

A Mathematical Investigation on Active Release in Soil and its Validation in Greenhouse Studies

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Abstract

Encapsulation can be used to mitigate the degradation of pesticides in a hostile environment. The design of the right release profile, however, is critical to tune the balance between the degradation and uptake into plants for pest control. In this paper, we use Compound A, an insecticide that undergoes rapid degradation in soil, as an example to demonstrate how mathematical modeling combined with a greenhouse study, can be utilized to suggest an optimal release profile and help the design of controlled-release formulation for an active ingredient (AI). First, a mathematical model was constructed to understand the minimum dosage requirement to meet the 1-month insect control target. Then a spiking greenhouse test with the defined use rate was designed to validate the model. Combination of these data led to the determination that the minimum dosage in microbiologically active soil for 1-month insect control was 0.03-0.045 µg AI/g soil. The spiking test demonstrated that controlled-release of Compound A achieved through appropriate encapsulation technology would deliver sufficient insect control for 1 month, with at least 9 times reduction on its use rate if applied without encapsulation. This information can serve as a guide in selecting the composition of the polymeric encapsulant as well as improving the translation from lab screening to greenhouse test, and eventually helping to improve the translation to field performance.

Keywords: Controlled-release; Soil pesticide; Modeling; Degradation; Pest control

Introduction

Pesticides are chemicals intentionally released into the environment to control unwanted pests including weeds, fungi and insects. Many pesticides are intrinsically stable and so can be formulated without using a formulation mechanism to protect them. In some cases where the pesticide's physical and chemical properties result in instability, suboptimal movement, and undesired loss in storage or use, encapsulation technologies are often used to prevent unwanted effects of the environment on the pesticide. For example, clomazone volatility was reduced to 50% by interfacial microencapsulation [1]. Tefluthrin movement in soil was improved and translated into enhanced bio efficacy against soil-borne pests [2]. In other cases, encapsulation can be used to reduce the exposure hazard [3,4]. Microencapsulated *lambda*-cyhalothrin showed much reduced eye and skin irritation when compared to the emulsion in water [5]. With incorporation of base trigger in the polymeric shell, the pesticide formulations can be selectively effective against certain undesirable insects while not harmful to beneficial insects or insects which do not feed on the capsule materials [4]. The microcapsules of cadusafos were demonstrated to reduce mammalian toxicity without affecting efficacy [6]. In addition, actives can also be encapsulated to eliminate or retard chemical degradation due to incompatible chemicals [3]. It is expected that degradation due to pH [3], temperature, UV-light or microbial bio-degradation would also be mitigated or minimized with encapsulation. Usually this protection is achieved by controlled-release of the active, therefore providing pest control for the desired duration.

Soil applied pesticides are exposed to a range of complicated processes including binding to soil, exposure to chemical and microbial decomposition or conjugation, as well as leaching into ground water (Figure 1). The portion that is taken up into the plant may be subjected to plant metabolism before it can reach the insects and control them. These processes may affect the activity of the applied pesticide before it can eventually reach the insects. Compound A is a systemic insecticide

which undergoes rapid metabolism by microbes in soil (see Results and Discussion) and results in its short half-life. Therefore, to achieve long term pest control, a continuous feeding of active ingredient (AI) is required. It was hypothesized that encapsulation technology could control release of Compound A and thus deliver the right balance between degradation, uptake, metabolism, and insect control. We first designed a mathematical model to predict the minimum dosage requirement to meet the pest control for one month. Subsequently greenhouse tests were used to validate the model. An ideal release profile was then established to provide guidance in selection of the encapsulant. This information will serve as a guide to select the composition of polymeric encapsulant as well as improve the translation from lab screening to greenhouse test, and eventually help improve the translation to field performance.

Materials and Methods

Mathematical model

The model was developed using Microsoft Excel software with the Solver add-in function.

Soil degradation test

Three hundred grams Brookston silt clay loam (Hancock Co. Indiana) soil was agitated by a KitchenAid K5SSWH Heavy Duty Series 5-Quart Stand Mixer. A suspension of Compound A was added

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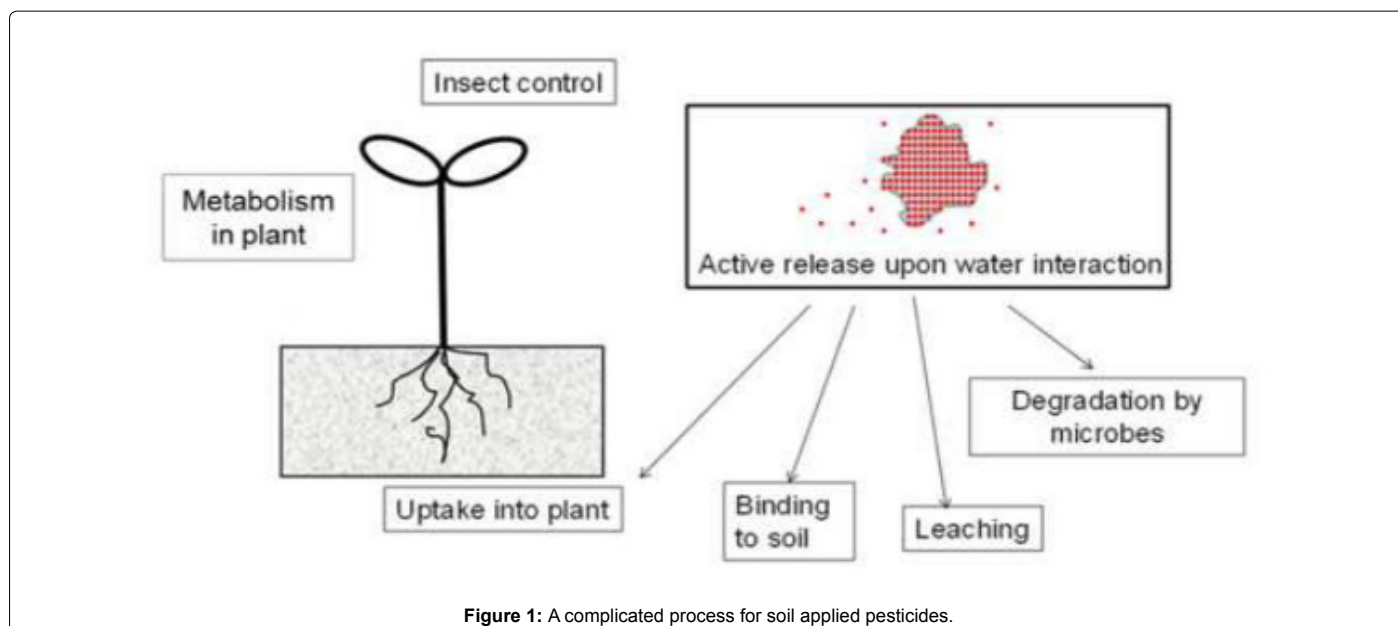


Figure 1: A complicated process for soil applied pesticides.

together with water. The mixture was blended thoroughly to achieve a final concentration of $\sim 8 \mu\text{g}$ Compound A /g soil and 26 wt % water. Persistence of parent material (Compound A) was analyzed across a range of time points by mixing a 15-g sample of soil with 10 mL acetonitrile for 1 hour. The mixture was then centrifuged at 3500 rpm for 10 minutes, and the supernatant was filtered with $0.2 \mu\text{m}$ PTFE syringe filter. The filtrate was analyzed by HPLC to determine the concentration of Compound A.

Water capacity in soil

A soil sample was prepared by blending water into soil with 5 stainless steel beads (3 mm) as the mixing aide. The mixture was then mixed at low speed on a reciprocal shaker for 2 minutes until completely homogenized. The soil sample was centrifuged at 1500 rpm for 10 minutes and the supernatant was removed with a fine tip pipette. The process was conducted repeatedly until no supernatant was observed. The remaining soil was placed in a weighing pan of an OHAUS model MB45 moisture determination balance. The sample was heated to 110°C until the weight change was $<1 \text{ mg}$ for 90 s. The weight loss was the moisture level of the soil and was used as the soil capacity.

Greenhouse test to determine minimum concentration requirement for Compound A

The soil was sterilized by layering $<7.5 \text{ cm}$ thick in a pan and then heating to 100°C for 60 minutes in an autoclave. This process was repeated to the same soil on consecutive days to ensure complete sterilization. Sterile soils were transferred to cups in a laminar flow hood using sterile techniques. Bioassay were conducted using 1 oz cup filled with ~ 30 grams of sterilized soil, to which 1 ml of experimental solution was pipetted. Compound A test solutions were prepared by adding 2 ml of acetone to 2 mg active compound and then adding 198 ml of pure water. Lower doses were prepared by serial dilution with pure water. After treating, the soil was manually mixed and watered to a uniform water volume that approximated field capacity. The tested concentration was 0.2137, 0.0267, 0.0033, and $0.0004 \mu\text{g AI/g}$ soil. All cups were capped and a pin hole was made in each cap to allow for air exchange. The cups were incubated in a growth room at 25°C for 14 days, at which time the lids were removed and a single, 1-2 leaf cabbage

plant (*Brassica oleracea capitata*) was transplanted into each cup. Each plant was infested with ~ 20 -30 mixed stage green peach aphids (GPA, *Myzus persicae*). These plants were held in a growth room (16:8 L:D, 25°C) for three days and watered as needed using distilled water. At the end of this period the aerial portion of the plants were cut and the total number of live GPA per plant counted. Surviving aphid numbers were percent control transformed using the average number of aphids remaining in the blank treatment where no Compound A was used. At least 4 replicate cups were used to evaluate each rate. Data were analyzed using Minitab.

Greenhouse spiking test

All treatment solutions were prepared by diluting Compound A to a specified weight of deionized water. The concentration was defined in the Results and Discussion. Each 1 oz cup was filled with ~ 30 grams of soil and there were 4 replicates per treatment. For spiking treatments, 1 ml of solution was added on Monday morning and Thursday afternoon for 4 weeks (8 times in total). Cups were covered with lids and a small hole was poked in the lid to allow for air flow. Trays with cups were covered with a black plastic bag to prevent light from entering and placed in an environmental chamber set at 25°C . Cabbage seedlings were transplanted at day 7, 14, or 28. Cups were watered as needed after plants were added. Each plant was infested with ~ 20 -30 GPA and evaluated 3 days after infestation. Plants were graded 3 day-after-treatment (DAT) by cutting the plant at the base and counting the number of aphids on each replicate. GPA control% was defined by dividing the number of live aphids with the total number of aphids in a blank treatment where no Compound A was used.

Control experiments with single dosages were also included. All other set-ups were the same. For the second spiking test a day 0 treatment was introduced and the spiking dosage was used 7 times.

Results

Mathematical model assumptions

Compound A half-life in soil sample: After incubating the Compound A in microbially active soil, the remaining concentration of Compound A was plotted versus the time incubated in soil (Figure 2). The data were

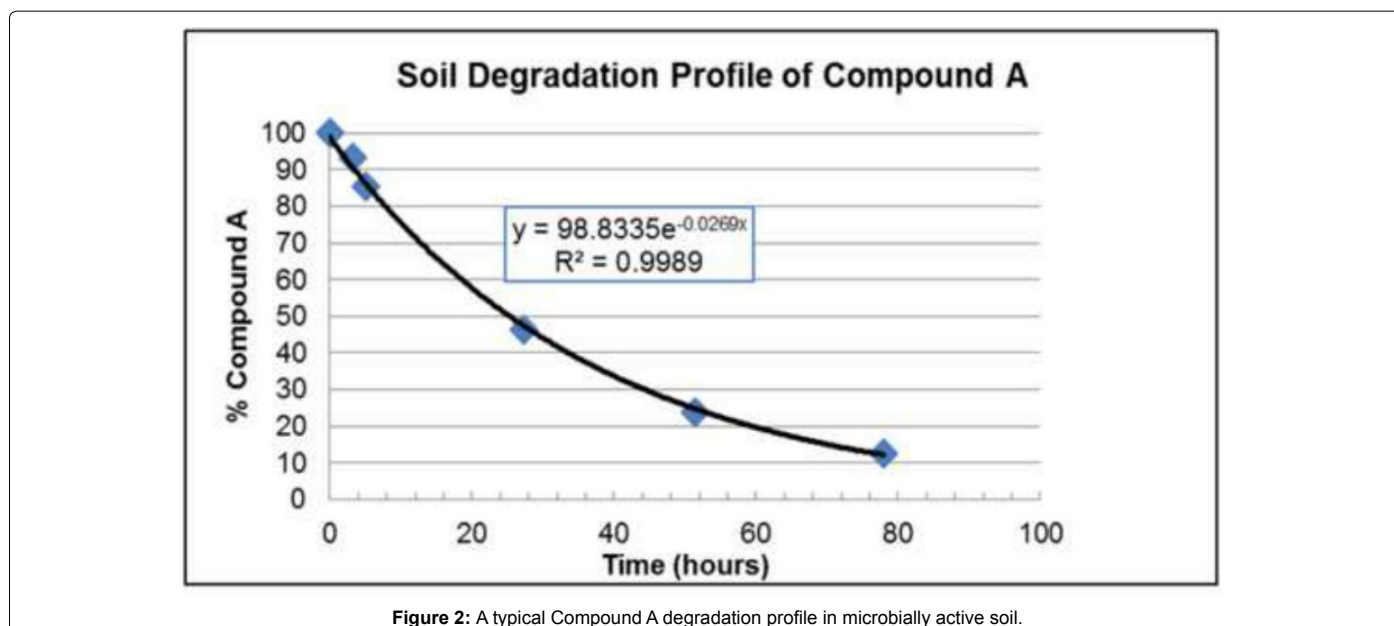


Figure 2: A typical Compound A degradation profile in microbially active soil.

fit by an exponential decay with $R^2=0.9989$. The decay rate constant from the fit resulted in the half-life $t^{1/2}=\ln 2/0.0269=25.77$ h. Similar experiments were conducted and the half-life obtained thereof was in the range of 25-60 h. Therefore, $t^{1/2}$ of 24 h (1 day) was used in the model to evaluate a worst-case scenario. This value was used throughout the remaining study unless otherwise noted.

Minimum concentration requirement C_{min} for sufficient insect control: We determined minimum concentration requirement C_{min} by dosing incremental amount of Compound A into 30 g sterile soil. Since in most greenhouse (GH) tests the soil would be kept at its field capacity, it was decided to calculate concentrations that included water content. The control on green peach aphids suggested a soil concentration of 0.003-0.027 $\mu\text{g AI/g}$ soil would provide sufficient GPA control up to 14 days in sterile soil. This range was used as the C_{min} . In a typical GH test, a cup with 30 g soil was used per treatment. Therefore, the required active per cup is $30 \times C_{min}$ μg , which was defined as M_{need} . Taking C_{min} of 0.005 $\mu\text{g AI/g}$ soil, M_{need} per cup is 0.15 μg . Taking C_{min} of 0.03 $\mu\text{g AI/g}$ soil, M_{need} per cup is 0.9 μg .

Spiking interval: Due to the soil capacity, water available to release Compound A in soil is limited. Therefore, the active release is highly dependent on watering frequency. Drip irrigation frequency in vegetable market depends on several factors, such as soil, crop type, and climate.

There is no fixed frequency for all farms. Hanson et al. studied the effect of irrigation frequency on subsurface drip irrigated vegetables in Central Valley of California and suggested drip irrigation frequencies of 1/day or 2/week were appropriate in medium to fine texture soils for the soil and climate of the project site [7]. Therefore, the spiking frequency of twice a week (interval of 3.5 Day) was selected for the model and subsequently suggested for GH test, corresponding to the active release upon irrigation.

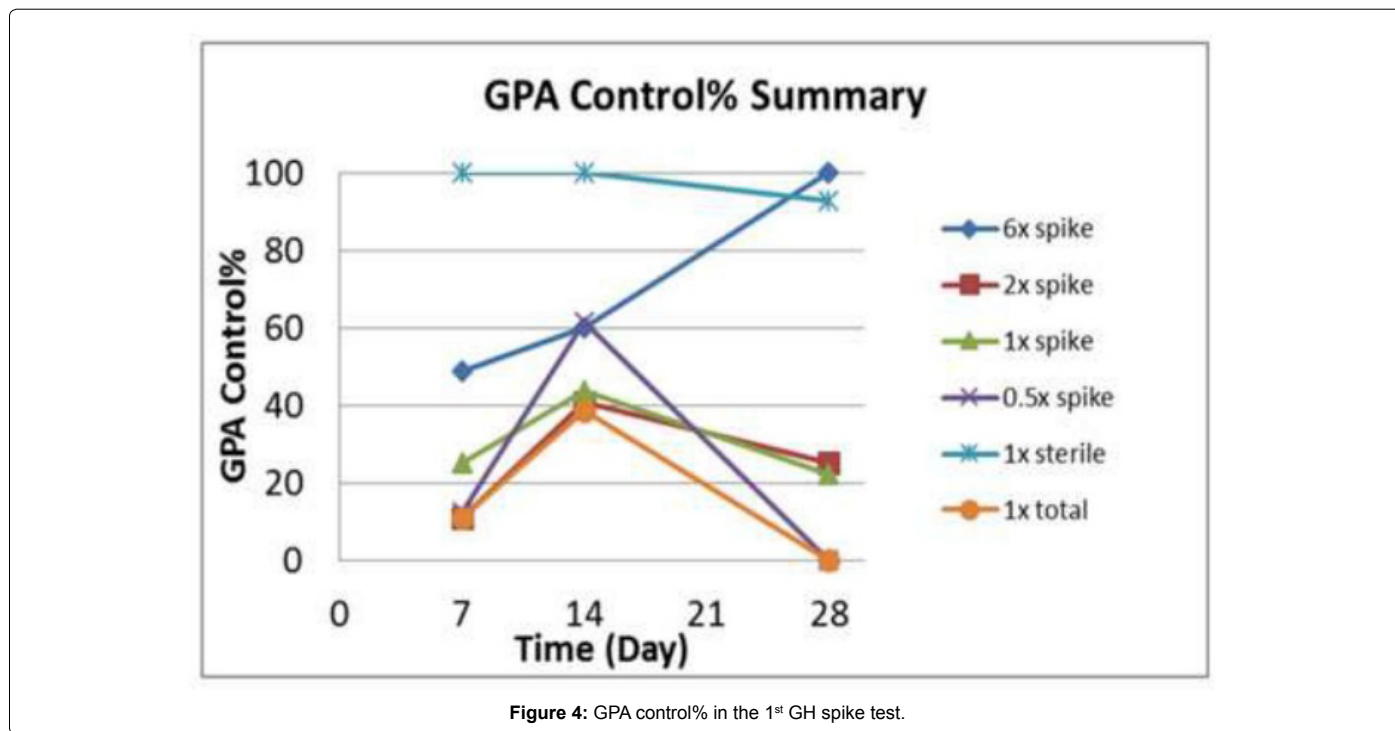
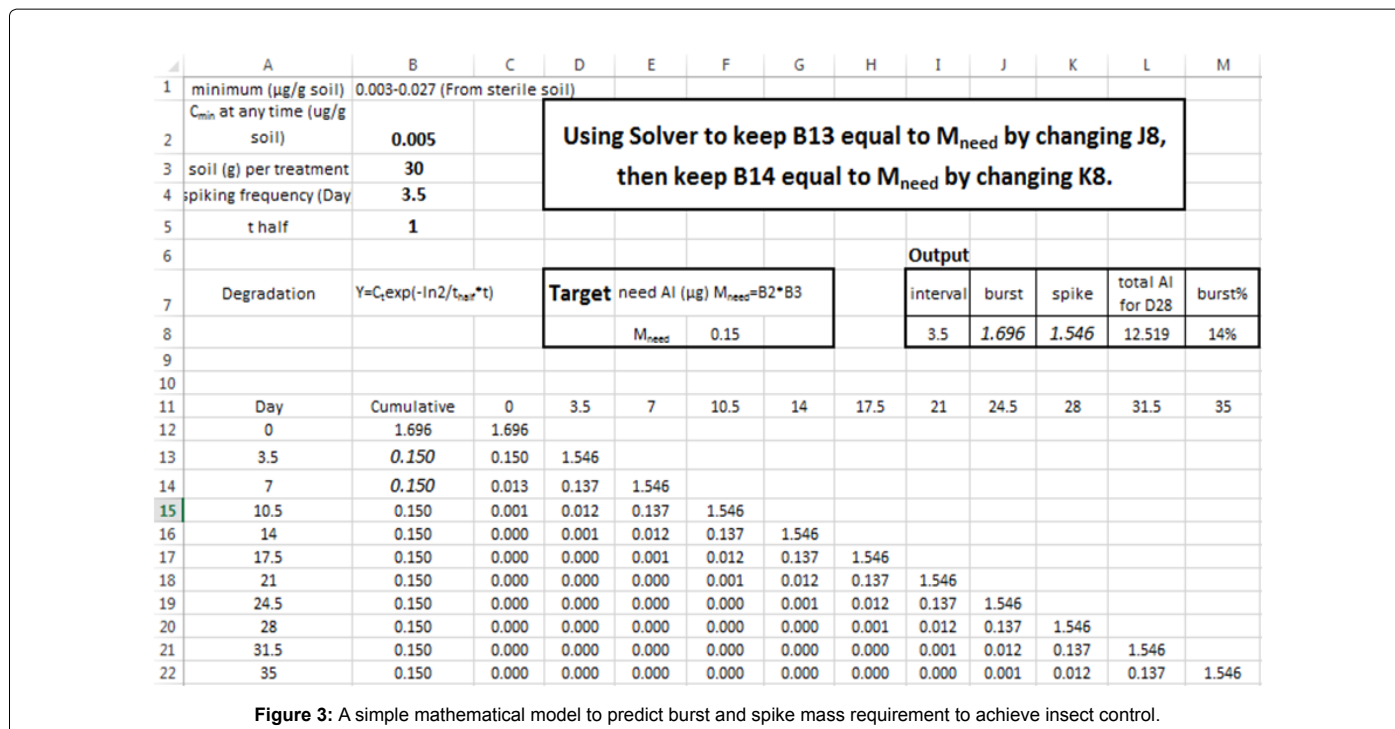
Using the model to predict use rate

A snapshot of the model is shown in Figure 3. The upper left side listed all assumptions in bold: C_{min} , soil weight per treatment, spiking interval, and Compound A soil half-life. Cell A12 to A24 listed the

days of spiking treatment which will change automatically depending on the input of spiking frequency. Cell C12 was the treatment at day 0 (D0) (burst rate), which equals to cell L8. Cell C13 and beyond showed the corresponding Compound A concentration in soil following an exponential decay with a half-life of 1 day and starting concentration of C12. Cell D13 was the first spiking treatment, which is equal to cell M8, so were E14, F15, G16 and others. The exponential decay with a half-life of 1 day applied for cells afterward in each column. Cells B12 and beyond summarize the cumulative Compound A concentration in soil at each time point right before the spiking treatment. This is when soil has the lowest active concentration. The goal is to have the cumulative concentration in B12 and beyond at least equal to the required active M_{need} as marked by Target in F8. The solver function was used to identify the needed burst and spike mass of Compound A to achieve the target. The operation was indicated in the right upper corner of the spreadsheet. In Solver function, by setting objective cell B13 to the value of M_{need} by changing variable cell L8, we could find the least burst requirement to have the active concentration in soil greater than M_{need} at any time before the first spike treatment. Subsequently, by setting objective cell B14 to the value of M_{need} by changing variable cell M8, we could find the least spike requirement to have the active concentration in soil greater than M_{need} at any time before the second spike treatment. The math works the way if cell B14 is satisfied, the cell B15 and beyond would have the same value as B14. In the output table, total active mass for 28-day insect control was calculated by $\text{burst} + 7 \times \text{spike}$. The burst % was calculated by $\text{burst}/\text{total}$ which is an important factor to design the release profile. Figure 7 showed the model with C_{min} of 0.005 $\mu\text{g/g}$ soil and 30 g soil. The calculations predict that a burst of 1.696 μg and 7 times of spikes of 1.546 μg would result in good insect control for 28 days.

Effect of spiking interval

The output for scenarios at various spiking intervals is summarized in Table 1. Increased spiking intervals resulted in an increase in both burst and spike mass requirements. The mass increment was getting larger to compensate for the exponential decay of the active. The data suggested an increased use rate in the GH or field with reduced watering frequency. So, if we used watering twice a week as the worst-



case scenario, the use rates derived there should be able to result in acceptable control for more frequent irrigation frequencies. The burst % was between 13 to 22%, suggesting a burst release of ~20% would be a good assumption for the design of the controlled-release formulation.

1st Spiking Test in GH

C_{min} of 0.005 µg/g soil was selected in the first GH spiking test. The corresponding 1x spike use rate was determined to be 1.55 µg. To simplify

the test, a spike use rate of 8 times was used. The applications were done twice a week. Treatments are summarized in Table 2. Treatment 3 used the rate of 1.55 µg (1x). Based on this, 1/2x, 2x, and 6x use rate were tested in this protocol. The 6x use rate (9.3 µg) corresponded to C_{min} of 0.03 µg/g soil as well. Therefore, most of the range of C_{min} was covered. Treatment 5 is a control experiment where the 1x rate was applied to sterile soil only once. Treatment 6 is a control experiment where the total active used in treatment 3 (1.55 × 8=12.4) was used once. Figure 4

summarizes the GPA control result in this test. The 1x rate treatment in sterile soil showed 100% GPA control for 14 days and >90% control at D28. The data was consistent with previous reports and confirmed the assumption of C_{min} of 0.005 $\mu\text{g/g}$ soil for sterile soil. The 1x total (12.4 μg) control, however, showed unexpected lower control % at D7 than that of D14. Since the active was treated at D0, a higher GPA control % at D7 than D14 would be expected. The mean live aphid counts were 34 ± 16 among all 4 replicates for this treatment and the high standard variation might cause the lower control in D7.

All spiking treatments showed insufficient GPA control except the 6x rate at D28, which would suggest the C_{min} in active soil is close to 0.03 $\mu\text{g/g}$ soil. As the model predicted, a burst rate which is higher than the spike rate was needed to meet M_{need} . When a spike rate of 9.3 μg was used instead of the burst rate, as shown in Figure 5, the soil concentration was less than M_{need} by D7. Typically, the insect dose response curve has a sharp transition. Once below the threshold, insect control would be <80% and randomly distributed. This could partially explain the insufficient GPA control at D7. The spike rate was not added at exactly every 3.5 days. As shown in Table 1, a nearly 50% rate increase was needed if the spike interval was increased from 3.5 days to 4 days. This time variation would cause insufficient GPA control as well. As time elapsed, the soil concentration would build up and eventually pass the M_{need} to show good insect control.

Prediction of single dosage requirement of compound A for 1 month insect control

Taking the soil half-life of 1 day and C_{min} of 0.03 $\mu\text{g/g}$ soil, it was predicted that $2.7 \times 10^9 \mu\text{g}$ (2.7 Kg) of Compound A was needed to control insects for 1 month in the GH soil cup test. This was based on assumptions that all of Compound A was available for microbial degradation. Research on microbial degradation of aromatic compounds has suggested solubility affects the degradation rate [8]. Therefore, we hypothesized that only molecular Compound A (e.g., soluble in water) would be subjected to degradation. The particulates remaining in solid

form within the soil would still be intact. The available water capacity in soil is the amount of water that a soil can store that is available for use by plants [9] and is highly dependent on the soil property. We determined the water capacity in soil to be 26%. The limited water in turn can result in much reduced solubility. An average solubility of 9.8 ppm was determined in soil with 33-35% water by centrifuge and analysis of the supernatant, assuming this range was the average during the irrigation duration. Taking 9.8 ppm solubility in 30 g soil with 26% water capacity, the soluble active is 76.44 μg . Thus, only this amount (or less) of active would be subjected to degradation at any point in time. Any additional active added to the soil would precipitate out and form particulates. In the equilibrium state, active substance was dissolved gradually at the rate to match the decomposition. As shown by the purple line in Figure 6, Compound A in soil water would remain constant until no further dissolution. At that point, the exponential decay curve would appear in the graph. Assuming that the active dissolves at the same rate as it decays, mathematical calculations were used to determine the dissolution rate to be 52.95 $\mu\text{g/day}$, which resulted in a total use rate of ~1300 μg with 30 g soil for 31 D control. In comparison, the spike treatment (shown in blue line in Figure 6) resulted in 84.59 μg total. To validate this prediction, a single dosage experiment with three use rates was conducted in the GH.

2nd Spiking test in GH

To verify the previous experimental results, the 9.3 ppm sample was set to 1x use rate in this test (Table 3, Treatment 1). The burst rate was also used, which was listed under D0 Concentration. The 1.5x and 2x use rates are treatment 2 and 3, respectively. Treatment 4, 5 and 6 were the single dosage controls to evaluate the previous prediction of ~1300 μg discussed previously. Given the many assumptions used to predict the rate, we felt a range between 1000 to 2000 μg active substance should provide a reasonable conservative estimation.

Compared with the 1st spike test, the increased rate at D0 indeed resulted in an improvement in GPA control (80% in 2nd test vs 49% in 1st

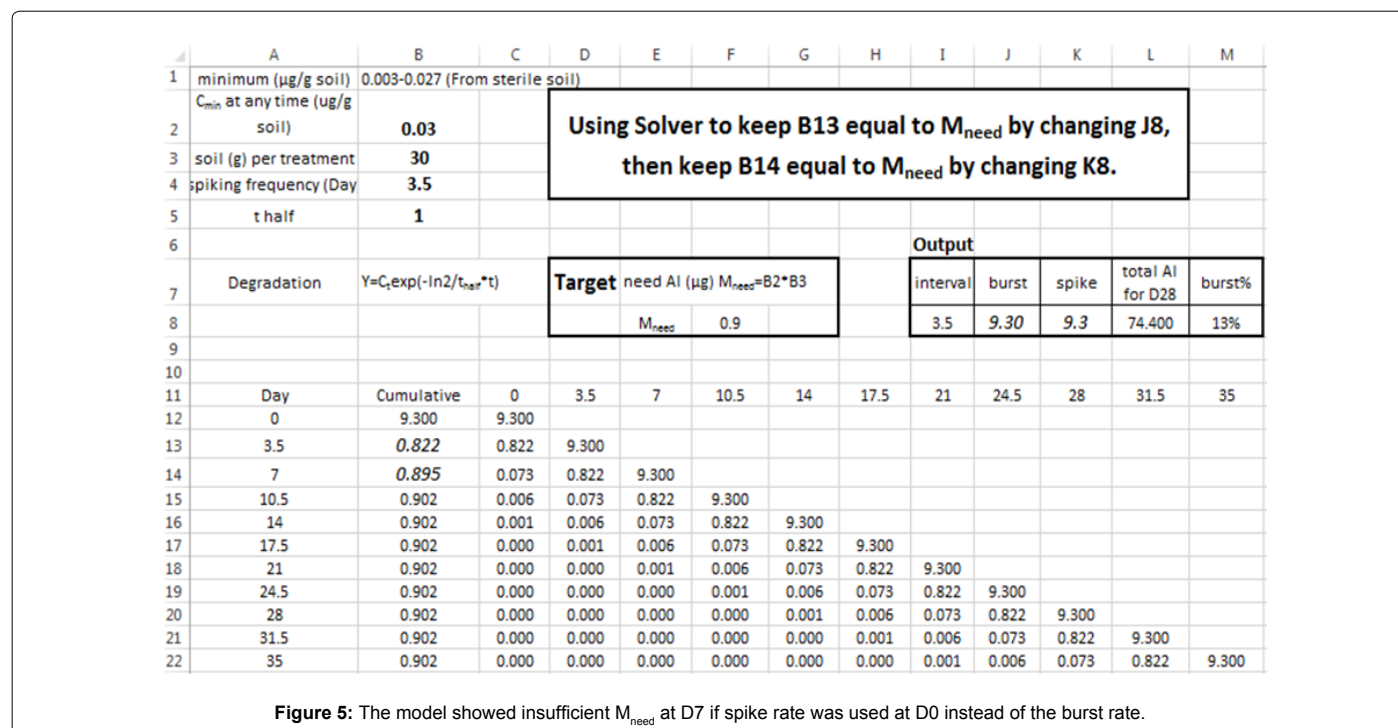


Figure 5: The model showed insufficient M_{need} at D7 if spike rate was used at D0 instead of the burst rate.

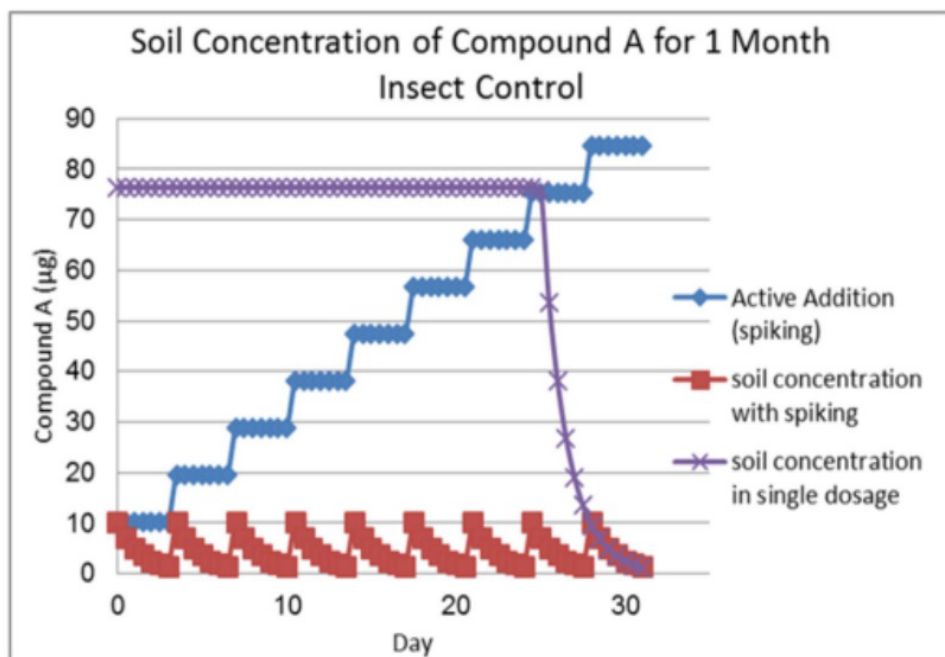


Figure 6: Predicted Compound A single dosage and spiking treatments for 1-month insect control.

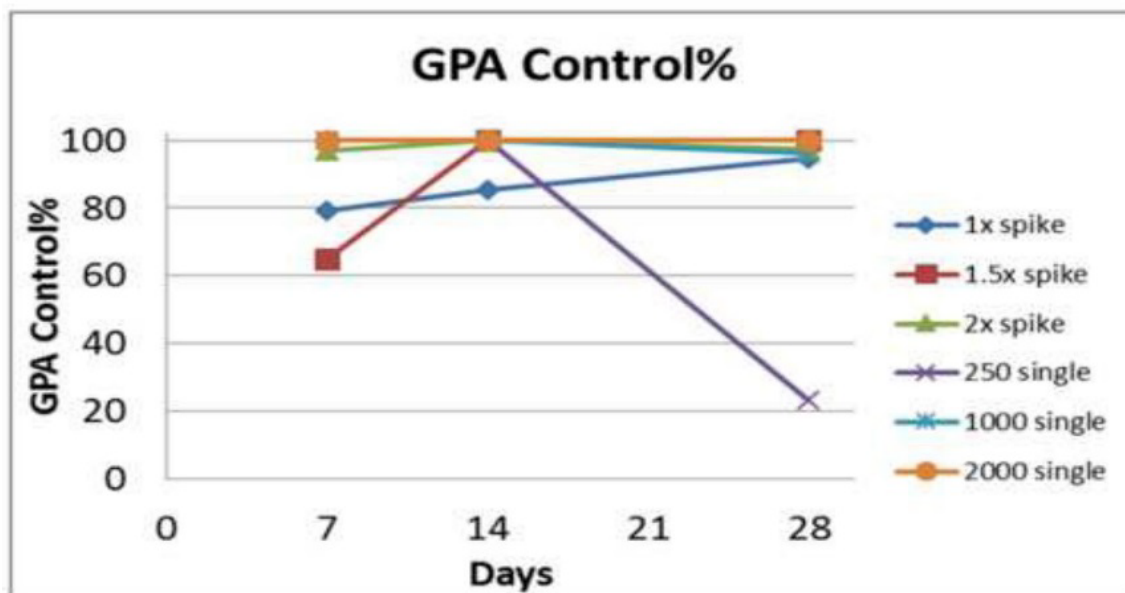


Figure 7: GPA control% in the 2nd GH spike test.

one at D7, 85% in 2nd test vs 60% in 1st one at D14) (Figure 7). The lower control% for 1.5x rate at D7 was attributed to the high variation in GH test. 2x spike rate achieved >97% control for all three evaluation dates. The data suggested the desired spiking rate is between 1x and 1.5x, and it could be close to 1x giving the time variation in spiking. This also suggested that the minimum needed dosage of Compound A in active soil for 1-month insect control to be 0.03-0.045 µg AI/g soil.

For the single dosage treatments, the 250 µg rate showed 100%

control up to 14 days and began to break down at D21, suggesting the lost control due to degradation of Compound A. The 1000 µg rate held at 100% for 3 weeks and began to break at D28 (96% control). The 2000 µg rate held at 100% control for all evaluation times. The data suggested the single dosage required for 1-month control was close to 1000 µg, which was quite similar as the 1300 µg we predicted previously. The data suggested the benefit from encapsulation would be to reduce use rate at least 9 times (1000 µg in single dosage vs 113 µg in spike).

Spiking interval	Burst	Spike	Total AI for D28	Burst %
1	0.30	0.15	1.35	22%
2	0.60	0.45	3.75	16%
3	1.20	1.05	8.55	14%
3.5	1.70	1.55	12.52	14%
4	2.40	2.25	18.14	13%

For 0.005 µg/g soil, 30 g soil

Table 1: Output summary with various spiking intervals.

S. No.	Treatment Name	Spike Concentration (ppm)	Volume (ml)	Spike times	Total Use Rate (µg)
1	6x spike	9.3	1	8	74.4
2	2x spike	3.1	1	8	24.8
3	1x spike	1.55	1	8	12.4
4	0.5x spike	0.775	1	8	6.2
5	1x sterile	1.55 (control)	1	1	1.55
6	1x total	12.4 (control)	1	1	12.4
7	Water	0 (blank)	1	1	0

Table 2: Treatment summary for the 1st GH spiking test.

S. No.	Treatment name	D0 Concentration (ppm)	D0 Volume (mL)	Spike Concentration (ppm)	Spike Volume (mL)	Spike Times	Total use rate (µg)
1	1x spike	10.19	1	9.3	1	7	75.29
2	1.5x spike	15.29	1	13.96	1	7	113.01
3	2x spike	20.38	1	18.6	1	7	150.58
4	250 single	31.25	8	N/A	N/A	N/A	250
5	1000 single	125	8	N/A	N/A	N/A	1000
6	2000 single	250	8	N/A	N/A	N/A	2000
7	Water	0 (Blank)	8	N/A	N/A	N/A	0

Table 3: Treatment summary for 2nd spike GH test.

Conclusions

Soil applied pesticides can undergo complicated breakdown and uptake processes and care must be taken to achieve desirable pest control. The rapid degradation of Compound A by soil microbes increased the hurdle for its residual control. A mathematical model was developed to facilitate our understanding on the feasibility of controlled-releasing Compound A to deliver sufficient insect control over the designated time period. Several assumptions were made based on experimental data and known farmer practices. Through theoretical calculations and greenhouse testing we could determine the minimum needed dosage of Compound A in active soil for 1-month insect control to be 0.03-0.045 µg AI/g soil. This loading was higher than the corresponding number of 0.003-0.027 µg AI/g required for sterile soil.

For soil application, no clear correlation is understood between GH use rate and field use rate therefore a field test is needed to determine whether the proposed use rate would be economically viable. Taken together, the data suggest that through appropriate encapsulation technology, controlled-release Compound A can deliver sufficient insect control for 1 month. Encapsulation would reduce the use rate at least 9 times relative to non-encapsulated Compound A formulations. The corresponding use rate in GH testing is 75-113 µg per cup (2.5-4.3 µg AI/g soil), which would be greatly reduced if irrigation was more frequent.

Challenge exists to find a technology that can control release Compound A exactly according to the projected release profile, a highly desirable zero-order constant rate of release [10-13]. However,

the obtained information through this study offered a starting point in selecting the polymeric encapsulant.

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