 4.8b
Inoculated, irradiated 30 minutes	7.0 ± 1.2b
Inoculated, irradiated 360 minutes (6 hours)	6.2 ± 1.4b
Inoculated, no fumagillin	19.2 ± 3.0a
Uninoculated, no fumagillin	6.0 ± 2.0b

Means followed by the same letter are not significantly different ($P > 0.05$) from each other (ANOVA; F = 5.050; df = 6, 28; $P = 0.0013$).

4.2. Activity of irradiated fumagillin

Fumagillin treatments of bees inoculated with *Nosema apis* produced significant decreases in spore counts over the course of the experiment, without regard to the duration of irradiation. In the two treatments not involving fumagillin, no such decrease occurred.

The bees used in this study were from a field colony. The high spore titer at three days (before the production of new spores by a new infection) indicates that some were already infected to various degrees with nosema disease. Since the whole guts were removed for spore counts, the only ways for spore counts to decrease are defecation in the cage, which would reduce remaining spores in the bees, or death of the heavily infected individuals. The second possibility seems more likely, since only live bees were chosen for spore counts. Bee mortality in cages during the five day treatment period was not significantly different for all treatments and the uninoculated control. The inoculated control without fumagillin treatment had significantly higher mortality that could have been caused by heavy infection of nosema disease. These results showed that reduced bee mortality and reduction of spore counts resulted from feeding fumagillin treatments in the surviving bees in comparison to non-fumagillin fed bees.

Time and personnel constraints did not allow the use of the more exact procedure of individually inoculating newly-emerged bees (Fries, 1988). Instead, the more expeditious technique of Webster (1994) was used, with the exception that isolated guts were removed before homogenization rather than homogenizing the whole bees. Shimanuki and Knox (2000) indicate that cleaner preparations are obtained from isolated guts than from abdomens, which is hardly surprising. In turn, homogenization of whole bees, as used by Webster, would be expected to be even messier.

Significant decreases in spore counts for all fumagillin treatments regardless of irradiation time were found. There seems to be no major decrease in activity as fumagillin is transformed into its various photoproducts. Webster (1994) reported equally good activity from fumagillin at 50, 25, and 10 mg/L in his tests. Our syrup after 6 hours' irradiation still contained 8 µg/mL actual fumagillin, so any decrease in activity in the photoproducts may not be apparent under our conditions. Some combination of the two is certainly also possible.

Since fumagillin has been reported to be fairly stable in hives (in the dark; Furgala, 1962) the reported failures of fumagillin in the field when bees fed on bulk syrup seem to be due to some other cause than photolysis or thermal decomposition in the dark.

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Résumé – Étude en laboratoire de la photostabilité de la fumagilline, matière active du Fumidil B. La fumagilline est la seule substance autorisée aux États-Unis et au Canada pour traiter la nosémose de l'Abeille domestique. Elle est utilisée sous forme de Fumidil B en sirop depuis le début des années 50, mais des observations récentes suggèrent que la décomposition par la lumière pourrait expliquer certains échecs lors de son utilisation dans les nourrisseurs couvre-cadres.
La fumagilline a été dissoute dans de l'éthanol à 50 % ou dans du sirop de sucre et exposée au soleil. On a observé une série de décompositions (Fig. 2), avec des demi-vies de l'ordre de 4–5 s, 30 s, 4 min et 16 h. En 6 h la concentration en fumagilline est passée de 40 µg/mL à 5,5 µg/mL et six autres produits qui absorbent la longueur d'onde de 280 nm sont apparus. La somme de tous ces pics d'absorption, exprimée en fumagilline, a baissé également (Fig. 3). Après une diminution exponentielle de 42 à 37 µg/mL ($t_{½}$ 4 s), la somme de tous les pics d'absorption a ensuite diminué de façon linéaire sur 6 h jusqu'à une valeur équivalant à 15 µg/mL. Des résultats semblables ont été obtenus en irradiant des solutions de Fumidil B dans de l'éthanol à 50 % ou dans du sirop de sucre. L'exposition au soleil de la fumagilline dans l'éthanol à 50 % durant trois jours a entraîné la disparition, à l'exception de traces, de toute la substance de départ ou de tous les produits de dégradation qui absorbent la longueur d'onde de 280 nm. Une photolyse semblable, quoique plus lente, s'est produite sous lumière fluorescente ($t_{½}$ 29,9 min, Fig. 4). Le fait qu'au moins certaines de ces modifications correspondaient à des réactions d'équilibre a pu être montré en séparant un échantillon irradié durant 4 min en une fraction « substance de départ » et une fraction « produits » puis en irradiant à nouveau les deux fractions. Après irradiation les deux fractions étaient constituées

d'un mélange semblable de composés, ce qui indique une photoréversion des produits vers la substance d'origine. On pense que des réactions d'isomérisation *Z:E* sont à la base de ce processus dans le groupe ester tétraénédioïque de la molécule.
Des abeilles, auxquelles on avait inoculé *Nosema apis* et qui avaient reçu en nourrissement de la fumagilline dans du sirop de sucre irradié pendant 0, 0,5, 5, 30 ou 360 min, ont présenté pour tous les traitements une baisse du nombre de spores (Fig. 5). On n'a pas observé de différences significatives pour les traitements sans fumagilline. Le nombre initialement élevé de spores indique que certaines abeilles utilisées étaient déjà infectées par la nosémose. Des expositions de courte durée (6 h ou moins) de la fumagilline au soleil provoquent des réarrangements moléculaires mais n'ont pas d'effets négatifs apparents sur l'activité contre *N. apis*.

fumagilline / stabilité / photolyse / nosémose / Apis mellifera

Zusammenfassung – Laborstudien zur Photostabilität von Fumagillin, der aktiven Substanz von Fumidil B. Fumagillin ist die einzige in den USA und Kanada zur Behandlung der Nosematose bei Bienen zugelassene Substanz. Sie kam seit den frühen fünfziger Jahren als Fumidil B in Zuckersyrup zur Anwendung. Neuere Beobachtungen weisen darauf hin, daß der Zerfall der Substanz durch die Einwirkung von Licht in einigen Fällen das Versagen der Behandlung bei der Verwendung von Sammelfütterern erklären könnte. Fumagillin wurde in Alkohol (50 %) oder Zuckersyrup aufgelöst und dem Sonnenlicht ausgesetzt. Es wurden Zerfallserien mit Halbwertszeiten im Bereich von 4–5 Sekunden, 30 Sekunden, 4 Minuten und 16 Stunden registriert. Die Konzentration des Fumagillins nahm über einen Zeitraum von 6 Stunden von 40 µg/ml auf 5,5 µg/mL ab, und es traten sechs andere Substanzen mit Absorptionen bei 280 nm auf. Die als Fumagillin bezeichnete Summe aller dieser Absorptionsspitzen nahm ebenfalls ab (Abb. 3). Nach einer anfänglichen exponentiellen Abnahme von 42 auf 37 µg/mL ($t_{½}$ 4 Sekunden), nahm die Summe aller Absorptionsspitzen über 6 Stunden linear auf das Äquivalent von 15 µg/mL ab. Ähnliche Resultate wurden bei der Bestrahlung von Lösungen von Fumidil B in entweder 50 % Ethanol oder Zuckersyrup erhalten. Die Sonnenexposition von Fumagillin in 50 % Ethanol über drei Tage führte zum Verschwinden des Ausgangsmaterials oder seiner bei 280 nm absorbierenden Zerfallsprodukte bis auf Spuren. Eine ähnlich obgleich langsamere Photolyse erfolgte auch bei Fluoreszenzbeleuchtung im Labor ($t_{½}$ 29,9 Minuten, Abb. 4). Dass zumindest einige dieser Änderungen Gleichgewichtsreaktionen darstellen, konnte durch eine Auftrennung des Ausgangsmaterials und der Mischung der Zerfallsprodukte einer 4 Minuten lang bestrahlten Probe, gefolgt von einer Nachbestrahlung der aufgetrennten Proben gezeigt werden. Nach Bestrahlung zeigten beide Fraktionen eine ähnliche Produktzusammensetzung, was auf eine Photoreversion der Produkte in Richtung Ausgangsmaterial hinweist. Vermutlich liegen diesem Prozess *Z:E*-Isomerisierungsreaktionen in der Tetraendiol-Estergruppe des Moleküls zugrunde.
Die Verfütterung von unbestrahlten oder 0, 0,5, 5, 30 oder 360 Minuten lang bestrahlten Fumagillinlösungen in Zuckersyrup (es wurde keine 3-Tage Probe zubereitet oder verfüttert) an mit Nosema infizierte Bienen zeigten bei allen Fumagillinbehandlungen signifikante Abnahmen der Sporenzählungen (Abb. 5). Behandlungen ohne Fumagillin zeigten keine Abnahme. Hohe anfängliche Sporenzählungen wiesen darauf hin, dass einige der Bienen bereits zuvor mit *Nosema apis* infiziert waren. Eine kurze Sonnenlichtexposition (6 Stunden oder weniger) hat daher keinen offensichtlichen mindernden Effekt auf die Wirksamkeit gegen Nosematose, obwohl sie molekulare Umordnungen verursacht.

Fumagillin / Stabilität / Photolyse / *Nosema apis* / Honigbienen

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