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Phytotoxicity of *Beauveria bassiana* Oil Carriers to Selected Crops

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ABSTRACT

Beauveria bassiana (Balsamo) Vuillemin, is an insect specific fungus which can control sweetpotato whitefly and the boll weevil. An oil carrier is necessary to maintain the *B. bassiana* conidia until delivery to the insect host. Oil carriers act as an anti-desiccant for the conidia of the fungus and also may increase the retention time on the leaf after application. The objective of this study was to evaluate three oil carriers used as application vehicles for the *B. bassiana* fungus, for phytotoxicity to seven crops. The effect of the *B. bassiana* fungus on whiteflies was not included in the study since evaluations of sweetpotato whitefly control by the *B. bassiana* fungus have been conducted in separate studies. Crops were treated with either cottonseed, soybean, or petroleum oil, every five days for ten successive treatments. Crop phytotoxicity, height, weight, leaf number, and leaf area were measured. Successive oil treatments did not adversely affected growth or development of broccoli, honeydew, peanut, bell pepper, tomato, cabbage, or lettuce indicating that the oil carriers would be safe for use on these crops under similar environmental conditions. These results are important, since multiple applications of *B. bassiana* formulated in oil at three to five day intervals may be required to prevent economic damage to these crops by the sweetpotato whitefly. Lack of phytotoxicity to these vegetable crops is an important criterion when using oil carriers with this fungus for insect control.

RESUMEN

El hongo *Beauveria bassiana* (Balsamo) Vuillemin, es un parásito especffico de insectos capaz de controlar a la mosca blanca del camote y al picudo del algodón. Es necesario que los conidios de *B. bassiana* se conserven en un vehículo oleaginoso hasta su liberación en el insecto hospedero. Los vehiculos oleaginosos actuan como un antidesecante para los conidios del hongo y también pueden incrementar el tiempo de retención en la hoja después de la aplicación. El objectivo de este estudio fué evaluar la fitotoxicidad de tres aceites usados como vehículos de aplicación para el hongo *B. bassiana* sobre siete cultivos. El efecto del hongo *B. bassiana* sobre las moscas blancas no fué incluido en este estudio ya que este se evaluó en un experimento separado. Los cultivos recibieron cada cinco dias diez tratamientos suscesivos de alguno de los siguientes aceites: algodón, soya o petroleo. Se evaluó la fitotoxicidad al cultivo, el peso, la altura, el número de hojas y el área foliar. Los tratamientos suscesivos de aceite no afectaron adversamente el crecimiento o desarrollo en el brócoli, melón, cacahuate, pimiento morrón, tomate, repollo ó lechuga lo cual indicó que el uso de estos vehiculos oleaginosos puede ser inofensivo en estas plantas bajo condiciones ambientales similares. Estos resultados son importantes, ya que puenden ser necesarias aplicaciones múltiples de *B. bassiana* formuladas en aceite a intervalos de 3 a 5 días para prevenir los daños económicos causados por la mosca blanca del camote. La ausencia de fitotoxidad a estos cultivos es un criterio importante que debe considerarse cuando se usen vehiculos oleaginosos junto con este hongo para el control de insectos.

The sweetpotato whitefly became a serious pest in the Rio Grande Valley on cotton and vegetables in 1990 and insecticides were ineffective for control, thus there is a need for a whitefly control agent other than those currently available. The biology of the sweetpotato whitefly makes it difficult to kill because its scale-like larvae reside on the underside of the host leaves and the adults are highly mobile (Wright, 1992). The female may oviposit more than 300 eggs during a lifecycle which may be as short as 17 days. Few predators and parasites significantly impact this species, and whitefly resistance to insecticides may have already evolved.

In 1990 researchers found a proprietary petroleum-based horticultural oil (Sunspray 6E Plus, Sun Refining and Marketing Col, Philadelphia, Pa.) to be effective in repelling sweetpotato whitefly in a greenhouse study (Larew and Locke, 1990). This study also reported that the petroleum-based oil reduced the hatch of whitefly eggs that were laid on the treated leaf surface by 50%. While this oil is an one alternative for reducing whitefly infestations a need exists for a whitefly control rather than a repellant.

An insect specific fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has been reported to be effective in controlling the boll weevil, *Anthonomus grandis* Boheman (Wright and Chandler, 1992) and the sweetpotato whitefly, *Bemisia tabaci* Gennadius (Wright, 1992). The formulation, not the fungus, is effective against these insects. The Fungus only attacks and causes some pathogenesis. The widespread insect control provided by the formulated product goes beyond the effect of the fungus alone (Wright, 1992). To be effective, *B. bassiana* fungus must be transported in conidia form to the insect host. The transport mechanism must act as an anti-desiccant for the fungal conidia, be fluid enough for application through existing spray equipment, and increase the retention time on the plant leaf after application so that the fungus can contact an insect host. Some spray additives, oils, and adjuvants can be phytotoxic to plant leaves and can reduce crop growth and production (McWhorter, 1985). Oil carriers for the fungus should have little or no phytotoxic effect on crop leaves or crop growth.

The fungal strain on *B. bassiana*, FTCC 74040, causes pathogenesis in all life stages of the sweetpotato whitefly (by contact activity). Formulation and application technology of *B. bassiana* are important considerations in getting the fungus to the target pest on the underside of the plant leaves. Oil carriers act as an anti-desiccants for the fungal conidia and may increase the retention time on the leaf after application (Freed and Witt, 1969; Foy and Smith, 1969). Although oil may aid in enhancing conidial contact with the foliage, the primary purpose is conserve spore moisture and provide an environment favorable to extend conidial viability.

Insect control is proportional to the degree of contact between the spores and the insect cuticle, and an oil carrier facilitates this process. Compatibility between the oil and the fungus is of primary importance and three oil carrier candidates from preliminary laboratory evaluations (Wright, J. E. per comm.) were identified for potential use in a commercial formulation against the sweetpotato whitefly in vegetables. Three oils were chosen for this study because they can act as an anti-desiccant and sustain the fungus conidia in a viable condition, until it can be delivered to the insect host. Additionally, an oil carrier itself may act to kill or repel whiteflies from host plants. Crop phytotoxicity for these oils had not previously been documented under similar conditions for the vegetable crops included in this study. The objective of this study was to evaluate these oil carriers at two dosages each, for phytotoxicity on seven crops. Evaluations of effectiveness of the *B. bassiana* fungus for insect control was carried out in separate studies.

Table 1. Oil phytotoxicity to broccoli (var. Waltham 29 L-5814 W) after ten applications at five day intervals starting at one leaf stage of plant growth.

Treatment	concentration ml/L water	plant height (cm)	leaf stage (no.)	leaf area (cm^2)	fresh weight (g)	dry weight (g)	visual injury (%)
cottonseed oil	0.01	15.4 a	6.8 a	339 a	17.8 a	3.4 a	0 a
	0.02	22.6 a	7.1 a	536 a	32.6 a	4.9 a	0 a
soybean oil	0.01	18.2 a	5.6 a	442 a	25.6 a	4.4 a	0 a
	0.02	17.1 a	6.0 a	549 a	20.7 a	3.7 a	0 a
petroleum oil	0.01	18.9 a	6.1 a	500 a	30.2 a	5.3 a	0 a
	0.02	18.4 a	6.5 a	488 a	29.3 a	5.5 a	0 a
water	------	16.5 a	5.4 a	332 a	19.8 a	3.5 a	0 a
untreated	------	20.0 a	6.8 a	364 a	22.5 a	3.7 a	0 a

Numbers within a column followed by a different letter indicate a significant difference (alpha = 0.05) using a Waller/Duncan K-ratio T-test. Eight replications of each treatment were included in the experiment. Injury was evaluated on a percentage (0-100) scale where 0 = no visible injury and 100 = complete death of the plant.

MATERIALS AND METHODS

Glass house experiments were conducted in 1991 and 1992. The experiments were arranged as a split-plot, randomized complete block design with eight replications. Circular containers, 16 cm in diameter, 16 cm tall, and with a taper to 13.5 cm base, were used for growing the vegetables. Approximately 2.2 kg (kilograms) of a 2:1 heat sterilized soil mixture (Willacy silt loam

Table 2. Oil phytotoxicity to honeydew-melon (var. TAM DEW improve) after ten applications at five day intervals starting at one leaf stage of plant growth.

Treatment	concentration ml/L water	plant height (cm)	leaf stage (no.)	leaf area (cm^2)	fresh weight (g)	dry weight (g)	visual injury (%)
cottonseed oil	0.01	50.5 a	6.6 a	534 a	27.3 a	2.8 a	1 a
	0.02	58.0 a	7.3 a	557 a	28.8 a	2.8 a	0 a
soybean oil	0.01	51.3 a	8.2 a	735 a	30.7 a	4.3 a	0 a
	0.02	65.1 a	7.9 a	615 a	33.8 a	3.4 a	0 a
petroleum oil	0.01	53.9 a	8.4 a	512 a	27.9 a	2.6 a	0 a
	0.02	57.3 a	7.0 a	599 a	32.8 a	3.0 a	0 a
water	------	58.8 a	7.1 a	508 a	24.8 a	2.4 a	0 a
untreated	------	69.0 a	9.0 a	649 a	34.6 a	3.0 a	0 a

Numbers within a column followed by a different letter indicate a significant difference (alpha = 0.05) using a Waller/Duncan K-ratio T-test. Eight replications of each treatment were included in the experiment. Injury was evaluated on a percentage (0-100) scale where 0 = no visible injury and 100 = complete death of the plant.

plus organic peat) of pH 6.0 were added to each of the containers and four to six seeds of each vegetable were planted in containers. Immediately following emergence, plants were thinned to two plants per container except for tomato which was thinned to one plant per container. Plants were fertilized with a soil-applied fertilizer solution (20% nitrogen, 20% phosphorous, and 20% potassium) at an equivalent dosage of 5.6 kg ha^{-1} for N, P, and K every 14 days for the duration of the study. Adequate fertility was provided for optimal growth of the plants throughout the duration of the study. Illumination of plants was provided by natural sunlight with an average mid-day exposure of 900 W m^{-2}. Supplemental artificial lighting was provided to extend the day length to 12 hours. Six halogen 1000 W m^{-2} lights were placed over approximately 56 m^{-2} of floor space and approximately 1.3 m above the vegetable crops.

Vegetables included in the study were: honeydew (var. 'TAM DEW Improve'); broccoli (var. Waltham 29 L-581400); bell peppers (var. 'Grande Rio 66', Lot #1561104); tomato (var. 'Homestead' 24, 231-3330); cabbage (var. 'Early Round Dutch' 33-079); lettuce (var. 'Great Lakes' 200-1048); and peanuts (var. 'Runner'). Main plots for the experiment consisted of the vegetables listed above. Subplots consisted of eight individual treatments sprayed onto the leaves of each vegetable. The following eight treatments were included: cottonseed oil applied at 0.01 or 0.02 ml/L water; soybean oil at 0.01 or 0.02 ml/L water; refined petroleum oil applied at 0.01 or 0.02 ml/L water; water applied at a similar dosage as the oil carriers, and an untreated control. Treatments were applied using a track-mounted, laboratory pot sprayer calibrated to deliver an equivalent dosage of 93.5 L ha^{-1} at 125 KPa pressure for all crops except peanut, where the rate of 47 L ha^{-1} was used at 125 KPa pressure. Timing and dosage level of treatments to be made in commercial treatments with B. bassiana are currently under investigation. Treatments were applied ten times, once every five days commencing at the appearance of the first true leaf for each of the seven crops. The experiment was repeated, with the second experiment being initiated 21 days after initation of the first experiment.

Crops were watered with rain water every morning as needed to provide adequate moisture for plant development. Every seven

Table 3. Oil phytotoxicity to peanuts (var. Runner) after ten applications at five day intervals starting at one leaf stage of plant growth.

Treatment	concentration ml/L water	plant height (cm)	leaf stage (no.)	leaf area (cm^2)	fresh weight (g)	dry weight (g)	visual injury (%)
cottonseed oil	0.01	31.8 a	40.7 a	1156 a	65.5 a	10.3 a	1 ab
	0.02	29.8 a	42.8 a	1125 a	60.8 a	9.7 a	1 ab
soybean oil	0.01	28.5 a	41.7 a	1072 a	57.9 a	8.6 a	0 a
	0.02	29.6 a	36.0 a	824 a	40.2 a	7.5 a	3 ab
petroleum oil	0.01	31.1 a	40.3 a	1176 a	52.1 a	10.3 a	0 a
	0.02	29.1 a	37.3 a	967 a	42.1 a	8.0 a	2 ab
water	------	34.4 a	39.6 a	905 a	61.5 a	9.6 a	0 a
untreated	------	33.7 a	45.1 a	1047 a	62.8 a	10.0 a	1 ab

Numbers within a column followed by a different letter indicate a significant difference (alpha = 0.05) using a Waller/Duncan K-ratio T-test. Eight replications of each treatment were included in the experiment. Injury was evaluated on a percentage (0-100) scale where 0 = no visible injury and 100 = complete death of the plant.

Table 4. Oil phytotoxicity to bell peppers (var. Grande Rio 66; Lot #156 1104) after ten applications at five day intervals starting at one leaf stage of plant growth.

Treatment	concentration ml/L water	plant height (cm)	leaf stage (no.)	leaf area (cm^2)	fresh weight (g)	dry weight (g)	visual injury (%)
cottonseed oil	0.01	23.3 a	17.9 a	887 a	66.6 a	5.4 a	0 a
	0.02	25.8 a	17.1 a	1079 a	50.4 a	6.7 a	0 a
soybean oil	0.01	21.6 a	16.1 a	799 a	36.1 a	4.6 a	0 a
	0.02	23.1 a	16.3 a	837 a	39.5 a	5.1 a	0 a
petroleum oil	0.01	26.1 a	13.1 a	1920 a	45.2 a	6.0 a	0 a
	0.02	29.3 a	14.1 a	1231 a	57.1 a	7.8 a	0 a
water	------	25.3 a	17.7 a	1109 a	52.6 a	6.8 a	0 a
untreated	------	25.4 a	18.3 a	1154 a	53.3 a	7.0 a	0 a

Numbers within a column followed by a different letter indicate a significant difference (alpha = 0.05) using a Waller/Duncan K-ratio T-test. Eight replications of each treatment were included in the experiment. Injury was evaluated on a percentage (0-100) scale where 0 = no visible injury and 100 = complete death of the plant.

days following the first application of oil treatments the glasshouse was treated with either acephate (O, S-Dimethyl acetylphosphormidothioate) or resmethrin ([5−(phenylmethyl) -e- furanyl] methyl 2, 2-dimethyl-3- (2 methyl-1-propenyl) cyclopropane] fog spray (using an aerosol generator) to prevent insect or mite damage from confounding the phytotoxic effect of the oils. Insecticides were applied at a dosage of 0.06 kg per 56 m^{-2} of floor space.

Table 5. Oil phytotoxicity to tomato (var. Homestead 24 #231-3330) after ten applications at five day intervals starting at one leaf stage of plant growth.

Treatment	concentration ml/L water	plant height (cm)	leaf stage (no.)	leaf area (cm^2)	fresh weight (g)	dry weight (g)	visual injury (%)
cottonseed oil	0.01	45.3 a	6.8 a	967 a	54.8 a	8.8 a	0 a
	0.02	33.0 a	7.1 a	511 a	29.3 ab	4.4 a	0 a
soybean oil	0.01	37.7 a	5.6 a	714 a	53.4 a	7.8 a	0 a
	0.02	39.0 a	6.0 a	680 a	42.8 ab	6.8 a	0 a
petroleum oil	0.01	40.5 a	6.1 a	445 a	28.0 ab	5.5 a	0 a
	0.02	38.5 a	6.5 a	608 a	38.4 ab	5.6 a	0 a
water	------	48.0 a	5.4 a	1062 a	71.6 a	11.8 a	0 a
untreated	------	46.0 a	6.8 a	872 a	60.4 a	9.8 a	0 a

Numbers within a column followed by a different letter indicate a significant difference (alpha = 0.05) using a Waller/Duncan K-ratio T-test. Eight replications of each treatment were included in the experiment. Injury was evaluated on a percentage (0-100) scale where 0 = no visible injury and 100 = complete death of the plant.

Plant height and leaf number were measured and counted four days following the first, third, fifth, seventh, and tenth application. Plant injury was evaluated visually on a 0 to 100 scale, where 0 equals no visible injury symptoms and 100 equals complete plant necrosis. Observable injury appeared as spotting or discoloration of the leaf surface and the values given are for the percent of plants with injury. This discoloration did not become necrotic over the course of the experiment. Visible injury was evaluated four days after the first, fourth, eighth, and tenth application. Six days following the last application plants were clipped at soil surface and removed from the container. Plant height or length was measured, leaf number was counted, and fresh weight of each plant was recorded. Six days following the last application leaves were removed from the stem of each plant and leaf area of each plant was measured using a LI-COR LI-3100 area meter (Li-Cor Inc., Lincoln, NE). Plants which could not be measured immediately were placed in a polyethylene bag and placed on ice until measurements could be made later in the day. Plants were then placed in a paper sack (stems and leaves) and dried at 70°C for 72 hours in a forced air oven with circulating air. After 72 hours of drying, the samples were allowed to equilibreate to room temperature for approximately two hours, and the dry weight of each plant was measured and recorded.

Statistical analysis. Data were analyzed as a split plot with crops as the main plots and treatments as the subplots. Analysis was done using General Linear Models (GLM) analysis of variance. Mean separation of subplots (treatments to individual crops) were performed using a Waller-Duncan K-ratio T-test where alpha =

Table 6. Oil phytotoxicity to cabbage (var. Early Round Dutch #33-079) after ten applications at five day intervals starting at one leaf stage of plant growth.

Treatment	concentration ml/L water	plant height (cm)	leaf stage (no.)	leaf area (cm^2)	fresh weight (g)	dry weight (g)	visual injury (%)
cottonseed oil	0.01	16.0 a	9.0 a	2702 a	33.6 a	3.5 a	0 a
	0.02	16.0 a	11.0 a	769 a	44.8 a	4.2 a	0 a
soybean oil	0.01	14.3 a	9.8 a	1076 a	61.7 a	6.2 a	0 a
	0.02	19.5 a	9.5 a	877 a	44.5 a	5.0 a	0 a
petroleum oil	0.01	12.3 a	7.3 a	423 a	34.7 a	4.4 a	0 a
	0.02	18.0 a	11.7 a	1039 a	67.6 a	7.4 a	0 a
water	------	12.3 a	6.5 a	498 a	22.9 a	5.1 a	0 a
untreated	------	14.0 a	11.3 a	822 a	44.4 a	5.1 a	0 a

Numbers within a column followed by a different letter indicate a significant difference (alpha = 0.05) using a Waller/Duncan K-ratio T-test. Eight replications of each treatment were included in the experiment. Injury was evaluated on a percentage (0-100) scale where 0 = no visible injury and 100 = complete death of the plant.

0.05. Means of variables measured after the tenth application of oil treatments are presented in Tables 1 through 7. Data collected for plant height, leaf stage, or leaf injury prior to the tenth application did not indicate statistical differences between treatments and therefore are not presented.

Table 7. Oil phytotoxicity to lettuce (var. Great Lakes 200-1048) after ten applications at five day intervals starting at one leaf stage of plant growth.

Treatment	concentration ml/L water	plant height (cm)	leaf stage (no.)	leaf area (cm^2)	fresh weight (g)	dry weight (g)	visual injury (%)
cottonseed oil	0.01	22.3 a	5.4 a	1043 a	52.7 a	4.0 a	0 a
	0.01	21.9 a	5.6 a	763 a	34.9 a	3.3 a	0 a
soybean oil	0.02	20.2 a	5.3 a	893 a	44.9 a	3.7 a	0 a
	0.01	20.9 a	5.8 a	994 a	48.5 a	4.6 a	0 a
petroleum oil	0.01	20.7 a	5.1 a	689 a	32.7 a	2.8 a	0 a
	0.02	22.6 a	5.8 a	1644 a	81.9 a	8.1 a	0 a
water	------	21.2 ab	5.4 a	932 a	41.4 a	3.9 a	0 a
untreated	------	20.2 a	5.3 a	983 a	48.8 a	3.8 a	0 a

Numbers within a column followed by a different letter indicate a significant difference (alpha = 0.05) using a Waller/Duncan K-ratio T-test. Eight replications of each treatment were included in the experiment. Injury was evaluated on a percentage (0-100) scale where 0 = no visible injury and 100 = complete death of the plant.

RESULTS AND CONCLUSIONS

By the tenth application, honeydew plants were approximately 65 cm long and had 8 to 9 leaves; broccoli plants were 20 cm tall and had 6 to 7 leaves; bell peppers were 32 to 36 cm in height and had 14 to 17 leaves; tomato plants were from 50 to 68 cm in height and had 9 to 10 leaves; cabbage were 19 cm tall and had 10 to 11 leaves; lettuce plants were 20 cm tall and had 6 to 7 true leaves; and peanuts were 39 cm tall and had 34 to 36 fully expanded leaves.

Plant height was significantly different between crops after the tenth application, however within a particular crop there were no detectable differences (alpha = 0.05) (Tables 1-7). Some broccoli plants were one to two leaf stages behind in growth when compared to the most advanced broccoli plants, however, there were no relationship (correlation data not included) between oil treatments and leaf stage. Broccoli leaf stage and plant height effects appeared to be random variation between plants (Table 1). With eight replications and relatively low coefficient of variation values the statistical power of the experiment was such that any real differences between treatments should have been detectable.

Leaf area following the tenth application of treatments had the greatest amount of variability with tomato (Table 5) and cabbage (Table 6), however there were no detectable differences between individual treatments for any of the crops. Crop fresh and dry weights indicated that there were no negative effects associated with oils (either cottonseed, soybean, or refined petroleum oil) or dosage applied (either 0.01 or 0.02 ml/l oil to water ratio).

These studies indicate that in a glass house environment, the oil carriers; cottonseed oil, soybean oil, or refined petroleum oil were not phytotoxic to broccoli, honeydew, peanut, bell pepper, tomato, cabbage, or lettuce even after ten applications. The results herein are important in that multiple applications at three to five day intervals of *B. bassiana* formulated in oil may be required for prevention of economic damage to these crops by the sweetpotato whitefly. Lack of potential phytotoxicity by the oil carrier to vegetable crops certainly is a positive criterion for use with this fungus for insect control.

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