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Evaluation of Chrysodeixis includens Nucleopolyhedrovirus (ChinNPV) formulations for Soybean Looper (Chrysodeixis includens) Control in Soybean

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Evaluation of *Chrysodeixis includens* Nucleopolyhedrovirus (*Chin*NPV) formulations for Soybean Looper (*Chrysodeixis includens*) Control in Soybean

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ABSTRACT

Chrysodeixis includens Nucleopolyhedrovirus (*Chin*NPV) specifically Chrysogen, has been commercially available in South America since 2016 but is not current available in the U.S. Chrysogen targets soybean looper (*Chrysodeixis includens*) (SBL), the major defoliator of soybean (*Glycine max* (L.) Merrill) in the mid-southern region of the U.S. Experiments were conducted during the 2020 and 2021 growing seasons to evaluate effectiveness of three formulations of Chrysogen at multiple rates for control of SBL. Soybean looper larvae used for following experiments were kept in an insect incubator at the University of Arkansas Lonoke Research and Extension Center. The first objective was to evaluate the efficacy of *Chin*NPV formulations at multiple rates on selected instars. Purified *Chin*NPV provided greater control of SBL when compared to the commercial formulation with no difference being observed between rates of *Chin*NPV. Purified *Chin*NPV provided the greatest level of control when applied to third instar larvae or smaller. The second objective was to determine if commercial Chrysogen combined with insect growth regulators (IGRs) could reduce defoliation between virus exposure and SBL morality. Leaf disks were dipped into solution of Chrysogen combined with 0.5x rate of selected insect growth regulator. Insect growth regulators in combination with Chrysogen provided 100% control when applied to first and third instars. Treatments containing Intrepid 2F and Dimilin alone provided the greatest control compared to all other treatments when applied to fifth instar larvae. The addition of Chrysogen to any insect growth regulator did not improve control. The third objective was to determine chrysogen and purified formulation #1 efficacy on SBL and defoliation levels of V3 growth stage soybean when applied with selected rates of two Chrysogen formulations. All rates of Chrysogen failed to reduce defoliation on soybean, exceeding the defoliation threshold (25%) and never exceeding 50% larval mortality. Soybean

plants treated with purified formulation #1 had lesser defoliation (<10.5%) while observing 100% control between 6 and 7 DAT. The fourth objective was to compare experimental techniques implemented in evaluating *Chin*NPV. Higher mortality was observed in diet overlay techniques, with closest related results between diet overlay and leaf dip techniques compared to greenhouse rate response. The last research objective was to evaluate purified formulation #3 capabilities of reducing defoliation and SBL densities when applied in a field setting. Field locations were located in Tillar and Marianna, AR with randomized complete block and strip trial design being used respectively. Intrepid Edge was the only treatment to significantly reduce SBL density when compared to the untreated check for all three observation dates.

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TABLE OF CONTENTS

Chapter One: Literature Review

Introduction

Soybean looper (SBL) is a major pest of soybean in the Mid-Southern U.S. (Arkansas, Mississippi, Louisiana, Tennessee) (Musser et al 2021). SBL larvae feed on foliage and can cause major defoliation in soybean. The defoliation threshold in Arkansas for vegetative stage soybean is 40% and 25% defoliation in the reproductive stage (Studebaker et al. 2021). These levels of defoliation are commonly reached by SBL in reproductive stage soybean resulting in yield losses (Hensley et al. 1964). In combination with defoliation, SBL larvae must be present to warrant insecticide applications. When treatment is necessary, synthetic insecticides are the most commonly used control option for this pest. However, SBL has develop resistance to multiple modes of actions including pyrethroids (Felland et al., 1990; Boethel et al., 1992), organophosphates (Leonard et al. 1990), and diamides (Owen 2012) resulting in alterative control measures to be explored.

Since the 1900's, Nucleopolyhedrovirus (NPV) has been used to manage insects (Inceoglu et al. 2006). *Chin*NPV solely targets SBL, which is similar to other NPVs used in agriculture such as *Hear*NPV used to control corn earworm (Black 2017). *Chin*NPV is applied to soybean in the same way as synthetic insecticides. After SBL ingests the virus, *Chin*NPV will multiply causing reduced feeding activity and movement. Postmortem, the larvae liquifies spreading occlusion bodies to into the environment resulting in epizootic events in following generations, providing control reminiscent of the residual activity provided by some synthetic foliar insecticides.

Soybean Production in the U.S.

Soybean (*Glycine max* (L.) Merrill) originated from Eastern Asia formerly recognized as *Glycine ussuriensis* (Piper and Morse 1955). The domestication of wild soybean occurred during

the Zhou Dynasty in Northeast China. The original annual soybean (*Glycine soja*) is the first soybean to be distributed throughout East Asia. The domestication of soybean resulted in smaller seed size along with alterations of plant height and quality traits. In the late 1990s, soybean production in North and South America greatly increased leading to rapid developments in the soybean industry. Increased growth of the industry can be contributed to soybean being implemented into food, oil, and feed supplements.

Earliest records suggest that soybean introduction in the United States occurred in 1765 by Samuel Bowen (Hymowitz and Harlan 1983). In 1829, Thomas Nuttall introduced multiple additional soybean into the United States to produce descriptions of the newly discovered plant. Since the introduction of soybean, many research institutes have experimented with soybean variety selection (Piper and Morse 1955). In 1926, P.H. Dorsett and W. J. Morse conduct multiple explorations to Japan to find additional soybean varieties. Discovery of soybean uses in production of manufactured goods such as adhesives and plastics increased interest in soybean. During War World II, soybean production increased roughly 77% due to the limited accessibility to butter and oils. Soybean production continued to increase and surpassed sorghum production in 1942 along with cotton production in the mid-1950s (Shurtleff and Aoyagi 2017).

Today, soybean production ranks second overall for total planted acres in the U.S> and is the country's most exported row crop. A 14.6% reduction in soybean planted acres occurred in 2019, when the price of soybeans decreased to $0.33 per kilogram in January and hit its lowest price since December 2001 at $0.16 per kilogram. During 2021, 35.3 million hectares of soybean were planted in the U.S., which is an 11% increase since 2012 (USDA -NASS). In 2013, a yield increase of 940.8 kg/ha was observed for irrigated soybean when comparing non-irrigated, rain-fed soybean, however only 9.7% of total U.S. soybean acres are irrigated (USDA – NASS 2013).

Continued research and development have led to soybean cultivars with increased yield potential. In 2020, average yield in the United States exceeded 3427 kg /ha, a 73% yield increase when compared to average yield in 1992 (USDA – NASS 2020).

Soybean Production in Arkansas

Soybean is the widest produced row crop in Arkansas, with 1.2 million planted hectares in 2021. A large portion of soybean acres are found within the Mississippi River Alluvial Plain or Delta region. Counties located in the Arkansas Delta are responsible for 85% of the total planted acres of soybean in Arkansas with an average yield of 3279.4 kilograms/ha (USDA - NASS 2020). Highly productive soils such as Alfisols, Inceptisols, and Entosols are the primary soil classifications present in the Arkansas Mississippi River Alluvial Plain (USDA-NRCS 2022). Soils located in Arkansas are acidic to neutral requiring additional applications of lime in comparison to mid-west soils which are more alkaline. In 2020, 51% of soybean acres were fertilized with phosphate at an average rate of 77.28 kg/ha and potash at an average rate of 107.5 kg/ha was applied to 54% of soybean planted acres (USDA - NASS 2020). Arkansas soybean production contributes 22% to the total production acres in the Midsouth U.S (Arkansas, Louisiana, Mississippi, and Tennessee). Mid-South soybean management programs differ from high producing Mid-West states (Iowa, Illinois, Indiana, Minnesota, Nebraska) were dryland and center pivot acres are dominant. Arkansas implements irrigation practices primarily on precision leveled fields enabling row watering or surge flooding practices. Irrigated soybean production in Arkansas accounts for 83% of total soybean planted acres with an average yield of 3575 kg/ha compared to 2412.5 kg/ha for non-irrigated soybean in 2018 (USDA - NASS 2018).

Longitudinal location of soybean production plays a pivotal role in soybean variety selection. Early maturity group (MG) soybean is utilized in northern states. Soybean production

in Michigan utilizes maturity group II (MG II) soybean varieties where short maturity and reproductive timing fits within shortened growing season. Soybean MG's that take longer to mature are implemented in Arkansas production areas when compared to Midwest production areas. Salmeron et al. (2016) conducted studies in the Mid-South U.S. to evaluate yield when related to maturity groups (MG) at different planting dates. MG IV soybean planted at any timing, resulted in the greatest yield when compared to MG III, V, and VI. Planting date ranges from late March until early July depending on weather conditions and planting practices with MG III – VI being best suited for the variable conditions. Decreased yield potential was observed for plantings after 17 May for MG 3-5 with an average loss of 17.5 kg/ha per day.

Soybean and rice crop rotation is a popular practice in Arkansas and increased yields have been observed when soybean is implemented into a rotation when compared to continuous soybean (Dabney et al. 1990, Bradley et al. 2004). Increased yields with less nitrogen inputs have been observed when rotated with corn (Peterson and Varvel 1989c) or grain sorghum (Peterson and Varvel 1989b). This can be attributed to soybean's ability to fixate nitrogen. Soybeans implemented into a three-crop rotation (Wheat-Soybean-Corn) provided high yields with a reduction of soybean cyst nematode (SCN) when compared to continuous soybeans and soybean-corn rotation (Edwards et al. 1988).

Soybean Growth and Development:

As soybeans gained popularity in the U.S., plant breeders started breeding soybean with distinct maturity groups along with other desired traits (Johnson and Bernard 1962). Maturity groups (MG) are divided into 13 categories depending on time to maturity with time to flowering corresponding with length of dark hours (Hymowitz and Bernard 1991). Early MG soybean varieties are designed for shorter growing seasons allowing for proper timing of flowering and

maturity occurring within the growing season. MG 000 are grown in Northern Canada due to the short growing season, while MG VI and VII are planted in Southeastern U.S. (Florida, Georgia, Alabama, South Carolina, North Carolina) because the growing season is much longer (Scott and Aldrich 1970). Planting date in the Southeastern United States has a wide range from mid-April for full season crops until late June for wheat-bean cropping rotations. Along with MG, there are determinate and indeterminate varieties. Determinate varieties produce very little or no new vegetative growth after flowering begins. Indeterminate varieties continue to produce vegetative structures after flowering if the plant has a sufficient amount of nutrients and moisture to support further development. At the time of flowering, soybean has reached half its final plant height, increasing in pod production with each growth stage.

Soybean development begins at germination, when soil temperatures reach 10•C or greater. At this temperature, seed size increases to double its original size and initiates the development of the radical. Rate of emergence is the highest at 30•C with reduced germination occurring above 40•C (Hatfield and Egli 1974). As development increases, two cotyledons penetrate the soil surface. Vegetative stages are determined by trifoliate nodes that are branched from the main stem. Vegetative growth stage 1 (V1) is the first trifoliate that branches off the mainstem. With the addition of each trifoliate the vegetative stage increases correspondingly, for instance, a plant with two trifoliates is V2, a plant with three trifoliates is V3 (Fehr et al. 1971). Once the plant begins to bloom the growth stage changes based primarily on reproductive structures which range range from R1 (first flower) to R8 (95% of the pods develop their mature pod color). The R2 growth stage is when soybean is in full bloom. The R3 growth stage is when a 0.48-centimeter pod is present in the four upper nodes of the main stem (Fehr et al. 1971). The R4 growth stage is a pod 1.9 cm long present in the four uppermost nodes of the main stem. The

R5 growth stage is pod fill with a 0.32-centimeter seed being present in four of the upper nodes of the main stem. The R6 growth stage contains a pod in the four uppermost nodes of the main stem containing green seed filling the pod cavity. Exceeding R6.5 growth stage, continuation of watering events and most pesticide applications fail to increase yield. The R7 growth stage is physiological maturity with one mature color pod at any node of the plant (Fehr et al 1971). The R8 growth stage is 95% total pods being brown also known as harvest maturity.

Early Soybean Production System (ESPS)

Conventional soybean production systems in the midsouth U.S. involve planting maturity group V, VI, and VII soybean during May and June. Planting date and maturity group played a key role in decreased yield due to soybean entering a reduced rainfall period during July through September (Bowers 1995), as well as potentially increasing insect and disease infestations. Combining early maturing varieties and early planting dates will help avoid possible drought events that occur during these months. Heatherly and Hodges (1999) determined that maturity group IV and V planted in April produced yield equal or greater than traditional MGs planted during May. The early soybean production system (ESPS) can be implemented to allow for early harvest with equivalent yields to conventional soybean practices. Early harvest reduces the risk of shattering from delayed harvest due to rain events that usually occur during October and November. Early harvest will also allow for fall field preparations to be made, providing reduced spring field preparations as well as reducing weed populations. Implementation of ESPS allows for greater weed control when using narrow row spacing and post-emerge herbicide applications in combination with decreased insect pressure (Heatherly and Spurlock 2002). Combination of early planting date and maturity group will allow for soybean maturity to occur prior to large infestations of late season soybean pests resulting in less yield reduction from insect damage

when compared to conventional soybean production systems (Heatherly and Hodges 1999). McPherson et al. (2001) observed similar yields between both production practices with higher defoliation in conventional practices and peak populations of SBL occurring after ESPS harvest. ESPS has the potential to avoid late season defoliators but may be attractive to stink bugs (Pentatomidae) (Baur et al., 2000). Increased implementation of ESPS can increase net returns in soybean. Greater net returns were observed for April planted soybeans when compared to seeding events occurring in May. Difference in net return can be contributed by obtaining higher yields, lower production costs, as well as fluctuating commodity unit price (Heatherly and Spurlock 1999).

Soybean Looper

Soybean looper (SBL), Chrysodeixis includens, (Lepidoptera: Noctuidae) is the third most damaging insect pest of soybean (Glycine max (L.) Merrill) in the mid-south region of the U.S., behind corn earworm, Helicoverpa zea, (Boddie) (Lepidoptera: Noctuidae) and the stinkbug complex (Hemiptera: Pentatomidae) (Musser et al., 2021). SBL typically infest soybean during the months of August and September. Larvae of SBL feed on the leaves of soybean, reducing leaf area and photosynthesis. SBL has a host range consisting of 31 plants, and of these, soybean has the most economic importance (Herzog 1980). Other economic crops which SBL can complete its lifecycle are cowpea (*Vigna unguiculata*), sweet potatoes (*Ipomoea batatas*), soybean (*Glycine max* (L.) Merrill), and non-*Bt* cotton (*Gossypium hirsutum*) (Moonga 2016). Historically, cotton was a major secondary host for SBL due to high source of carbohydrates, and increased fertility and fecundity can be observed in SBL adults when exposed to nectaried cotton cultivars when compared to nectariless cultivars (Beach et al. 1985). However,

implementation of *Bt* technology in cotton production has reduced the need for additional control of SBL in cotton (Tindall et al. 2009).

Morphology and Life Cycle

SBL belongs to the Noctuidae family in the order Lepidoptera. Larvae are distinguished by two pairs of prolegs with the addition of three pair of black or green true legs (Smith et al. 1994). Larvae also have white stripes located sinstral and dextral of individual, running from cephalic to caudal side. Cabbage looper larvae resemble this description with a study in Mississippi resulted in 46% of larvae having black legs (Jost and Pitre, 1998). In addition, cabbage looper larvae commonly migrate prior to SBL populations. Due to the overlapping characteristics and life cycle, field identification is unreliable between loopers. SBL adults are small to medium sized moth, with an unevenly curved postmedial line and silver spot in the center of the fore wing (Eichlin and Cunningham 1969). Additionally, SBL adults' hind wing becomes gradually darker towards the border.

SBL life cycle can range from 27 to 34 days with the ability to produce four generations per year (Canerday and Arant 1966). SBL females can produce between 600-700 eggs throughout the season. Highest egg production (274 eggs/female) occurs at 26°C with an adult longevity of approximately 11 days. As temperature decreases to 17°C adult longevity is 17.9 days with a decrease in egg production averaging 162 eggs per female. At a temperature of 35°C adult longevity decreases to 6.5 days and moths produce an average of 100 eggs per female (Mason and Mack 1984). Female SBL moths have shown a preference of egg laying in soybean at any growth stage when compared to other row crops at similar growth stages. Oviposition location on leaf material was observed to be significantly higher on underside of leaf when compared to all other locations (Jost and Pitre 2002).

Freshly deposited SBL eggs are 0.58mm in diameter and appear white in color. As embryonic development occurs, egg coloration transitions to pale yellow, with longitudinal ridges forming (Barrionuevo and Blas 2016). In hot climates, SBL eggs hatch within 3 days. First instar of SBL requires between 3 and 4 days for development. Larval instar 2^{nd} -4^{th} only require 2 to 3 days of development for each instar and both 5^{th} and 6^{th} instar larvae require between 5 and 6 days to finish development (Shour and Sparks 1981). During early instar stages (1^{st} -2^{nd}) much of the feeding observed is targeted toward low-fiber tissue which is easy to digest. However, changes in feeding occurs during the later larval instars (3^{rd}-6^{th}) to a less digestible diet of high-fiber leaf content (Kogan and Cope 1974). Feeding is greatest in the 4^{th} through 6^{th} larval instars and is responsible for 96% of overall leaf consumption (Reid and Greens 1973). Pupation occurs underneath the soybean leaf and lasts approximately seven days.

Seasonal Distribution

SBL overwinters in Mexico, Caribbean, North and South America as well as in the southernmost portions of the U.S. where mild winters occur. Adult looper populations overwinter in southern areas where low temperatures exceed 15.6°C (Tingle and Mitchell 1977). In the Lower Rio Grande Valley, populations of SBL increased significantly during July and August when compared to June and September (Harding 1976, Buschman et al. 1981). As temperatures increase north of overwintering areas, SBL adults migrate north feeding on soybeans, cotton, sweet potato, etc. The Mid-Southern (Arkansas, Mississippi, Louisiana, and Tennessee) U.S. encounters multiple, large populations of larvae during the growing season. Hensley et al. (1964) observed highest populations occurred during August in Louisiana with 90% of loopers (*Trichoplusia ni*, *Pseudoplusia includens*, *Rachiplusia ou*) collected being SBL.

Similar results were observed in Mississippi with larger populations occurring during August (Allen et al 2021).

Soybean Damage

SBL damage most commonly takes place in reproductive stage soybean. Soybean damaged in the vegetative stages have the capability to compensate by increasing new leaf production from the axillary stems. (Haile et al 1998). Soybean between beginning bloom (R1) and full seed (R5.5) are most vulnerable to yield reductions from defoliation (Begum and Eden 1965). Owen (2012) observed less than 10% yield loss when 33% defoliation occurred during the R3 and R5 growth stage. Yield reduction of ~30% was caused by 66% defoliation. During the R6 growth stage, a 12 and 20% yield reduction was observed at 66 and 80% defoliation, respectively. Board et al (1994) observed yield reductions by reduced seed size when defoliation occurred during R3 -R6 growth stages. Kalton et al (1945) and Turnipseed (1962) observed no yield reduction when fully developed soybeans (R6) obtained high or complete defoliation. Weber (1955) observed a reduction in seed quality from complete defoliation situations in R6 soybean. Yield reductions due to seed quality are less severe when occurring closer to physiological maturity but can be observed as late as R6.6 until R7 when seed filling period and rate are affected (Board et al. 1994). Board et al. (2010) also observed significant yield losses when light interception was decreased 18% by defoliation during similar growth stages. From 2016 to 2019 SBL infested 57.3, 21, 19.8, and 20.8 % of the acres in the midsouth, respectively, and a total kg lost due to SBL feeding ranged from 248,640,000 to 275,520,000 kg (Musser et al., 2016-2019).

Economic Threshold

Economic thresholds for SBL can vary between states depending on seasonal occurrence. Arkansas's current SBL thresholds are set at 40% defoliation prior to bloom with SBL present and 25% defoliation after bloom with SBL present (Studebaker et al. 2021) (Table 1). Defoliation thresholds for SBL in Mississippi and Tennessee are the same with defoliation thresholds being 5% less than Arkansas prior to and after bloom. In addition to defoliation thresholds, Arkansas, Mississippi and Tennessee also have SBL population thresholds based on sweep net collections of 38 and 19 larvae (≥ 1.27 cm) / 25 sweeps in vegetative and reproductive stages, respectively (Crowe et al. 2021, Stewart et al. 2022, Studebaker et al. 2021). Louisiana SBL thresholds are not based on defoliation with treatment based off SBL populations present with recommended treatment occurring with 38 SBL/ 25 sweeps (Towles et al. 2022). Research conducted by Owens (2012) and Thrash (2018) reinforced the current defoliation thresholds in the mid – southern U.S. The Eastern U.S. (Georgia, Florida, South Carolina) has lower defoliation thresholds in vegetative and reproductive soybean compared to states in the mid – southern region of the U.S. (Greene 2022; Roberts 2022). Economic thresholds for SBL in soybean are largely based on visual estimations of percent defoliation. Through qualitative observation, percentages can be estimated incorrectly resulting in mistimed applications and unwarranted spending for soybean producers. In addition to defoliation estimates, SBLs must be present in sufficient numbers to justify control. Monitoring (Sweepnet, pheromone traps) of soybean pests was implemented on 56% of total acres in 2000 to aid in effective and efficient insecticide applications (USDA-NASS 2001). Turnipseed (1974) determined that sampling SBL populations using a standard shake sheet resulted in higher larval counts compared to sampling with sweep net or D-vac for both SBL and *Helicoverpa zea.* Although shake sheets result in greater larval counts, a sweepnet is the method primarily implemented due to sampling of

multiple other pests such as stink bugs at the same time. Research resulted in an economic threshold of 40% defoliation during the vegetative growth stages to allow for control applications to prevent reaching the economic injury level where yield reductions are observed. Caviness and Thomas (1980) studied yield loss from defoliation in irrigated and non-irrigated soybeans with greater yield reductions being observed during reproductive growth stages when compared to vegetative growth stages. Defoliation reaching 50% caused yield reductions of 11% with higher yield reductions from defoliation being observed in irrigated soybean than non-irrigated soybean (Caviness and Thomas 1980). Fehr et al. (1977;1981) observed defoliation reaching 25% in the reproductive growth stages can reduce yields due to the reduction of water/nutrients allocated to vegetative structures for future pod development resulting in inadequate recovery time. Additionally they found that defoliation of soybean during the R5 growth stage significantly reduces yield when compared to all other reproductive growth stages. Research conducted by Owen (2012) observed that R3 and R5 soybean had higher sensitivity to defoliation when compared to R6 growth stage soybean. Thrash (2018) also observed no difference in yield loss when continuous season long and one time defoliation (16.5%) occurred at R3 growth stage.

Cultural Control

Use of the ESPS helps to avoid exposing soybean in their most vulnerable growth stage to many defoliating pests, including SBL. Increased SBL oviposition was observed during early (R3) and full pod development (R4) when compared to late vegetative (V12) and full bloom (R2) growth stages (Mascarenhas and Pitre 1997). Because higher populations are observed in September than June through July, the early planting can reduce oviposition activity within a field by avoiding these vulnerable growth stages (Buschman et al. 1981). Oviposition preference

for the middle-upper portion of the canopy has been observed by Mascarenhas and Pitre (1997). Their results indicate that more leaf material was present in these sections of the canopy and increased protection from environmental factors that could reduce survivability. In addition, row spacing was observed to impact SBL populations. Hamadain and Pitre (2002) observed adults laying 2.1-fold as many eggs on soybean canopies that were planted in a narrow row spacing that had increased canopy density when compared to wide row spacing with less density. In 2000, 19% of total U.S. soybean acres implemented row spacing <76.2 cm. This is in comparison to 1997 previously where only 13% of U. S. soybean acreage used row spacing ≥ 76.2 cm. (USDA-NASS 2001).

Chemical Control

When SBL reaches economic threshold insecticides are the only available effective control option. Many insecticide classes have been used to control SBL, such as DDT, cyclodienes, organophosphates, carbamates, pyrethroids, diamides and insect growth regulators (IGRs). Organophosphates and carbamates make up the group one insecticides, the acetylcholinesterase inhibitors, and were broadly used for SBL control. By the 1960's, SBL was resistant for a ten-year span to all classes of available insecticides. Louisiana Cooperative Extension service published an insect control guide during this time period stating that "None of the insecticides currently registered on soybeans will give effective control." The main drivers of this resistance being a combination of high larval densities adjacent to cotton fields and selection pressure (Leonard et al. 1990). Jensen et al. (1974) observed ~ 320% increase in egg production when SBL adults fed on cotton blossoms compared to soybean foliage. In 1974, methomyl was recommended for the control of SBL along with *Bacillus thurgensis* products. Two years after methomyl was introduced for control of SBL, resistance was observed at a level of 5 to 7-fold.

Methomyl and acephate are still being used for control, but to improve efficacy and combat increasing insecticide resistance, multiple modes of actions are being used in combination with these group one insecticides (Boernel 1992).

Permethrin, a pyrethroid insecticide, was released in the early 1980's and was thought to be the solution for insecticide resistance. Pyrethroid insecticides alter the ability of voltage gated sodium channels inside the neuronal membranes of insects by discontinuing electrical signaling in the nervous system (Soderlund 2010). In 1981, applications of permethrin (Ambush 2EC) provided a high level of efficacy at 1 and 4 days after treatment (Herbert 1982). Portillo et al. 1993 observed the first permethrin resistance in SBL in Mississippi. Field studies conducted in 1988 by Leonard et al. found that permethrin provided 87% control, significantly more control than acephate, sulprofos, and Javelin (Leonard et al. 1990). Early season applications of pyrethroids to the first generation of soybean loopers in 1989 and 1991 resulted in higher levels of resistance in the second generation (Portillo et al. 1993) and populations of SBL collected near cotton producing locations in Louisiana had shown greater levels of resistance (Mink and Boethel 1992). Due to the increased likelihood of resistance in SBL, diagnostic techniques were developed to monitor suspected populations. These techniques involved treating glass vials with permethrin, introducing a SBL moth into the vial, and then checking for mortality after 24 hours. As of current, SBL populations remain highly resistant to pyrethroids. In one experiment, SBL treated with lambda–cyhalothrin, a pyrethroid, had 85% survival compared to 8% in a susceptible SBL population (Stacke et al. 2020). Additionally, the resistant SBL population exhibited additional cross resistance to deltamethrin and cypermethrin at 6.2-fold and 22.5-fold, respectively (Stacke et al. 2020). Although resistance has worsened in SBL, the closely related cabbage looper still remains susceptible to pyrethroids.

Organophosphate, carbamate, and pyrethroid insecticide applications for SBL have largely been replaced by insect growth regulators (IGR). Methoprene was the first Insect Growth Regulator (IGR) brought to market in 1975 commonly found in flea treatments in cats and dogs in addition to fly control in cattle. Advances in insecticide chemistry produced additional IGRs such as novaluron (Diamond), diflubenzuron (Dimilin), and methoxyfenozide (Intrepid 2F). Novaluron and diflubenzuron belong to insecticide group 15 responsible for inhibiting chitin biosynthesis. Methoxyfenozide makes up Group 18 insecticides which mimic the molting hormone ecdysone, forcing premature molting (O' Brien 1967). Methoxyfenozide was among the top treatments to reduce SBL density at 5 and 7 DAT when compared to available insecticides in 2006 for SBL control (Catchot et al. 2008). Increasing evidence of resistance to has led to increasing rates for single chemistry insecticides or switching to combination products such as Intrepid Edge (Group 5 and 18). Intrepid Edge, a combination of methoxyfenozide and spinetoram, was approved in 2013 for agriculture use and is quickly becoming industry standard for SBL control. In 2020, plots treated with Intrepid Edge had overall fewer SBL when compared to Intrepid 2F and Besiege (Chlorantraniliprole + Lambda – cyhalothrin) at 4 DAT (Cook et al. 2021). Even though several newer products provide good control of SBL, this pest has a long history of developing resistance to many insecticide classes and alternate control methods may help to reduce the likelihood of resistance.

Biological Control

Natural enemies can help suppress SBL populations. Richman et al. (1980) found that *Reduviolus roseipennis* and larvae of *Chrysopa* consumed the greatest number of SBL eggs, with a mean of 28.6 and 19.13 eggs consumed per individual/day, respectively. They also confirmed that *Chracanthium inclusum* (Hentz) is an egg predator, consuming 9.16 eggs per individual/day.

Consumption of both larvae and eggs occurred with *Tropiconabis capisformis* (Germar), *Hoplistoscelis deceptivus* (Harris), *Geocoris punctipes* (Say), and *Geocoris uliginosus* (Say). *Calleida decora* (F.) consumed a mean of 6.40 larvae per individual/day with a preference of small to medium sized larvae. Large portions of medium size larvae were consumed by *Stiretrus anchorago* (F.) and *Arilus cristatus* (F.) with a mean larval consumption of 5.57 and 5.91 larvae per individual/day, respectively. Daigle et al. (1990) found several parasitoids in soybean fields: *Cotesia marginiventris* (Cresson) (Braconidae), *Meteorus autograohae* (Braconidae), *Brachymeria ovata* (Say) (Chalcididae), *Euplectrus comstockii* (Eulophidae), *Pediobious facialis* (Giraud) (Eulophidae), *Mesochurus discitergis* (Say), *Chaetophlepsis plathypenae* (Tachinidae), *Lespesia aletiae* (Riley) (Tachnidinae), and *Copidosoma truncatellum* (Dalman) (Encyrtidae). Of these species, *C. truncatellum* was the most predominant. Three pathogens (*NPV*, *E. gammae, N. rileyi*) were also found within the soybean field with the most abundant being *NPV* with an infection rate of 63.7% of SBL. *Entomophthora sp.* and *N. rileyi* were also found within the field and had an infection rate of 27.3% and 9% respectively (Daigle et al. 1990).

Baculoviridae

Baculoviridae is a family of viruses that most commonly infect Lepidoptera: Noctuidae larvae, however, baculovirus infections have been observed in over 600 species within the orders of Lepidoptera, Hymenoptera, Diptera, Coleoptera, and Trichoptera. Two types of viruses: nucleopolyhedrovirus (NPV) and granuloviruses (GV) comprise the baculoviridae family. *Alphabaculoviruses* (NPV) and *Betabaculoviruses* (GV) are most commonly used for biological control of Lepidoptera species. Further separation of nucleopolyhedroviruses results in genera: *Alphabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus* in addition to genera *Betabaculovirus* belonging to granuloviruses (Herniou et al 2012). Classification is based upon

occlusion body shape, with NPVs consisting of polyhedral occlusion bodies and granular occlusion bodies in GV (Kroemer et al 2015). Baculoviruses have shown effective control for *Neodiprion sertifer* (Hymenoptera: Diprionidae) (Podgwaite et al. 1984), *Oryctes rhinoceros* (Coleoptera: Scarahaeidae) (Zelazny et al. 1990), and *Heliothis* spp. (Lepidoptera: Noctuidae) (Ignoffo et al. 1976). Baculovirus is categorized as an obligate parasite, incapable of reproducing without a host. The virion takes over the cells of the host body and reproduction of the virus continues until cell death occurs. Viral spreading occurs when infected cells rupture and as the infection worsens, cells continuously rupture leading to a liquified state for the insect. Insects contaminated with baculovirus appear shiny and oily, and become more fragile as infection persist. Due to the highly host specific characteristics, this enables the baculovirus and beneficial insects to co-exist within the same field. The specificity baculoviruses can also be a weakness, making targeting multiple species difficult (Blissard and Rohrmann 1990).

Nucleopolyhedrovirus (NPV)

Viruses belonging to NPV family are naturally occurring within the environment. Nucleopolyhedrovirus (NPV) has two phenotypes: occlusion - derived virus (OV), which is responsible for initial infection, and budded virus (BV), responsible for secondary infection of neighboring cells. The alkaline environment of caterpillar midguts dissolves the paracrystilline matrix causing the spread of occlusion bodies (OB). Virions of OV enter the midgut epithelium cells by fusion of microvilli. Transportation of the nucleocapsids enter the nucleus, where gene expression and viral replication occurs (Kawanishi et al. 1972). Production of budded virus (BV) results in secondary infection of additional tissue by viral replication. BV are nucleocapsids that are transported to the plasma membrane but lose nuclear derived membrane in the cytoplasm. Nucleocapsids acquire virus encoded glycoprotein by budding though the cytoplasm. Virions of

BV are specialized for secondary infection of alternative host cells. NPV have numerous numbers of virions occluded with single intranuclear crystals. Inclusion bodies under high alkaline environments such as the insect midgut will dissolve and release virions that infect the gut epithelium (Potter et al. 1976).

Nucleopolyhedrovirus (NPV) are the most common baculoviruses used for control of insects. NPVs have been used for insect control of many species with the majority belonging to the order Lepidoptera. *T. ni* NPV has shown to reduce cabbage looper populations in 1972 and 1973 with similar control to *B. thuringeinis* (Dipel 560 g/ha or Thuricide HPC 4.5 liter/ha) and methomyl (Lannate 1.12 kg/ha) (Silva and Moscardi 2002). Larvae infected with HearNPV and SfMNPV had greater mortality and shorter lifespans than untreated larvae (Black 2017, Behle and Popham 2012). Environmental factors impact efficacy of NPVs, such as application timing during low light situations and water quality. Application of NPVs during low light, applications with a water volume greater than 46.77 Liters /ha and neutral water pH all improved NPV efficacy (Jaques 1977).

Chrysodeixis includens Nucleopolyhedrovirus

Chrysodeixis includens Nucleopolyhedrovirus (*Chin*NPV) is a species-specific biological insecticide targeting soybean looper (*Chrysodeixis includens*). *Chin*NPV belongs to the Baculoviridae family, located in the genus *Alphabaculovirus. Chin*NPV is a double stranded DNA virus spread through oral consumption (Kawanishi et al. 1972). After consumption, liquefaction of internal organs and rupture of the caterpillar increases virus exposure to uninfected individuals.

ChinNPV is a naturally occurring virus persisting within the environment, capable of infesting SBL larvae under favorable weather conditions. Yearlong viral presence in soil was

observed with occlusion body populations decreasing throughout the growing season with the ability to cause epizootic events in subsequent years (Young and Yearian 1979). Fuxa and Richter (2001) observed a 230% increase in occlusion bodies present ten months after application when comparing naturally occurring NPV and genetically modified NPV used as a biocontrol insecticide. Peng et al. (1999) observed NPV persistence was higher in soils used for agriculture. Increased viral presence can be observed following tillage practices (Young and Yearian 1986). Results from Fuxa and Ritcher (2001) found increased levels of viral presence in 0 -2 cm of topsoil with NPV as deep as 26 – 38 cm. Jaques (1967) observed 25% (1.3×10^7 OB/g) of original NPV was present in the soil five years after treatment. Much higher than concentrations ($28.4 – 2.3 \times 10^3$ OB/ g) causing 2% mortality, believed to initiate epizootic events observed by Fuxa (2008). Increased findings of naturally occurring NPV occur under cold and rainy conditions. Young (1990) found that NPV can be transported to the lower canopy with as little as 0.25 cm of rainfall. Fuxa and Richter (2001) observed viral transportation through rain with no difference between rainfall amounts (5-55 mm).

In Tucuman, Argentina, *Chin*NPV infected specimens were collected in soybean fields during the 2017 growing season and reared in a laboratory (Arneodo et al. 2018). Due to the different isolates of *Chin*NPV, variable results were observed when monitoring infectiveness, speed of kill and OB production. Aguirre et al. (2019) obtained nine isolates, with *Chin*NPV – F, J, R, and V having the highest probability of providing control as a bioinsecticide. For *Chin*NPV to be used as a biopesticide, OB production must be high quality and cost-efficient. Increased OB production has been observed with mortality occurring during fourth instar larvae resulting in greater viral production causing increased viral spread. Mass production of *Chin*NPV can be achieved with lower input costs contributing from reduced handling and material usage due to

non-cannibalistic behavior of SBL (Sanchez et al. 2021). Applications of *Chin*NPV are compatible with current ground or aerial insecticide application practices.

Increased resistance prompted studies evaluating *Chin*NPV for control of SBL resistant to synthetic insecticides (lambda-cyhalothrin and teflubenzuron) (Godoy et al. 2019). McLeod et al. (1982) observed a 40% reduction in populations of SBL in fields containing foliar applied *Chin*NPV. *Chin*NPV applied to soil at high rates increased mortality at six weeks after application. However, the economic threshold for defoliation occurred three weeks prior. Distribution of NPV in soil was not affected when cultivation occurred. (Fuxa and Richter 1995).

Viral death occurs takes longer when compared to synthetic insecticides, resulting in greater feeding damage during this time (Harper 1973). Due to excessive defoliation and insufficient virus-based mortality, solutions to increase effectiveness of *Chin*NPV have been sought. Addition of fluorescent brightener increased mortality with 0.16 percent of total suspension reaching the highest mortality (91.8%). Similar results were observed in corresponding field studies increasing mortality between 1.3 and 1.9-fold with the addition of fluorescent brightener (0.1%) (Zou and Young 1996). Increased effectiveness can be contributed to radiation protectant provided by fluorescent brighteners (Shapiro and Robertson 1990). Differences in efficacy timing was observed between neonate populations extracted from various geographical locations showing differing susceptibility levels between populations (Muraro 2018). Ali et al. (1987) observed control of SBL caused by secondary transmission of NPV. Combination of larval size at the time of mortality and size of introduced uninfected larvae played a key role in mortality observed. Mortality of secondary infected larvae increased as initial infected larval instars increased as well as decreasing instar of secondary infected larvae.

Table 1. Soybean Thresholds of Soybean Looper in Selected States During Vegetative and Reproductive Growth Stage

	Vegetative	Reproductive
Arkansas	40%	25% with 6-8 SBL present
Mississippi / Tennessee	35% or 38 SBL/sweeps	20% or 19 SBL/25 sweeps
Louisiana	n/a	125 SBL/row meter or 38 SBL/ 25 sweeps
Georgia, Florida, South Carolina	30%	15% with SBL present

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Chapter 2 - Determining the Effectiveness of *Chrysodiexis includens* Nucleopolyhedrovirus Formulations Using Different Application Techniques and the Addition of Selected Insect Growth Regulators to Commercial Chrysogen

Abstract

Soybean looper (SBL), *Chrysodeixis includens* (Walker), infested an estimated 62.5% of soybean hectares in the Midsouth U.S. in 2020. Increasing resistance to pyrethroids, organophosphates, carbamates, diamides, and insect growth regulators (IGRs) has been observed in SBL. Because of the widespread insecticide resistance recorded in SBL alternative control measures have been explored. This has led to the production of a biocontrol insecticide specifically targeting SBL. Early formulations of *Chin*NPV had inconsistent control of SBL. Multiple rates of two *Chin*NPV formulations (Chrysogen and purified) were applied using different techniques (diet overlay, leaf dip, greenhouse rate response) where multiple larval instars were evaluated for defoliation and mortality. All studies were conducted at the University of Arkansas Research and Extension Center, Lonoke, AR. Commercial Chrysogen caused quicker mortality (7.9 days) in first instar larvae than all other instars. Purified *Chin*NPV mortality was quickest when applied to 3rd instar larvae or smaller. Results indicate that increased control is observed when *Chin*NPV is applied to early instars. An additional study was conducted to determine the effects of adding an IGR to Chrysogen as a tank mix partner. When an IGR was combined with commercial Chrysogen, no difference in control of 1st or 3rd instar larvae was observed compared to an IGR alone. Reduced in control of 5th instar larvae was observed when Dimilin (IGR) was tank mixed with Chrysogen. Increased control was observed for 5th instar larvae when Intrepid 2F was tank mixed with Chrysogen compared to Chrysogen alone. Results suggest that addition of IGRs with Chrysogen could provide beneficial effects when applied to SBL populations. In the rate response study, purified formulations provided greater control in addition to reducing defoliation when compared to commercial Chrysogen. While a reduction in defoliation was observed for the 292.4 ml/ha rate of Chrysogen compared to

all other rates, all rates of Chrysogen exceeded defoliation thresholds. Highest mortality was observed in diet overlay studies when compared to alternative experimental techniques (Leaf – Dip, Greenhouse Rate Response). Diet overlay and leaf dip bioassay techniques were closest related in third instar larvae. Results suggest that closely related techniques could be contributed to surface coverage.

Introduction:

Soybean (*Glycine max* (L.) Merrill) is the second most produced row crop in the United States (USDA – NASS 2022). In 2022, the United States planted nearly 37 million ha and produced 12 billion kg of soybean. There are multiple economically important insect pests of soybean in Arkansas. One of the most damaging of these insect pests is soybean looper (*Chrysodeixis includens,* Walker) (SBL), infesting 65% of the 1.1 million hectares planted in Arkansas in 2020 (Musser et al. 2021; USDA-NASS 2020). Soybean looper is a late season pest of soybean in the southern U.S. that can cause high yield reductions through defoliation. Soybean looper adults will begin migrating into soybean during the later months (July, August, September) of the growing season (Harding 1976, Hensley et al. 1964). During this period, most soybeans will be in the vulnerable reproductive growth stages (Fehr et al. 1977). Defoliation results in lower yield due to decreased nutrient uptake and photosynthetic activity by reducing the amount of chlorophyll present (Alderfer and Eagles 1976). Soybean looper has ranked in the top three most damaging soybean insects in the Mid-South U.S. since 2010, except for 2014, averaging 14.7 % total loss plus the cost of control (Musser et al., 2010-2020).

Widespread resistance has been documented to pyrethroids, organophosphates, carbamates, diamides, and insect growth regulators (IGRs) in SBL (Leonard et al.1990; Owen 2013). Portillo et al. (1993) observed that as SBL populations were exposed to insecticide

applications each successive generation had higher survival rates in each successive generation. Portillo et al. (1997) also observed that early season applications of pyrethroids for control of SBL in cotton increased resistance in following generations originating from surviving adults. Increased resistance to synthetic insecticides has led to closer monitoring of resistance in SBL and several new insecticides have been tested for baseline susceptibility. Owen (2013) found that Louisiana and Mississippi populations had a 6.25- and 9.2-fold difference in chlorantraniliprole and flubendiamide resistance across seven SBL populations. Increased resistance has primarily been combated with new synthetic insecticides (Besiege, Intrepid Edge, Prevathon), but another potential solution is the introduction of *Chrysodeixis includens* nucleopolyhedrovirus (*Chin*NPV).

Recently, a nucleopolyhedrovirus (NPV) targeting corn earworm, *Helicoverpa zea* (Boddie), has proven effective and has seen widespread use across the Midsouth (Black, 2017; B. Thrash, Personal Communication, 2022). Several other NPV's, including one that targets SBL (*Chin*NPV) could help combat insecticide resistance and protect natural enemies. *Chin*NPV belongs to the Group II alphabaculoviruses, specifically targeting SBL. Evidence of epizootic events are delayed when compared to synthetic insecticide. Mortality occurred < 5 days after treatment (DAT) for synthetic insecticides, while viral mortality occurred between six and seven DAT, with a 21.1% population reduction at 11 DAT (McLeod et al. 1982). Beach and Todd (1988) observed a 78% reduction in soybean loopers at 14 days after exposure to NPV. Additionally, because of the delayed mortality, defoliation of soybean plants may continue to increase, resulting in further yield loss. Because of the extended amount of time needed for control, timely applications are essential.

Application of NPVs on various substrates has resulted in inconsistent performance. Young and Yearian (1974) observed the lowest mortality when NPV was applied to cotton. NPV had a higher viral persistence on tomato compared to cotton or soybean. Viral presence on tomato and soybean at 24 and 48 hours after application was higher than viral presence on cotton, with little viral presence being observed 96 hours after application for all plant types. Similar results were observed by Forschler et al. (1992) with NPV having less activity on cotton when compared to tomato and diet substrate. Acidity of plant foliage and condition of larval midgut were suggested for reduced activity on cotton. Viral activity is reduced three DAT due to ultraviolet (UV) irradiation (McLeod et al. 1977; 1982). Temperature alone failed to reduce viral activity until 45°C was exceeded. The delayed mortality and poor control have been seen in previously tested *Chin*NPV strains and would not be of much use in most modern row crop systems.

The primary objective of this study was to evaluate and compare effectiveness of commercially available Chrysogen and purified *Chin*NPV when applied at multiple rates and across several larval instars. The second objective was to evaluate the efficacy of commercial Chrysogen when combined with multiple IGRs. This objective was conducted to determine if Chrysogen in combination with IGRs would provide increased mortality and reduced defoliation. For the third objective, multiple rates of Chrysogen (Commercial *Chin*NPV) and purified *Chin*NPV were tested on third instar SBL larvae for efficacy and impacts on defoliation to V3 soybean. Additionally, comparisons between experimental techniques used were made.

Materials & Methods:

SBL larvae were obtained from Mississippi State University Insect Rearing Lab (Starkville, MS) and were reared on a diet substrate (Southland Products Inc., Lake Village, AR)

consisting of wheat and soybean germ in 29 ml insect rearing cups (Solo P100N, Lake Forest, IL). Larvae were allowed to feed until trial initiation and stored in an insect incubator (Percival I-36VL, Percival Scientific Inc., Boone, IO) with 14:10 light:dark ratio set at 29.4•C during light hours and 25.6•C during the dark hours

Soybean Looper Diet Overlay Trial:

Two formulations (Commercial and Purified) of *Chin*NPV were acquired from AgBiTech (AgBiTech Corporation, Queensland, Australia) with the same occlusion body amounts (7.5 x 10^9 OB/mL) for both formulations. "Commercial" *Chin*NPV consists of the combination of virus infested SBL cadavers and the diet substate they were reared on. All material present inside the container at mortality is combined and mixed thoroughly with the result of the *Chin*NPV isolate #460 with 65.8% diet substrate. The "Purified" formulations of *Chin*NPV are further processed, to remove the diet substrate. Commercial Chrysogen treatments were arranged as a 5 x 6 factorial with the two factors being: larval instar (1^{st}, 3^{rd}-6^{th}) and rate of treatment (0, 146.2, 182.8, 219.3, 255.9, 292.4 ml/ha). Purified *Chin*NPV treatments were arranged as a 6 x 6 factorial with the two factors being: larval instar (1^{st} - 6^{th}) and rate of treatment (0, 146.2, 182.8, 219.3, 255.9, 292.4 ml/ha). Multiple replications were conducted for both commercial and purified formulations at selected instars (Table 2.1). To reach the corresponding field rates both commercial and purified products were diluted into a 1:1 ratio of virus: water. Desired measurements (625, 781.25, 937.5, 1093.75, 1250 µl) of *Chin*NPV mixture were added to 200 mL of water solution to represent corresponding field rates (146.2, 182.8, 219.3, 255.9, 292.4 ml/ha). Diet substrate (Southland Products Inc., Lake Village, AR) consisting of wheat and soybean germ was placed inside insect rearing cups (29 ml) (Solo P100N, Lake Forest, IL). Diet substrate surface was contaminated with 100 µl of *Chin*NPV solution and allowed to air dry prior

to infestation. Larvae were placed individually onto the contaminated diet substrate and allowed to feed for fourteen days. Larvae were stored in an insect incubator (Percival I-36VL, Percival Scientific Inc., Boone, IO) with 14:10 light:dark ratio set to 29.4•C during the light hours and 25.6•C during the dark hours. Each replication consisted of 30 worms per treatment and were arranged in a randomized complete block design. Larval instars were individually observed for mortality and pupation until fourteen DAT. Mortality of larval instars was declared when darkened epidermis and liquification was observed.

All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences between days to 50 and 80% mortality were determined by utilizing the Tukey's HSD at a= 0.05. Formulation and instar were considered fixed effects while replication was considered a random effect.

Soybean Looper IGR Leaf-Dip Bioassay:

Three soybean seeds (Asgrow 46x6) (Monsanto, Creve Coeur, MO) were placed into potting soil (The Scotts Miracle-Gro Company, Marysville, IL) within individual pots (10.2cm x 10.2cm x 8.9cm) (Greenhouse Megastore, Danville, IL). Upon soybean reaching V1 growth stage, two out of three soybean plants were removed from the pot, leaving the healthiest, most uniform plant. Soybeans were kept at the University of Arkansas Lonoke Research and Extension Center. Growing soybean were placed under a grow light set to 14:10 light:dark ratio with a greenhouse temperature setting between 25.6 and 29.4•C.

Six hours prior to experiment, leaves were clipped from plant with clippings occurring before each trifoliate position. Each trifoliate was then separated into individual leaflets and soaked in a 1:20 ratio of bleach:water solution. Leaflets were allowed to soak for 30 minutes to decrease viral contamination on leaf surface. Clean leaflets were air dried until bleach:water

solution was no longer present on leaf surface. Multiple similar sized leaflets were stacked, never exceeding fifteen leaflets at one time. Disks were then cut from stacked leaflets using a one-inch hole punch.

Chrysogen acquired from AgBiTech (AgBiTech Corporation, Queensland, Australia) was diluted into a 1:1 ratio of virus:water mixture. Desired measurement (625 µl) of the Chrysogen mixture was added to 200 mL of water to represent a field rate of 146.2 ml/ ha. Designated IGR products were then added to 200 mL solution at designated measurements (468, 312, 703 µl), respectively. Treatments were a 3 (larval instar :1^{st}, 3^{rd}, 5^{th}) x 6 (Insecticide treatment) full factorial. Insecticide treatments include *Chin*NPV [Chrysogen (146.2 ml/ha)], methoxyfenozide [Intrepid 2F (219.3 ml/ha)], diflubenzuron [Dimilin 2L (146.2 ml/ha)], novaluron [Diamond (329 ml/ha)], with combinations of each IGR and Chrysogen. Each instar (3) by insecticide treatment (6) contained thirty SBL larvae were considered a replication.

Individual leaflets were submerged into designated treatments and placed on paper towels and allowed to air dry to prevent drowning mortality of smaller SBL instars. Cotton pads were placed into the bottom of each petri dish with the addition of 100 µl of water to prevent leaflet from drying out. Singular leaflets were then introduced to separate petri dishes. Thirty SBL larvae were placed into individual petri dishes containing treated leaf disks. Treatment separation consisted of corresponding petri dishes being placed on trays. Lunch trays (25.4cm x 35.6cm) (Choice, Clark Associates Inc., Lancaster, PA) containing thirty petri dishes with one larval instar per petri dish represented one replication per treatment, were placed within an insect incubator (Percival I-36VL, Percival Scientific Inc., Boone, IO) with 14:10 light:dark ratio set to 29.4•C during the light hours and 25.6•C during the dark hours. Larvae were allowed to feed on leaf disk until mortality was observed or 14 DAT occurred, and then leaf area consumption was

determined. Mortality of larvae was determined by the darkening of the epidermis and liquification of the individual. Leaf area consumed was calculated daily by using LeafByte (Adam Campbell). Remaining leaf material and frass accumulation was removed after calculating leaf area consumption and replaced with fresh leaf disks daily.

All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences between days to 50 and 80% mortality were determined by utilizing Tukey's HSD at a= 0.05. Formulation and instar were considered fixed effects while replication was considered a random effect.

Greenhouse Rate Response Study

Soybeans were planted at the University of Arkansas Lonoke Research and Extension Center greenhouses on 14 May, 21 May, and 11 June during 2020 and in 2021 on 18 May and 24 May. Soybeans were grown the same as IGR Study under identical environmental conditions.

At 24 h before trial initiation, two hundred third instar larvae were placed into a sanitized container filled with soybean leaves that had been sterilized by being dipped into a 1:20 bleach-water solution and allowed to air dry prior to infestation. Precautions prior to and during ongoing experiments were taken to reduce spread and contamination. Handling of SBLs within a designated disinfected area and use of UV lightbulbs between experiments in designated areas and inside insect incubator were utilized to reduce viral presence. Upon combining leaflets and uninfected larvae, the container was then placed into the insect incubator. Two days prior to initiation of rate response study, 60 V3 growth stage soybeans were placed outside of the greenhouse to allow UV degradation of any present viral contamination. *Chin*NPV was kept inside a refrigerator between applications of mixed cans to insure *Chin*NPV remained at a cool temperature (< 25•C) to allow for occlusion body survival. During the study, untreated check

replications were separated from virus treatments by utilizing a neighboring greenhouse set to matching environmental conditions. Separation of untreated check from viral treatments were utilized to reduce aerial spread of *Chin*NPV. When analyzing defoliation and mortality, observations were always taken from greenhouse containing the untreated check first. Reentry into uncontaminated greenhouse after leaving was not authorized to reduce the spread of *Chin*NPV to healthy larvae.

Two formulations (Commercial and Purified) of *Chin*NPV were acquired from AgBiTech (AgBiTech Corporation, Queensland, Australia) with the same occlusion body amounts (7.5 x 10^9 OB/mL) of both formulations. Production of Commercial Chrysogen© consists of the SBL cadavers caused by virus exposure and diet substate. For ease of production, all material present inside the container at the time of mortality is combined and mixed thoroughly with the result of the *Chin*NPV isolate #460 with 65.8% diet substrate. Purified formulations of *Chin*NPV have been further processed, allowing for the removal of filler substances resulting in the end product of *Chin*NPV isolate #460 with diet substrate removed.

Treatments for commercial rate response trial were arranged as a randomized complete block design with a one (3rd instar larvae) by five (Chrysogen rates) factorial arrangement. Commercial Chrysogen© rates were 182.8, 219.3, 255.9, 292.4 ml/ha. Each run (2) consisted of 10 cages per rate, for a total of 40 treated cages and 10 untreated cages. The purified rate response trial treatments consisted of purified *Chin*NPV at 146.2, 182.8, 219.3, 255.9, and 292.4 ml/ha arranged in a randomized complete block design, with a one (3rd instar) by six (purified *Chin*NPV treatment) factorial arrangement. Each run (1) consisted of 10 cages per treatment, for a total of 50 treated cages and 10 untreated cages. For both studies *Chin*NPV was mixed in 7.6-liter spray cans and applied to ten V3 growth stage soybean plants using a CO_2 backpack sprayer

fitted with hollow cone spray nozzles (TeeJet TX-VK6) (Teejet Technologies, Glendale, IL) on 49.5cm spacing at 93.54 L/ha and 275.6 kPa. After airdrying, soybeans were brought inside and a 20.3 x 40.6cm insect cage (BioQuip Products, Rancho Dominquis, CA) was placed around the soybean to contain the larvae. Three healthy third instar larvae that had successfully transitioned onto soybean from diet were placed within each cage. Larvae were individually observed for mortality and pupation until fourteen DAT. Larvae were considered dead when darkened epidermis and liquification was observed. LeafByte (Zoe Getman – Pickering, Cornell University) was used to determine percent defoliation of each individual leaflet that were fed upon until fourteen DAT. All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences of defoliation, mortality, pupation, and days to mortality, and days to pupation were determined by utilizing the Tukey's HSD at a= 0.05. Formulation of *Chin*NPV and rate of *Chin*NPV applied were considered fixed effects. Random effects consisted of replication and run.

Throughout each experiment, precautions were taken to reduce contamination. UV lightbulbs (Coospider UV, Paris, France) were utilized three nights per week within virus designated areas between experiments to reduce viral presence within area of experimentation. During preparation of experiment, liquified diet substrate was kept in designated nonviral areas. All virus contaminated instruments were soaked in a bleach solution for a 24-hour period. During observations, the untreated check was separated from *Chin*NPV treatments and observed first.

Results:

Soybean Looper Diet Overlay Trial:

In the diet overlay study, there was a formulation and instar response, but no rate response was detected (Table 2.2). Commercial Chrysogen applied to diet substrate exceeded 50% mortality for all instars between 6.9 and 9.75 DAT but no instar ever reached 80% mortality (Table 2.3). Purified Chrysogen treatments reached 50% mortality between 5.1 and 9.1 DAT for all instars. Larval instar 1st – 3rd reached 80% mortality 5.8-6.4 DAT, with 4th-6th instar larvae not reaching a mean mortality of 80%.

In the commercial formulation first instar larvae had quicker mortality when compared to 3rd, 4th, and 5th larval instars. Quicker mortality was observed in 6th instar (7.9 d) when compared to 4th and 5th instar (9.25 and 9.75 d respectively). First instar larvae reached 80% mortality (7.9 d) one day after reaching 50% mortality while all other instars reached 80% between 10.4 and 10.7 DAT. No difference of days to 80% mortality for 3rd through 6th instar were observed. Final mortality of instars treated with commercial Chrysogen ranged from 67.2% in 4th instar to 76.9% in 1st instar larvae.

The purified formulation resulted in 1st-4th larval instars exceeding 50% mortality quicker than 5th and 6th instars while no difference was observed between 1st and 3rd instar larvae. Larvae less than 4th instar observed 80% mortality within a day or less of exceeding 50% mortality, with 1st instar larvae exceeding 80% mortality within the same day as 50% mortality. Quicker mortality was observed for first instar larvae (5.8 d) when compared to larvae greater than 3rd instar. Mortality of instars greater than 3rd instar ranged from 8 DAT for 4th instar to 11.2 d in 5th instar. Purified *Chin*NPV applied to 2nd and 3rd instars had quicker mortality than 5th and 6th instar, but no difference was observed when compared to 1st and 4th instar larvae. Mean final mortality of 1st - 3rd instars were the only larval instars to have exceeded 80% mortality (80.8 to 83.1%), while 4th - 6th instars ranged from 73.1 to 79.3% mortality.

Soybean Looper IGR Leaf-Dip Bioassay:

In 1st and 3rd instar larvae all treatments containing IGR's had less feeding than the UTC or Chrysogen alone (Table 2.4). Higher mortality (100%) was observed in larvae introduced to treatments containing IGR's compared to 20 and 60% mortality of Chrysogen alone applied to 1st and 3rd instar, respectively. With 5th instar larvae, Intrepid and Chrysogen + Intrepid had the least amount of consumed leaf area in addition to the highest mortality (100%). The mixture of Chrysogen + Dimilin had an antagonistic effect where 5th instar larvae consumed greater amounts of leaf area than treatments of Chrysogen or Dimilin alone. Diamond + Chrysogen was also antagonistic where the amount of leaf area consumed was greater than Chrysogen alone. Reduced mortality was observed with the addition of Dimilin and Diamond to Chrysogen when compared to Dimilin or Diamind alone.

Greenhouse Rate Response Study

In greenhouse rate response studies, there was a response to both formulation and rate in defoliation, mortality, days to mortality, and pupation (Table 2.5). The interaction for formulation by rate was also significant for defoliation, mortality, and pupation. Increased mortality was observed as Chrysogen rates increased resulting in decreased defoliation. All rates of commercial Chrysogen reduced defoliation when compared to UTC (Table 2.6). Defoliation percentages ranged from 39.2% to 48.1%. Chrysogen applied at 292.4 ml/ha reduced defoliation when compared to the 182.8 and 219.3 ml/ha rates. No difference was observed in percent mortality or days to mortality between Chrysogen rates with the UTC having significantly lower mortality when compared to all Chrysogen rates. Mortality in Chrysogen treatments ranged from 45 to 48.3% with mortality occurring between 7.4 and 8.3 DAT. SBL exposed to Chrysogen had a lesser percent pupation compared to the UTC (Table 2.6). No significant difference between

Chrysogen rates was observed with percent pupation, which ranged from 51.7% in 255.9 ml/ha to 55% in 146.2 ml/ha, with pupation occurring between 9 and 9.7 DAT.

All rates of purified *Chin*NPV reduced defoliation compared to the untreated check (Table 2.6). There was no rate response observed for defoliation or percent mortality with purified *Chin*NPV. All rates of purified *Chin*NPV had higher (100%) and faster mortality (6.0-6.4 d) compared to the UTC which only achieved 20% mortality at 8.1 days (Table 2.6) Pupation of UTC reached 80% pupation (9.25 d) while no pupation was observed for purified *Chin*NPV treatments (Table 2.7)

Discussion:

The addition of alternative control methods for SBL is greatly needed due to increasing development of insecticide resistant SBL in midsouth soybean production (Felland et al. 1990) Similar results were observed in 1st instar larvae by Milks et al. (1998) with reduced efficacy occurring with increasing larval instar. When applications of Commercial Chrysogen are applied to larger instars (3rd -6th), 80% mortality is observed between 10.4 and 10.7 days after treatment. McLeod et al. (1982) found similar results with 60% more mortality occurring at 11 days when comparing treated and untreated fields.

Applications of purified *Chin*NPV resulted in early instars (1st – 3rd) reaching 80% mortality quicker than late instar (4th – 6th) SBL. Alam et al. (1987) observed similar results with early instars being more susceptible to *Chin*NPV with reduced susceptibility occurring as larvae mature. These results suggest that sufficient control is provided only for early instar larvae (1st - 3rd). In addition to targeting early instar larvae (≤3rd), applications should target lower SBL population in field scenarios. Results observed in the diet overlay suggest that purified *Chin*NPV provided greater control than commercial Chrysogen. Soybean looper larvae life cycle is

completed within fourteen days, for both formulations of *Chin*NPV, mortality was observed for 5th and 6th instars approximately 10-12 DAT. With *Chin*NPV mortality being observed as late as 12 days for 5th and 6th instar larvae, results suggest a delay in life cycle completion when larvae are exposed to *Chin*NPV. Observations of extended life cycle seemed to correlate to instar size at time of treatment, with an increase in development time as larval instar increases, which is similar to Patil et al (1989). These results are also compared to those of Abbas and Young (1991), where a decrease in mortality and increase in time to death was observed for large larvae treated with purified *Chin*NPV.

With commercial Chrysogen providing insufficient control of later instars (3rd -6th) the addition of insect growth regulators was implemented to evaluate the mortality and decrease in foliar feeding. Treatments containing IGRs provided 100% control when larvae were between 1st and 3rd instar, and less than 0.2 cm^2 of leaf area was consumed. IGR containing treatments provided control between 2-3 and 3-4 DAT to 1st and 3rd instars, respectively. Increased uptake of occlusion bodies from consumed leaf area can be contributed to an increase in 3rd instar larvae mortality. Lasa et al. (2007) observed similar results when insect growth regulators were introduced to NPV resulting in > 90% mortality in late larval instars (5th-6th). The delayed control between instars can likely be correlated to instar size at time of treatment. Further research needs to be conducted to determine if insect growth regulator group plays a major role in control, due to group 18 insecticides (Intrepid 2F) observing greater control than group 15 insecticides (Dimilin 2F, Diamond).

Due to slower control when compared to synthetic insecticides (1-2 d), applications of *Chin*NPV should consider current SBL population density before defoliation percentages, giving adequate time to achieve control as to not exceed the current defoliation threshold (25%)

(Studebaker et al., 2020). Due to SBL control occurring between 6-9 DAT, *Chin*NPV applications should occur with small amounts of defoliation present. Based on this and the data from the greenhouse rate response studies, *Chin*NPV applications need to target early instars (<3rd) to increase chances of achieving adequate control and avoiding later instars (5th – 6th) where 84% of total larval consumption occurs (Reid and Greene 1973). Commercial Chrysogen applications provided a 45-48% mortality in SBL within 7-9 DAT. Greater mortality was observed in Chrysogen applications when compared to McLeod et al. (1982) findings of 21.1% control eleven DAT. These results were similar to Young and Yearian (1979) findings of all rates of *Chin*NPV exceeded 33% defoliation and less than 20% mortality occurring three weeks after treatment. While all rates reduced the amount of defoliation occurring when compared to the UTC, Chrysogen applied at 292.4 ml/ha reduced defoliation when compared to lower rates (182.8, 219.3 ml/ha). Results suggest that higher rates of Chrysogen reduce the amount of defoliation occurring. This could be contributed to increased occlusion body presence resulting in increased opportunity for viral uptake causing sickness with a decreased uptake of leaf material. Milks et al. (1998) observed similar findings of decreased pupal weight when cabbage looper (*Trichoplusia ni*) larvae were infected with sublethal rates of TnSNPV, with no rate response being observed for all three instars tested.

Purified *Chin*NPV provided 100% control with mortality occurring an average of 6 DAT for all rates when applied to third instar larvae. Similar results were observed by Young and Yearian (1979) with control reaching 100% with the highest rate of NPV applied to soil. Although percent control was similar, the time to mortality was slower to reach 100% control (6 weeks) while exceeding 33% defoliation, three weeks after treatment. All treatments of purified *Chin*NPV reduced defoliation, with all treatments remaining below 10.5% defoliation. Although

no difference in defoliation between purified rates were observed, each defoliation consistently trended downward as rate increased.

Preliminary findings from leaf dip data suggest that purified formulations of *Chin*NPV could provide greater control with quicker mortality and reduce defoliation (Appendix A). Purified *Chin*NPV provided >50% more mortality when compared to commercial Chrysogen, and this could be contributed to the removal of additional diet substate. Nucleopolyhedroviruses (NPVs) effectiveness is dependent on exposure to ultraviolet light and have the capability of surviving up to three days with UV light exposure (Ignoffo et al. 1989; McLeod et al. 1977, Shapiro et al. 2002). During this period, NPVs have the capability of spreading easily (Black 2017). Increased mortality (20%) was observed in the UTC of purified *Chin*NPV, this could be contributed to higher levels of effectiveness in combination with spreading to uninfected SBL larvae. Some formulations show improvement within further prcessing of *Chin*NPV resembling findings of Erlandson et al. (2007) on *Trichoplusia ni* with multiple formulations capable of infection with select isolates having greater compatibility with higher efficacy when introduced within an environment. Future studies should evaluate the effectiveness of additional formulations.

Comparison of techniques used in the evaluation of *Chin*NPV formulations resulted in higher efficacy in purified *Chin*NPV when compared to Chrysogen, in both the diet overlay and rate response studies. Increased mortality was observed in diet overlay studies when compared to all alternative techniques (leaf dip bioassay, greenhouse rate response) in first - fifth instar SBL larvae. Shapiro et al (1981) observed increased virus production with the consumption of diets containing higher levels of wheat germ. In third instar larvae, higher mortality was observed in leaf dip bioassay compared to greenhouse rate response studies, with a minimum of 13%

increase in mortality when compared to all rates within Chrysogen greenhouse rate response studies. Forschler et al. (1992) observed similar results with the addition of NPV to different diet substrate. Additionally, a 4% increase in mortality was observed by Zou and Young (1996) when comparing diet bioassay to leaf material within a spray table technique. Young et al. (1977) also observed higher levels of viral activity in soybean foliage compared to cotton. Additionally, higher levels of defoliation were observed in greenhouse rate response studies compared to leaf dip bioassay. Closely related mortality was observed between diet overlay and leaf dip bioassay techniques in third instar mortality. Similarity between these two techniques could be contributed to closely related experimental procedures of complete substrate coverage of *Chin*NPV compared to soybean canopy coverage provided by spray nozzles. Immaraju et al. (1990) observed similar results when comparing leaf dip techniques to field applications, with 2-fold increased mortality in citrus thrips (*Scirtothrips citri*) (Moulton). Further development of *Chin*NPV, resulting in increased mortality, would allow Arkansas soybean producers to implement an efficient and cost-effective control method for SBL.

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Table 2.1 List of instars and rates for each formulation for diet overlay studies conducted at Lonoke Research and Extension Center in 2020 and 2021

Formulation	Instar	Rate (ml/ha)	Replication	n=
Commercial	1	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	2	360
	3	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	2	360
	4	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	1	180
	5	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	1	180
	6	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	2	360
Purified	1	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	2	360
	2	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	2	360
	3	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	2	360
	4	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	3	540
	5	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	3	540
	6	(0, 146.2, 182.8, 219.3, 255.9, 292.4)	3	540

Table 2.2 Analysis of Variance statistics for days to 80% mortality by formulation, treatment, instar, and their interactions for diet overlay studies conducted during 2020 and 2021

	F	DF	P1
Formulation	17.70	1	<0.01
Instar	46.85	1	<0.01
Rate	0.53	4	0.71
Formulation*Rate	0.77	4	0.55
Formulation*Instar	11.03	1	<0.01
Rate*Instar	0.75	4	0.56
Formulation*Rate*Instar	0.14	4	0.97

Data is considered significantly different if $p < 0.05$

Table 2.3 Impact of SBL larval instars and *Chin*NPV formulation on days to 50 and 80% mortality at University of Arkansas Research and Extension Center, Lonoke,AR during 2020 and 2021

Formulation	Instar	Days to 50% mortality	Days to 80% mortality	Final Mortality at 14 DAA	% Pupation
Commercial	1	6.9 a ‡	7.9 a	-	23.1 a
	3	8.6 bc	10.7 b	-	30.3 a
	4	9.8 c	10.7 b	-	32.8 a
	5	9.3 c	10.5 b	-	30 a
	6	7.9 ab	10.4 b	-	23.7 a
		F=10.1 df= 4 p=<0.01			F=2.7 df=4 p= 0.03
Purified	1	5.5 ab	5.8 a	83.1	16.9 b
	2	5.1 a	6.2 ab	80.8	19.2 ab
	3	5.4 ab	6.4 ab	82.5	17.5 b
	4	6.5 b	8 b	-	20.7 ab
	5	9.1 c	11.2 c	-	24.6 ab
	6	8.1 c	10.7 c	-	26.9 a
		F=28.3 df=5 p= <0.01	F=26.2, df=5 p=<0.01		F=4.3 df=5 p= <0.01

‡ denotes values within column followed by the same letter are not significantly different

Table 2.4 Efficacy of Chrysogen in combination with insect growth regulators (IGRs) to evaluate leaf area consumption, percent mortality, and days to mortality of three larval instars of *Chrysodeixis includens* (Lepidoptera: Noctuidae).

Instar	TRT	Cumulative Leaf Area Consumed	% Mortality	Days to Mortality	% Pupation
1	UTC	1.8 ± 0.14 a ‡	6.7 ± 4.6 b	5.5 ± 0.5 b	-
	Chrysogen	0.35 ± 0.03 b	20 ± 7.4 b	5.5 ± 0.34 b	-
	Intrepid	0.01 ± 0.001 c	100 a	2.56 ± 0.09 a	-
	Dimilin	0.01 ± 0.001 c	100 a	2.43 ± 0.09 a	-
	Diamond	0.02 ± 0.001 c	100 a	2.66 ± 0.09 a	-
	Chrysogen + Intrepid	0.01 ± 0.001 c	100 a	2.53 ± 0.09 a	-
	Chrysogen + Dimilin	0.02 ± 0.001 c	100 a	2.66 ± 0.09 a	-
	Chrysogen + Diamond	0.02 ± 0.001 c	100 a	2.63 ± 0.09 a	-
		F= 161.01, df= 7, p= <0.01	F=169.39, df= 7, p= <0.01	F=36.7, df= 7, p=<0.01	
3	UTC	1.82 ± 0.14 a	20 ± 7.4 c	5.67 ± 0.33 b	-
	Chrysogen	0.35 ± 0.03 b	60 ± 9.1 b	5.72 ± 0.19 b	-
	Intrepid	0.01 ± 0.001 c	100 a	3.66 ± 0.09 a	-
	Dimilin	0.01 ± 0.001 c	100 a	3.46 ± 0.14 a	-
	Diamond	0.02 ± 0.001 c	100 a	3.7 ± 0.12 a	-
	Chrysogen + Intrepid	0.01 ± 0.001 c	100 a	3.23 ± 0.08 a	-
	Chrysogen + Dimilin	0.02 ± 0.001 c	100 a	3.78 ± 0.15 a	-
	Chrysogen + Diamond	0.02 ± 0.001 c	100 a	3.7 ± 0.15 a	-
		F=161.01, df= 7, p= <0.01	F= 51.37, df= 7, p=<0.01	F=29.9, df= 7, p= <0.01	
5	UTC	16.25 ± 0.67 a	6.7 ± 4.6 c	4.0 ab	93.33
	Chrysogen	5.46 ± 0.37 d	10 ± 5.6 c	4.0 ab	90
	Intrepid	2.17 ± 0.07 e	100 a	3.56 ± 0.09 ab	-
	Dimilin	6.35 ± 0.57 d	60 ± 9.1 b	3.61 ± 0.12 ab	40
	Diamond	13.04 ± 0.67 bc	33.33 ± 8.8 bc	3.7 ± 0.15 ab	63.33
	Chrysogen + Intrepid	1.93 ± 0.07 e	100 a	3.33 ± 0.12 b	-
	Chrysogen + Dimilin	11.34 ± 0.67 c	26.67 ± 8.2 c	4.0 a	73.33

Chrysogen + Diamond	15.08 ± 0.67 ab	13.33 ± 6.3 c	3.5 ± 0.29 ab	86.67
	F=114.99, df= 7, p= <0.01	F= 37.35, df= 7, p= <0.01	F=2.17, df= 7, p= 0.04	

‡ denotes values within a column followed by the same letter are not significantly different

Table 2.5 Analysis of Variance statistics for defoliation, mortality, days to mortality, and pupation by formulation, rate and their interactions for greenhouse rate response studies conducted during 2020 and 2021

		F	df	p value
Defoliation	Formulation	27.12	1	<0.01
	Rate	58.56	4	<0.01
	Formulation*Rate	7.36	4	<0.01
Mortality	Formulation	44.05	1	<0.01
	Rate	10.31	4	<0.01
	Formulation*Rate	1.84	4	0.01
Days to Mortality	Formulation	64.23	1	<0.01
	Rate	18.94	3	<0.01
	Formulation*Rate	8.63	3	0.12
Pupation	Formulation	6.05	1	<0.01
	Rate	8.21	4	<0.01
	Formulation*Rate	2.42	4	<0.01

Data is considered significantly different if $p < 0.05$

Table 2.6 Impact of selected rates of *Chin*NPV formulations on defoliation and mortality for Greenhouse Rate Response trials conducted at University of Arkansas Research and Extension Center, Lonoke, AR in 2020 and 2021.

Treatment	Rate (ml/ha)	% Defoliation	% Mortality	Days to Mortality
Chrysogen	UTC	63.4 ± 1.8 a ‡	0 b	-
	182.8	47.8 ± 1.6 b	45.0 ± 0.06a	8.3 ± 0.31 a
	219.3	48.1 ± 1.6 b	46.6 ± 0.07a	8.2 ± 0.3 a
	255.9	45.2 ± 1.5 bc	48.3 ± 0.07 a	7.4 ± 0.31 a
	292.4	39.2 ± 1.6 c	45.0 ± 0.07 a	7.4 ± 0.36 a
		F=21.95 df=4 p=<0.01	F=19.02 df= 4 p=<0.01	F= 2.91 df=3 p=0.03
Purified #1	UTC	45.3 ± 3.3 a	20.0 ± 0.07 b	8.1 ± 0.54 b
	146.2	10.5 ± 1.3 b	100 a	6.4 ± 0.12 a
	182.8	10.1 ± 0.9 b	100 a	6.1 ± 0.13 a
	219.3	7.7 ± 0.4 b	100 a	6.2 ± 0.13 a
	255.9	6.7 ± 0.4 b	100 a	6.2 ± 0.13 a
	292.4	5.4 ± 0.7 b	100 a	6.0 ± 0.01 a
		F=99.27 df=5 p=<0.01	F=116.0 df= 5 p= <0.01	F= 10.17 df= 5 p= <0.01

‡ denotes values within a column followed by the same letter are not significantly different

Table 2.7 Pupation response to selected rates of *Chin*NPV formulations applied to 3rd instar SBL in trials study conducted at University of Arkansas Research and Extension Center, Lonoke,AR in 2020 and 2021.

Treatment	Rate (ml/ha)	% Pupation	Days to Pupation
Chrysogen	UTC	95 ± 0.03 b ‡	9.4 ± 0.27 a
	182.8	55 ± 0.07 a	9.7 ± 0.4 a
	219.3	53.3 ± 0.07 a	9.4 ± 0.43 a
	255.9	51.7 ± 0.07 a	9 ± 0.36 a
	292.4	55 ± 0.07 a	9 ± 0.4 a
		F=9.69	F=0.7
		df=4	df=4
		p=<0.01	p=0.58
Purified #1	UTC	80 ± 0.07 b	9.25 ± 0.26
	146.2	0 a	-
	182.8	0 a	-
	219.3	0 a	-
	255.9	0 a	-
	292.4	0 a	-
		F=116	
		df=5	
		p=<0.01	

‡ denotes values within a column followed by the same letter are not significantly

Chapter 3 - In-Field Evaluation of purified *Chrysodeixis includens* Nucleopolyhedrovirus (*Chin*NPV) for Soybean Looper (*Chrysodeixis includens*) Control

Abstract:

Chrysodeixis includens Nucleopolyhedrovirus (*Chin*NPV) belongs to the Baculoviridae family and is used to specifically control soybean looper (SBL). *Chin*NPV efficacy in field scenarios has been well documented with control being inconsistent. Understanding *Chin*NPV's ability to manage SBL will determine its role in SBL management. The objective of this study was to evaluate purified *Chin*NPV for control of SBL in soybean. Trials were conducted in two locations with all treatments (UTC, Intrepid Edge, and *Chin*NPV) sampled at 7, 10, and 14 days after treatment. Soybean looper density and leaf area index (LAI) were evaluated using a standard black shake sheet and AccuPAR LP -80 for all observation dates. Results indicate that *Chin*NPV did not provide adequate control of SBL, and defoliation levels were similar to the UTC at all sample dates, while Intrepid Edge was the only treatment to reduce SBL populations.

Introduction:

Soybean (*Glycine max* (L.) Merrill) is the top row-crop commodity produced in the state of Arkansas. In Arkansas soybean is planted on 1.2 million hectares averaging 3,429 kg/ha yield, accounting for 1.95 billion dollars (USD) in exports (USDA-NASS 2021).

Soybean in the Midsouth observes numerous flights of soybean looper (SBL), *Chrysodeixis includens* (Walker), during the later months of the growing season where they can negatively impact yield (Harding 1976, Hensley et al. 1964). Early studies of the correlation between defoliation and yield by Begum and Todd (1965) found little to no yield reduction when defoliation occurred during early reproductive stages (R1-R2), but defoliation occurring during pod development and seed fill stages could result in major yield reductions. Defoliation occurring prior to pod development reduces yield by decreasing pod number (Goli and Weaver 1986) while defoliation occurring during pod fill reduces yield by decreasing seed size (Board et

al. 1994). Soybean defoliation (50%) occurring during the R3 growth stage had a 34.7% yield reduction when compared to 3.8% reduction for 50% defoliation occurring at V7 growth stage (Teigen and Vorst 1975). More recent research by Owens (2012) and Thrash (2018) resulted in greater yield losses occurring during R3 and R5 growth stage with no difference between defoliation event occurring once and continuous seasonal defoliation.

Insecticide resistance in SBL has been well documented with pyrethroids, organophosphates, carbamates, and insect growth regulators (IGR's) (Leonard et al. 1990; Felland et al. 1990; Minks and Boethel 1992), with a 15% decline in control of permethrin being observed between 1987 and 1988 in Louisiana (Leonard et al. 1990). Most recently, Reisig (2015) has observed control issues with diamides in the Eastern U.S. Increased insecticide rates and combinations of insecticide classes had been used to combat insecticide resistance. The addition of piperonyl butoxide (PB) to permethrin applications improved SBL control equivalent to thiodicarb at the time (Thomas and Boethel, 1994). Thomas and Boethel (1994) suggests that this combination may not be sustainable due to the high cost of application even though results show effectiveness by mixing insecticide chemistry. Increased resistance to synthetic insecticides has led to the need for alternative solutions while reducing costs for SBL control.

*Chin*NPV, also known as Chrysogen, (AgBiTech Corporation, Queensland, Australia), has been commercially available in South America since 2016 (AgBiTech 2020). *Chin*NPV provides control of SBL and can help manage resistant SBL populations (Godoy 2019). NPVs persist within environments capable of infecting the host when weather conditions (cold and rain) align resulting in epizootic events. Young (1990) and Fuxa et al. (2001) observed NPV remaining viable for 17 months within soils. *Chin*NPV is orally ingested by SBL larvae resulting

in initial infection. Secondary production of occlusion bodies infect healthy cells causing the rupture of the individual, spreading *Chin*NPV within the environment.

The objective for this study was to evaluate efficacy and defoliation reduction for purified *Chin*NPV applied at multiple rates to the industry standard, Intrepid Edge (Corteva Agriscience, Indianapolis, IN), to high and low populations of SBL larvae.

Materials & Methods:

Field trials were conducted near Tillar, AR on a grower's field, and in Marianna, AR at the University of Arkansas Lon Mann Cotton Branch Experiment Station during the 2021 growing season. *Chin*NPV trials were attempted during the 2020 growing season, however due to weather and naturally occurring virus the trials were unsuccessful. During the 2021growing season, significant weather events were not present resulting in environmental conditions unfavorable for epizootics. Experiments in Tillar, AR were planted at 370,500 seed/ha on 25 May using Asgrow 46XF2 (Monsanto, Creve Coeur, MO). The experiments in Marianna were planted on 25 June using DM45F61 (Don Mario, Gibson City, IL) at 296,400 seeds/ha.

Commercial Chrysogen consists of *Chin*NPV isolate #460 with 7.5 x 10^9 occlusion bodies per milliliter and 65.8% diet substrate. Purified *Chin*NPV formulations consists of *Chin*NPV isolate #460 with 7.5 x 10^9 occlusion bodies per milliliter with diet substrate removed. Previous experiments comparing *Chin*NPV formulations resulted in no differences between formulations. Purified formulation #2 was used in this study because it had the highest mortality and numerically the quickest time to death (Appendix A).

Trials located in Tillar were arranged in a randomized complete block design and replicated four times with a plot size of four rows wide (97-cm row spacing) by 12.2-m in length. Trials located in Marianna had a plot size four rows wide (97-cm row spacing) by 72.8-m in

length arranged in a strip trial design and replicated four times. Replications of *Chin*NPV treatments were blocked together to reduce viral contamination in non-viral treatments. In each trial there were four treatments: purified *Chin*NPV (AgBiTech Corporation, Queensland, Australia) at 146.2 ml/ha and 292.4 ml/ha, methoxyfenozide + spinetoram [(Intrepid Edge (Corteva Agriscience, Indianapolis, IN) (438.6 ml/ha)], and an untreated control. Trials were initiated when SBL densities exceeded 15 larvae (≥50% 1^{st} -2^{nd} instars) per 3.1 row meters. Treatments were applied to reproductive stage soybeans (R3 – R4) in Tillar on 29 Jul and 12 Aug. Trial initiation in Marianna occurred on 23 Aug at R4 growth stage soybean. All applications were made using a Mud Master Sprayer (Bowman Manufacturing, Newport, AR) using 93.5 L/ ha at 275.6 kPa fitted with Teejet TX6 nozzles (Teejet Technologies, Glendale, IL). SBL density was recorded using a 0.7-meter shake sheet. Leaf area index (LAI) readings were made using an ACCUPAR LP-80 (Meter Group, Inc., Pullman, WA). Plots were sampled twice per plot at 7, 10, and 14 days after treatment (DAT). To reduce spread of *Chin*NPV, designated shake sheets were used for each treatment. Sampling of untreated check was always conducted first, with sampling of Intrepid Edge and purified *Chin*NPV treatments following.

All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences in SBL density and LAI were determined by utilizing the Tukey's HSD at a= 0.05. Treatment and dates of observations were considered fixed effects. Random effects consisted of replication and location.

Results:

No interaction was observed between SBL density and LAI readings. Soybean looper density was significant at 7 ($F= 8.59$, $df=3$, $p=<0.01$), 10 ($F=6.95$, $df=3$, $p=<0.01$) , and 14 ($F=3.24$, $df=3$, $p=0.04$) days. Intrepid Edge was the only treatment to reduce SBL populations 7

and 10 DAT, with 1.6 and 6.5 SBL per 3.1 row meter, respectively (Table 3.1). At 7 and 10 DAT, no difference in SBL density was observed between either rate of *Chin*NPV when compared to UTC. Intrepid Edge was the only treatment to reduce SBL populations (5.2 SBL/3.1 row meter) at 14 DAT when compared to UTC, but no differences were observed when compared to *Chin*NPV treatments. No differences were observed for LAI readings for any of the sample dates (Table 3.2).

Discussion:

*Chin*NPV did not provide adequate control of SBL at any sampling date, and never differed from the UTC. When applying *Chin*NPV, viral presence may be observed but reduction of SBL populations should not be expected. McLeod et al. (1982) observed NPV obtained from Guatemala (Livinston and Yearian 1972) applied to SBL populations resulted in 49% mortality at fifteen DAT with first mortality occurring 6 and 7 DAT. Beach and Todd (1988) had contradicting findings with NPV obtained from Georgia. Similar mortality (45%) was observed at 9.7 DAT with highest effectiveness occurring before 7 DAT. Difference in results may be attributed to individual isotopes extracted from separate locations.

For studies conducted in 2021, applications of *Chin*NPV were made during the early afternoon under high UV light presence similar to current pesticide application tactics. The degradation of occlusion bodies due to UV light could be the reason for the delayed control observed. Young and Yearian (1974) and Botelho et al. (2018) observed a 70% reduction in efficacy due to UV degradation 4 DAT. Additionally, Ignoffo et al. (1977) also observed a 50% decrease in viral presence occurring 2 hours after application with minimal NPV presence at 24 hours due to UV exposure. To maximize control potential with *Chin*NPV, applications should be made in low light scenarios (dawn, dusk).

Field trials conducted in Tillar and Marianna consisted of mixed populations of SBL with ≥50% of all SBL present being small larvae (1st – 2nd instar) at the time of application. Results from previous studies on different instars suggest that applications of *Chin*NPV should be targeted toward early instar (<3rd) in field scenarios with lower SBL populations present (Alam et al. 1987). Silva Morgado et al. (2020) observed lower susceptibility to *Chin*NPV in the closely related cabbage looper (*Trichoplusia ni*) compared to SBL. The ability to correctly identify SBL versus cabbage looper would allow correct population estimates for warranted applications and control to occur without overwhelming the capabilities of *Chin*NPV. Comparisons between *Chin*NPV formulations resulted in purified formulations, not commercially available, having numerically higher control (Appendix A). With the current state of commercially available Chrysogen, applications for SBL control are not recommended due to inadequate mortality in addition to prolonged time to death. Future formulations of *Chin*NPV may provide sufficient control (80%) within a reduced period of time after application, allowing defoliation to remain below the economic injury level.

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Table 3.1 Mean soybean looper density across three trials at three sampling dates in 2021 at Tillar, AR and Marianna, AR

Treatment	7 DAT	10 DAT	14 DAT
	SBL / 3.05 row meters		
UTC	17.5 ± 0.22 a‡	20.3 ± 0.32 a	18.1 ± 0.16 a
Intrepid Edge	1.6 ± 0.21 b	6.5 ± 0.29 b	5.2 ± 0.29 b
*Chin*NPV (146.2 ml/ha)	16.6 ± 0.13 a	17.4 ± 0.28 a	14.3 ± 0.15 ab
*Chin*NPV (292.4 ml/ha)	20.2 ± 0.24 a	16.3 ± 0.27 a	15 ± 0.23 ab
	F=8.59 df=3 p=<0.01	F=6.95 df=3 p=<0.01	F=3.24 df=3 p=0.04

‡ values within a column followed by the same letter are not significantly different

Table 3.2 Mean LAI readings across three trials at three sampling dates in 2021 at Tillar, AR and Marianna, AR

TRT	7 DAT	10 DAT	14 DAT
UTC	5.7 ± 0.8	5.5 ± 1.1	4.7 ± 0.6
Intrepid Edge	5.7 ± 0.7	5.7 ± 1.0	4.8 ± 0.8
*Chin*NPV (146.2 ml/ha)	5.2 ± 0.5	5.8 ± 1.0	4.8 ± 0.5
*Chin*NPV (292.4 ml/ha)	5.6 ± 0.8	5.7 ± 0.9	4.8 ± 0.8
	F=1.31 df=3 p= 0.28	F= 0.36 df=3 p= 0.78	F= 0.13 df=3 p=0.94

‡ denotes values within a column followed by the same letter are not significantly different

Conclusion

Chrysogen, in its current formulation, will not be recommended for soybean looper control in Arkansas due to an inadequate and prolonged time to mortality. Additionally, defoliation was not kept to an acceptable level in these studies. However, it is encouraging that there were substantial differences, in terms of percent and time to mortality, between the tested formulations. Some of these formulations provided much better control than the currently commercialized Chrysogen formulation. Future formulations of *Chin*NPV may greatly improve control and allow defoliation to remain below the economic injury level. These studies did document improved efficacy across all formulations when applied to first or second instar larvae. This is similar to what has been recorded for other commercialized viruses. If future *Chin*NPV formulations have greater efficacy, they will likely need to be applied early in the infestation to provide a satisfactory control level. With increasing public scrutiny over pesticide use, biological control options must continue to be evaluated for control of economically damaging pests. Growers may also benefit from biological pesticide use if costs can be kept low and acceptable levels of control can be obtained. Overall, biological insecticides are another tool in the growers tool box and research needs to continue to find where they fit in modern agriculture.

APPENDIX A

EVALUATION OF DEFOLIATION AND MORTLAITY TIMING

OF *Chin*NPV FORMULATIONS

Objective

Evaluate whether selected *Chin*NPV formulations provide increased control while reducing defoliation.

Materials and Methods

Study was conduct at the University of Arkansas Research and Extension Center, Lonoke, AR during the 2021 growing season. Soybean plants were grown to the V3 growth stage upon which leaf trifoliate were extracted. Each trifoliate was then separated into individual leaves and allowed to soak for thirty minutes in a 1:20 bleach: water solution. Leaflets were airdried before separated into similar sizes and punched into leaf disks. Soybean leaf disks (116 cm^2) (Asgrow 46x6, Monsanto, Creve Coeur, MO) were submerged in a viral solution containing one of three *Chin*NPV formulations. Each *Chin*NPV formulation acquired from AgBiTech (AgBiTech Corporation, Queensland, Australia) was diluted into a 1:1 ratio of virus: water. Formulations were added to 200 mL of water equivalent of 146.2 ml/ha (625 µl). Treatments (Commercial, Purified #1, Purified #2) were arranged in a randomized complete block design consisting of one run. *C. includens* larvae were obtained from Mississippi State University Insect Rearing Lab. After introduction of virus contaminated leaf disk, one third instar larvae were placed into each petri dish. Petri dishes were placed onto trays containing thirty replications per treatment and then relocated to an insect incubator (Percival I-36VL, Percival Scientific Inc., Boone, IO) with a 14:10 Light: Dark ratio set to 29.4•C during the day and 25.6•C during the dark hours. Mortality was observed daily with mortality being declared when discoloration and rupture of epidermis. Total area of consumed leaf material was calculated by using LeafByte (Adam Campbell).

All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences were determined by utilizing the Tukey's HSD at a= 0.05. Formulation of *Chin*NPV was considered a fixed effect. Random effects consisted of cumulative leaf area consumed, percent mortality, as well as day of mortality.

Results

Mean cumulative consumption ranged from 25.5 to 37.1 cm^2 of leaf area. Leaf area consumption was reduced to 11.6 cm^2 when applied with purified #2 when compared to UTC. Purifed #2 reached the lowest amount of leaf feeding of both purified formulations. Mean % mortality ranged from 16.7 to 93.3, with formulation have no effect of percent mortality (Table 4.1). Although no differences were observed between formulations for percent mortality, purified #2 provided quicker time to mortality.

Appendix Table 1 Evaluation of *Chin*NPV formulations on leaf area consumption and mortality.

Treatment	Leaf Area Consumed	% Mortality	Day of Mortality
UTC	37.1 a ‡	16.7 b	4.2 a
Commercial Chrysogen	26.3 bc	76.7 a	5.4 b
Purified #1	30.2 b	83.3 a	5.4 b
Purified #2	25.5 c	93.3 a	4.9 ab
	F= 8.4 df=3 p=<0.001	F= 26.7 df= 3 p=<0.001	F= 4.1 df= 3 p= 0.009

‡ denotes values within a column followed by the same letter are not significantly different

APPENDIX B

EVALUATION OF COMMERCIAL CHRYSOGEN CONTROL OF CHRYSODEIXIS INCLUDENS EXTRACTED FROM TREATED FIELD

Objective

The objective was to evaluate mortality of soybean looper (SBL)caused when introduced to leaf material extracted from field applied with Chrysogen©.

Materials and Methods

Study was conducted at the University of Arkansas Lonoke Research and Extension Center during the 2020 growing season. Prior to treatment, 150 uninfected SBL larvae were removed from within trial replications and introduced to unsprayed leaf material. Soybean leaf material was removed from each treatment post application in fields located in Tillar, AR. Healthy larval instars were evenly distributed into containers holding leaf material of treatment and allowed to feed for 24 hours. After feeding for 24 hours, thirty of the healthiest SBL larvae were removed from leaf material and transferred to diet substrate (Southland Products Inc., Lake Village, AR). Diet trays consisted of 30 replications per treatment and placed inside an insect incubator (Percival I-36VL, Percival Scientific Inc., Boone, IO) with a 14:10 Light: Dark ratio set at 29.4•C during light hours and 25.6•C during dark hours. Treatments were arranged in a randomized complete block design consisting of one run. Mortality was observed for fourteen days, with death being declared when epidermis appeared liquified and discolored.

All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences were determined by utilizing the Tukey's HSD at a= 0.05. Treatment was considered a fixed effect. Random effects consisted of percent mortality as well as day of mortality.

Results

Soybean looper larvae introduced to leaf material previously sprayed within a field resulted in significant mortality (96.67%) when compared to Chrysogen treatments and UTC. No

difference was observed between rates of Chrysogen, with the 292.4 ml/ha rate having the highest mortality (30%) with Chrysogen treatments. Intrepid Edge provided quicker mortality when compared to all other treatments. Mortality of Intrepid Edge was observed two days sooner than Chrysogen© treatments (Table 5.1). Results suggest that commercial Chrysogen© fails to reach significant mortality levels to control SBL populations.

Appendix Table 2, Mortality of soybean looper larvae after feeding on field sprayed substrate containing Chrysogen and Intrepid Edge

Treatment	% Mortality	Days to Mortality
UTC	16.67 b ‡	4.4 b
Intrepid Edge	96.67 a	2.2 b
Chrysogen 146.2ml	20 b	4.16 a
Chrysogen 292.4ml	30 b	4.44 a
	F= 30.39 df= 3 p= <0.001	F= 21.89 df= 3 p= <0.001

‡ denotes values within a column followed by the same letter are not significantly different

APPENDIX C

EVALUATION OF CHRYSOGEN CONTROL OF FIRST INSTAR CHRYSODEIXIS INCLUDENS LARVAE ON V3 SOYBEAN

Objective

The objective of was to evaluate selected rates of Chrysogen when applied to 1st instar larvae to determine if increased efficacy could be observed on early instar SBL larvae.

Materials and Methods

Chrysodiexis includens larvae were obtained from Mississippi State University Insect Rearing Lab (Starkville,MS) were individually placed onto diet substrate and allowed to feed until initiation 24 hours before the trial. Upon reaching 24 hours before initiation of trial, two hundred third instar larvae were placed into a sanitized container filled with sterilized soybean leaves.

Potting soil (The Scotts Miracle- Gro Company – Landscaping, Marysville, OH) was filled one inch from the top of 10.2cm x 10.2cm x 8.9cm pots (Greenhouse Megastore, Dansville, IL). Three untreated Asgrow 46x6 soybean seeds (Monsanto, Creve Coeur, MO) were placed into potting soil and compacted to insure seed-soil contact. To ensure sufficient moisture for germination, pots were watered twice on day of planting and one watering event per day after. Upon soybeans reaching V1 growth stage, two out of three soybean plants were removed from the pot, leaving the healthiest, most uniform plant. Soybeans were kept at the University of Arkansas Lonoke Research and Extension Center. Growing soybeans were placed under a grow light set to 14:10 Light: Dark ratio with a greenhouse temperature setting between 25.6•C and 29.4•C.

Two formulations (Commercial and Purified) of *Chin*NPV were acquired from AgBiTech (AgBiTech Corporation, Queensland, Australia) with similar occlusion body amounts (7.5×10^9 OB/mL) of both formulations. Production of Commercial Chrysogen© consists of the combination of SBL cadavers caused by virus exposure and diet substate. For ease of production,

all material present inside container at time of mortality is combined and mixed thoroughly with the result of the *Chin*NPV isolate #460 with 65.8% diet substrate. Purified formulations of *Chin*NPV have had furthered processing, allowing for the removal of filler substances resulting in the end product of *Chin*NPV isolate #460 with diet substrate removed.

Treatments for Commercial Rate Response were designed as a randomized complete block design with a one (1st instar) by six (Chrysogen treatments) factorial arrangement. Commercial Chrysogen© treatments include applications applied at 146.2, 182.8, 219.3, 255.9, 292.4 ml/ha. Each run (1) consisted of 10 cages per treatment, for a total of 50 treated cages and 10 untreated cages. The rate response study was initiated by mixing Chrysogen acquired from AgBiTech (AgBiTech Corporation, Queensland, Australia) in 7.6-liter spray cans. Chrysogen was applied to ten V3 growth stage soybean plants (Asgrow 46x6) (Monsanto, Creve Coeur, MO) using a CO_2 backpack sprayer fitted with hollow cone spray nozzles (TeeJet TX-VK6) (Teejet Technologies, Glendale, IL) on 49.5cm spacing at 93.54 L/ha and 275.6 kPa. After airdrying, soybeans were brought inside the greenhouse and a wood steak was then placed into the soil to signify applied treatment. Randomization of treatments occurred with ten treatments per run. An 20.3x43.2cm insect cage (BioQuip Products, Rancho Dominquis, CA) was then introduced around the soybean to enhance the containment of the larvae. Once all cages were applied, three 1st instar larvae were applied to each soybean. Selection of only healthy SBLs that had successfully transitioned onto soybean substrate were used for study. This precaution would allow for a successful transplant and reduced non-viral mortality.

Precautions prior to and during ongoing experiments were taken to reduce spread and contamination. Prior to the study, SBLs were handled within a designated disinfected area. UV lightbulbs were utilized between experiments in designated areas and inside insect incubator

(Percival I-36VL, Percival Scientific Inc., Boone, IO) to reduce viral presence. Leaflets used to transition SBLs were cleaned by using a 1:20 bleach-water solution and allowed to air dry. Upon combining leaflets and uninfected larvae, the container was then placed into the insect incubator. Two days prior to initiation of rate response study, 60 V3 growth stage soybeans were placed outside of the greenhouse to allow UV degradation of any present viral contamination. Chrysogen was kept inside a refrigerator between application of mixed cans to insure Chrysogen remained at a cool temperature (< 25°C) to allow for occlusion body survival. During the study, untreated check replications were separated from virus treatments by utilizing neighboring greenhouse set to matching environmental conditions. Separation of untreated check from viral treatments were utilized to reduce aerial spread of Chrysogen. When analyzing defoliation and mortality, observations were always taken from greenhouse containing untreated check first. Reentry into uncontaminated greenhouse after leaving was not authorized to reduce the spread of Chrysogen to healthy larvae.

Larvae were individually observed for mortality and pupation until fourteen DAT. Mortality of larvae were declared when darkened epidermis and liquification was observed. LeafByte was used to determine percent defoliation of each leaflet until fourteen DAT. All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences were determined by utilizing the Tukey's HSD at a= 0.05. Rate of Chrysogen applied was considered as a fixed effect. Random effects consisted of percent defoliation, percent mortality, and day of mortality.

Results

Chrysogen© rates greater than 146.2 ml/ha resulted in decreased defoliation when compared to the UTC (55.7%). Although rates greater than 146.2 ml/ha reduced defoliation, all

rates failed to keep defoliation below defoliation threshold (25%). All rates of Chrysogen observed significant amounts of mortality when compared to the UTC, ranging from 50% to 76.7%. No difference was observed for time to mortality for all rates of Chrysogen, with mean mortality occurring between 7.7 and 9.4 DAT.

Appendix Table 3. Defoliation and mortality results of first instar soybean looper larvae when introduced to soybean plants treated with different rates of Chrysogen.

Treatment	Rate (ml/ha)	% Defoliation	% Mortality	Days to Mortality
Chrysogen	UTC	55.7 b ‡	0 b	-
	146.2	43.6 ab	53.3 a	9.4
	182.8	39.5 a	66.7 a	9.1
	219.3	36.8 a	76.7 a	9.2
	255.9	34.1 a	66.7 a	7.7
	292.4	38.1 a	50.0 a	7.7
		F= 6.15 df= 5 p=<0.001	F=11.62, df= 5 p= <0.001	F= 1.63 df=4 p= 0.17

‡ denotes values within a column followed by the same letter are not significantly different

APPENDIX D

EVALUATION OF FOURTH INSTAR LARVAE INTRODUCED TO *CHIN*NPV CONTAMINATED LEAF SUBSTRATE

Objective

The objective was to evaluate efficacy of *Chin*NPV when soybean looper (SBL) were introduced to contaminated soybean leaf disks

Materials and Methods

Chrysodeixis includens larvae were obtained from the Mississippi State University Insect Rearing Lab (Starkville, MS). Prior to experiment, larvae were introduced to soybean leaf material for 24 hours to ensure successful transitioning from diet provided by Southland Products inc (Lake Village, AR). Chrysogen© acquired from AgBiTech (AgBiTech Corporation, Queensland, Australia) was diluted into a 1:1 ratio of virus: water. Desired measurements (625, 781.25, 937.5, 1093.75, 1250 µl) of *Chin*NPV mixture were added to 200mL of water solution to represent corresponding field rates (146.2, 182.8, 219.3, 255.9, 292.4 ml/ha). A randomized complete block design was implemented to determine the time to mortality of *C.includens* caused by Chrysogen©. Commercial Chrysogen© treatments were arranged as a 1 x 6 factorial with two factors: larval instar (4^{th}) and rate of treatment (0, 146.2, 182.8, 219.3, 255.9, 292.4 ml/ha) consisting of one run.

Six hours prior to study initiation, leaflets were extracted from soybean plants and allowed to soak in a 1:20 bleach solution for 30 minutes. Leaflets were airdried on paper towels until no presence of solution was observed. Upon drying, similar sized leaflets were stacked and cut using a one-inch hole punch. Sanitized leaf disks were dipped into designated treatment. Thirty replications of each treatment were placed onto a labelled paper towel and allowed to air dry. Cotton pad was introduced into the bottom of the petri dish and soaked with 100 ml of water to ensure leaf disk from dry out. Thirty desired larvae instars were individually placed onto leaf disk before petri dish lid was secured into place. Petri dish trays containing thirty replications per

treatment were placed within an insect incubator (Percival I-36VL, Percival Scientific Inc., Boone, IO) with 14:10 Light: Dark ratio set to 29.4•C during the light hours and 25.6•C during the dark hours. Daily observations were taken for leaf area consumed and mortality for each larval instar. Leaf area consumed was calculated by using LeafByte (Adam Campbell). Previous fed leaf material and frass accumulation was removed and replaced with fresh leaf disks daily. Mortality of larvae was determined by observations of darkening epidermis and liquification of individual.

All data was analyzed using JMP Pro v16 (JMP, Version 16, SAS Institute Inc., Cary, NC). Differences were determined by utilizing the Tukey's HSD at a= 0.05. Rate of Chrysogen and days after treatment (DAT) were considered as fixed effects with the random effect consisting of cumulative leaf area consumed.

Results

Chrysogen© applied at 292.4 ml/ha reduced leaf area consumption when compared to UTC, while rates less than 292.4 ml/ha were not significantly different from UTC at 3 DAT. Prior to 3 days DAT, all treatments remained below 4.1 cm^2 of leaf area consumed. Decreased leaf area consumption was seen in rates 146.2 and 292.4 ml/ha in days following 3 DAT, with significantly reduced leaf consumption occurring at 7 DAT for 182.8 ml/ha. At 8 DAT, leaf area consumption ranged from 19.1 cm^2 (Chrysogen© 255.9 ml/ha) to 5.7 cm^2 (Chrysogen© 292.4 ml/ha).

Appendix Table 4. Cumulative leaf area consumption of fourth instar soybean looper larvae introduced to leaf disks contaminated with rates of Chrysogen.

	Cumulative Leaf Area Consumed (cm2)							
	Days After Treatment (DAT)							
Treatment	1	2	3	4	5	6	7	8
UTC	1.3 ab ‡	3.9 abc	6.9 ab	10.3 a	13.1 b	14.6 ab	15.6 ab	16.3 ab
Chrysogen 146.2ml	0.7 b	2.4 c	4.5 bc	5.9 bc	6.7 c	6.8 c	6.9 cd	6.9 cd
Chrysogen 182.8ml	1.3 ab	3.3 abc	6.3 abc	8.2 abc	9.2 bc	9.4 bc	9.5 cd	9.5 cd
Chrysogen 219.3ml	1.8 a	4.1 a	7.9 a	10.6 a	12.0 b	12.3 b	12.3 bc	12.3 bc
Chrysogen 255.9ml	1.7 a	4.0 ab	7.2a	9.4 ab	18.9 a	19.0 a	19.1 a	19.1 a
Chrysogen 292.4ml	1.0 b	2.5 bc	4.0 c	5.5 c	5.7 c	5.7 c	5.7 d	5.7d
	F=6.3 df=5 p<0.01	F=4.2, df=5, p<0.01	F=5.97 df=5 p<0.01	F=5.3 df=5 p<0.01	F=14.4 df=5 p<0.01	F=13.9 df=5 p<0.01	F=13.7 df=5 p<0.01	F= 13.9 df=5 p<0.01

‡ denotes values within a column followed by the same letter are not significantly different

Figure 1. Chrysodeixis includens (Lepidoptera: Noctuidae) infected by *Chin*NPV in early stage of pupation

Figure 2. Healthy Chrysodeixis includens (Lepidoptera: Noctuidae) larvae feeding on remains of liquefied larvae

Figure 3. Chrysodeixis includens (Lepidoptera: Noctuidae) infected by *Chin*NPV in early stage of pupation

Figure 4. Liquefaction of *Chin*NPV infected *Chrysodeixis includens* (Lepidoptera: Noctuidae) larvae

Figure 5. Spine soldier bug (Hemiptera: Pentatomidae) feeding on the remains of *Chrysodiexis includens* (Lepidoptera: Noctuidae) infected with *Chin*NPV

Figure 6. Spined soldier bug (Hemiptera: Pentatomidae) feeding on the remains of *Chrysodiexis includens* (Lepidoptera: Noctuidae) infected with *Chin*NPV

Figure 7. Early stage of *Chin*NPV infection of late instar *Chrysodeixis includens* (Lepidoptera: Noctuidae) larvae with addition mortality of early instar larvae at top of leaf

Figure 8. Unruptured liquified *Chrysodeixis includens* (Lepidoptera: Noctuidae) larvae infected with *Chin*NPV