# Crop termination strategies to reduce *Neopestalotiopsis* spp. inoculum from infested fields and the continuous search for fungicide alternatives

**Adrian I. Zuniga, Juliana S. Baggio, and Natalia A. Peres**


## Summary

**We recovered *Neopestalotiopsis* sp. from soil and old crop residue in affected production fields, which proved that the pathogen can survive in Florida during the summer and might serve as a source of inoculum for disease development in the following season. In this project, we investigated different crop termination strategies to reduce *Neopestalotiopsis* inoculum at the end of the season and fungicide alternatives to reduce disease incidence during fruit production. Fumigants 1,3-dichloropropene, chloropicrin, and metam potassium were effective in reducing *Neopestalotiopsis* sp. inoculum in strawberry crowns. Contrary to previous reports, 1,3-dichloropropene seems to have a fungicidal effect. In a previous study, glufosinate-ammonium (Rely) at 170 oz/A significantly reduced inoculum of *Neopestalotiopsis* sp. However, that is twice the allowed label rate for weed control, and applications up to the maximum label rate (82 oz/A) did not significantly reduce inoculum. In additional field trials, applications of metam potassium (fumigant) and glufosinate-ammonium (herbicide) alone or in combination, as well as removing infected plants from the beds and exposing them to environmental conditions did not reduce *Neopestalotiopsis* inoculum in strawberry leaves or crowns. Among 32 fungicide products and programs evaluated, applications of Omega weekly and Switch tank mixed with paraffinic mineral oil or with Actigard and alternated with Thiram were the most effective in reducing fruit disease incidence and improving yield.**


## Methods

**Objective 1. To determine the effective rates of the fumigants 1,3-dichloropropene (Telone), chloropicrin (Pic), and metam potassium (K-pam) in reducing populations of *Neopestalotiopsis* spp. in strawberry crop residue.**

Pasteurized soil was placed in mason jars with two crowns of 'Florida Brilliance' strawberry plants inoculated with a mix *Neopestalotiopsis* sp. isolates. Field equivalency rates were calculated and adjusted based on the volume of soil contained within a 30.5-cm section of a standard raised bed used in Florida strawberry fields. Based on the volume, weight was calculated using soil density measurements for Myakka fine sand. Different rates of 1,3-dichloropropene (Telone® II, 1,3-dichloropropene 97.5%), chloropicrin (Tri-Pic 100, chloropicrin 99%), and metam-potassium (K-PAM® HL™, potassium N-methyldithiocarbamate 54%) (Table 1) were injected into soil contained in jars. Each treatment had three replicates. Fumigants 1,3-dichloropropene and chloropicrin were directly injected, whereas metam potassium was mixed with 30 ml of water before injection. Jars were immediately closed with TIF plastic and fastened with rubber bands. One day after fumigant application, holes were punched in the TIF plastic to let the fumigants vent. Crowns were retrieved in paper bags four to six days after fumigants were applied. Afterward, each crown was ground separately in a coffee grinder and *Neopestalotiopsis* spp. were enumerated using a semi-selective medium developed by our group.

**Objective 2. To evaluate the effect of the herbicide glufosinate-ammonium (Rely) applied at different rates for crop termination on the survival of *Neopestalotiopsis* spp. in strawberry debris.**
Field trials were conducted at the GCREC and in a commercial field in Plant City, FL. Both locations had high disease pressure at the end of the 2020-21 season. In these trials, glufosinate-ammonium was applied on strawberry plants at 22, 32, 42, 62, and 82 fl oz/A using two different water volumes, 40 and 100 GPA. Plants not sprayed with the herbicide were used as the non-treated control. Each treatment consisted of 20 plants per plot with 5 replications. Two weeks after herbicide application, 7 plants per plot were collected, brought to the lab, and leaves were separated and ground with a food processor. One gram of ground leaves was disinfested with a 0.05% solution of NaOCl and then processed using a semi-selective medium developed for the recovery and enumeration of *Neopestalotiopsis* spp. Colony-forming units (CFU) were counted 5 days after plating.

**Objective 3. To determine the efficacy of fumigant (metam potassium) injection in combination with glufosinate-ammonium application for crop destruction on the survival of *Neopestalotiopsis* spp. populations in strawberry debris.**
A field experiment was conducted in a previously infested area at GCREC at the end of the 2020-21 season. Metam potassium (K-pam) was injected through drip tape at 62 gpta and glufosinate-ammonium (Rely) was sprayed on strawberry plants at 40 fl oz/A using a $CO_2$ backpack sprayer calibrated to deliver 100 gal/A at 60 psi through a boom fitted with two hollow-cone T-Jet 8002 nozzles. Treatments consisted of metam potassium and glufosinate-ammonium alone or in combination and a non-treated control. Each treatment was composed of 12 plants per plot with 3 replications (beds). Plants were collected two weeks after treatment application and brought to the lab. Leaves and crowns were ground separately using a food processor. Samples were processed with a semi-selective medium as previously described.

**Objective 4. To evaluate the effect of crop removal from the bed and exposure to environmental conditions prior to soil incorporation on the survival of *Neopestalotiopsis* spp. in strawberry debris.**
After the 2020-21 season, plants from an area with high disease pressure were evaluated for the effect of plowing-out strawberry plants with a potato digger and placing them on the surface of the beds. Plants were exposed to the environment for 2 and 4 weeks to let them dry out at the end of the season. A control treatment consisted of plants that were not removed from the beds. Treatments were composed of 20 plants each and the experiment was performed twice. After plant retrieval, crowns were separated, ground, and processed individually. The semi-selective medium was used to process the crowns as described above.

**Objective 5. To assess the effect of pre-planting applications of glufosinate-ammonium (Rely) and fluazinam (Omega) in the row middles (bed alleys) on the survival of *Neopestalotiopsis* spp. in the soil.**
A field trial was conducted at the GCREC during the 2021-22 season. We intended to evaluate the soil application of glufosinate-ammonium (Rely) and fluazinam (Omega 500F) alone or in combination, and a non-treated control. Each treatment consisted of 12 plants per plot with 6 replications. Before treatment application, soil was collected from the field trial and processed with the semi-selective medium as previously described. Results showed zero *Neopestalotiopsis* CFU were recovered. Thus, we artificially inoculated the soil with a *Neopestalotiopsis* sp. suspension of $1x10^4$ spores/ml. Glufosinate-ammonium and fluazinam were then applied at 170 and 20 fl oz, respectively, on the row middles. Disease incidence on leaves was evaluated during early season (December to early January).

**Objective 6. To continue searching for fungicide alternatives for control of *Neopestalotiopsis* spp.**
The efficacy of different fungicides and programs for the management of Pestalotia leaf spot and fruit rot was evaluated in a field trial using Sensation® 'Florida127'. Four replications containing 12 plants each were used. Plants were inoculated by spraying a suspension of $10^4$ spores/ml. A non-inoculated control was not sprayed with the pathogen suspension. Fungicide treatments were applied

weekly (14 applications) from 29 Oct 2021 to 27 Jan 2022 using a $CO_2$ backpack sprayer calibrated to deliver 100 gal/A at 60 psi. The inoculated and non-inoculated control were not treated with fungicides. Several treatment programs included Switch 62.5WG or other fungicides of interest applied during weeks with high disease risk as determined by the Strawberry Advisory System (StAS) (http://agroclimate.org/tools/sas/) and Thiram SC or other fungicides of interest applied during weeks with low disease risk. In Table 5, we refer to the treatments applied during high risk as “alert” in the results table and “otherwise” for applications during weeks of low risk. StAS-based applications were made on 29 Oct, 4 Nov, 23 Nov, and 23 Dec 2021 (four applications). Fruit were harvested from 9 Dec 2021 to 10 Feb 2022 (16 harvests) to determine marketable yield and Pestalotia fruit rot incidence. Plants were also rated three times (1 Dec 2021, 3 Jan 2022, and 7 Feb 2022) for leaf spot severity using an ordinal scale with seven score levels (0 – no symptoms; 1 – slight infection, a few leaf spots; 2 – moderate infection, a few large spots on the leaf; 3 – moderate infection, leaf spots and wilting of leaves; 4 – moderate to severe infection, some dead leaves, and all leaves with at least one Neopestalotiopsis sp. spot; 5 – severe infection, several dead leaves and all leaves with spots; 6 – plant dead).

# Results

## Objective 1

A sustained decline in CFUs was observed with incremental doses of 1,3-dichloropropene and metam potassium (Table 1). For chloropicrin, a sharp decline in CFUs from doses of 0 to 81.86 liters/ha was observed, but doses above 81.86 liters/ha did not show significant differences among them. These results indicate that contrary to previous reports, 1,3-dichloropropene has a fungicidal effect and maybe be an important component in the efficacy of product formulations such as Telone C35.

**Table 1**. Effect of different rates of 1, 3-dichloropropene, chloropicrin and metam potassium on *Neopestalotiopsis* sp. in strawberry crowns after injection into soil in glass jars.

| **Rate of a. i. (liters/ha)** | ***Neopestalotiopsis* (CFU/ g crown)** |
|---|---|
| **1, 3- dichloropropene** | |
| 0 | 18465a |
| 28.46 | 7080a |
| 56.93 | 5153ab |
| 113.86 | 593bc |
| 170.78 | 726c |
| 227.71 | 90c |
| Probability of a greater F value | <0.0001 |
| **Chloropicrin** | |
| 0 | 18465a* |
| 40.93 | 2500b |
| 81.86 | 269c |
| 122.79 | 293c |
| 163.72 | 383c |
| 218.29 | 321c |
| Probability of a greater F value | <0.0001 |
| **Metam-potassium** | |
| 0 | 29162a |
| 116.82 | 12201b |
| 175.23 | 7040c |
| 233.64 | 2420d |
| 350.46 | 405e |
| 467.28 | 48f |
| Probability of a greater F value | <0.0001 |

*Treatments folowed by the same letter within a column are not significantly different according to the Fisher's Protected LSD test (p  0.05) on log transformed data. Non-transformed means are presented. Means of three replicateswith two crwons per replication for two experiments

## Objective 2

In general, 7766.7 to 17360.0 CFU/g leaves were recovered from the non-treated plants collected from the GCREC and a commercial field (Table 2). CFU reduction in response to the different glufosinate-ammonium (Rely) rates was not significantly different from the non-treated control, regardless of the water volume of 40 or 100 GPA. In a previous trial, we found that a high rate of 170 fl oz/A applied for crop destruction significantly reduced *Neopestalotiopsis* population in strawberry debris. Considering these results altogether, we conclude that glufosinate-ammonium can reduce *Neopestalotiopsis* spp. inoculum on leaves only when applied at 170 fl oz/A. However, this rate is double the legally allowed rate by the label (82 fl oz/A) for weed control and is likely to not be economical. Thus, alternative crop termination strategies are needed to reduce *Neopestalotiopsis* inoculum at the end of the season.

**Table 2.** Effect of glufosinate-ammonium (Rely) applied at different rates and volumes on *Neopestalotiopsis* spp. survival on strawberry leaves.

| Rely rates (oz/A) | *Neopestalotiopsis* spp. CFU/g leaves | | | |
|---|---|---|---|---|
| | 40 GPA | | 100 GPA | |
| | GCREC | Sizemore Farms | GCREC | Sizemore Farms |
| 0 | 10313.3 | 17360.0 | 7766.7 | 14793.3 |
| 22 | 10646.7 | 13173.3 | 7426.7 | 10213.3 |
| 32 | 10153.3 | 12546.7 | 8520.0 | 9660.0 |
| 42 | 11173.3 | 11760.0 | 6480.0 | 10080.0 |
| 62 | 11393.3 | 12100.0 | 7620.0 | 9160.0 |
| 82 | 10120.0 | 10153.3 | 7173.3 | 8866.7 |
| *P*-value: | 0.9973 | 0.7602 | 0.7321 | 0.9821 |

## Objective 3

The CFUs recovered from leaves and crowns were 55533.3 and 17944.4, respectively (Table 3). The applications of metam potassium (fumigant) and glufosinate-ammonium (herbicide) alone or in combination were not significantly different from the non-treated control. Thus, the treatments, at the rates tested, are likely not viable alternatives to reduce *Neopestalotiopsis* spp. inoculum on strawberry leaves and/or crowns.

**Table 3.** Effect of fumigant, herbicide, and their combination on *Neopestalotiopsis* spp. survival on strawberry debris.

| Treatments* | *Neopestalotiopsis* spp. CFU/g | |
|---|---|---|
| | Leaves | Crowns |
| Control | 55533.3 | 17944.4 |
| Herbicide | 53600.0 | 16477.8 |
| Fumigant | 50044.4 | 17311.1 |
| Herbicide + Fumigant | 49844.4 | 17255.6 |
| *P*-value: | 0.7734 | 0.9861 |

* fumigant: metam potassium (K-pam) at 62 gpta; herbicide: glufosinate-ammonium (Rely) at 40 fl oz/A.

## Objective 4

Our results showed that *Neopestalotiopsis* spp. inoculum for plants removed from beds and exposed to environmental conditions was significantly higher than the non-removed plants. We obtained 1379.0 and 908.3 CFU/g on plants collected at 2 and 4 weeks, respectively, from the non-removed treatment (Table 4). The respective CFU values from removed plants were 2173.5 and 1647.0. In both cases, lower *Neopestalotiopsis* CFUs were recovered from plants collected at 4 weeks. Even though the number of CFUs seem to go down over time, the numbers are still quite high and inoculum residue on these crowns can serve as a source of inoculum for the following season.

**Table 4.** Effect of crop removal from the bed and exposure to environmental conditions on the survival of *Neopestalotiopsis* spp. in strawberry crowns.

| Treatments | *Neopestalotiopsis* spp. CFU/g of crown | |
|---|---|---|
| | Collected at 2 weeks | Collected at 4 weeks |
| Non-removed | 1379.0 b | 908.3 b |
| Removed with potato digger | 2173.5 a | 1647.0 a |
| *P*-value: | 0.0011 | 0.0030 |

## Objective 5

Weather conditions during the 2021-22 season were not conducive for *Neopestalotiopsis* and disease incidence on leaves for every treatment in this trial was low. Thus, in addition to evaluating the leaves, we performed a second soil collection and processed the soil with the semi-selective medium. There were only 0 to 1.3 *Neopestalotiopsis* CFUs/g of soil, indicating the artificial inoculation was not successful. Therefore, due to the low inoculum in the soil, we were not able to determine the effect of applying glufosinate-ammonium (Rely) and fluazinam (Omega 500F) alone or in combination on *Neopestalotiopsis* spp. survival in the soil. We plan to repeat this study under controlled conditions during this season.

## Objective 6

Typical leaf spot symptoms started to appear about 8 days after inoculation with the *Neopestalotiopsis* sp. isolates. The treatment that received weekly Omega 500F applications as well as Switch + JMS and Switch + Actigard alternated with Thiram were the most effective in reducing fruit disease incidence and improving yield (Table 5). Omega is not currently labeled for commercial strawberry production but is in the process of registration for open-field strawberry nurseries. Interestingly, Switch alternated with Thiram was as effective in reducing fruit disease incidence, but the yield was not as high as the treatments mentioned above. Compared to the previous season, environmental conditions were not very conducive for the disease. Nevertheless, fruit rot incidence reached 55% in the inoculated control, whereas the best-performing treatments had less than 10% symptomatic fruit. Pestalotia leaf spot severity followed the trends observed for fruit rot incidence. In general, treatments with the least leaf spot severity were also Omega as well as all treatments including Switch or Thiram in addition to Bravo Weather Stick. Yield in the inoculated control was 12,281 lb/A, whereas yield in the Omega 500F and Switch alternated with Thiram was 53,802 and 31,104 lb/A, respectively.

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## Contact

Dr. Natalia A. Peres
UF/IFAS Gulf Coast Research and Education Center
P: 813. 419-6602
E-mail: nperes@ufl.edu

**Table 5.** Treatment effects on strawberry yield and fruit disease incidence caused by *Neopestalotiopsis* sp.

| Treatment[w] | Yield (lb/A)[x,z] | | Pestalotia fruit incidence (%)[x,z] | | Pestalotia leaf severity[y,z] | |
|---|---|---|---|---|---|---|
| Omega 500F 20 fl oz, weekly | 53802.5 | a | 6.5 | p | 1.1 | hi |
| Switch 62.5WG 14 oz + paraffinic mineral oil 1.6 pt (2%) alerts, alt Thiram SC 2.5 qt + paraffinic mineral oil 0.8 pt (1%) other weeks | 50775.9 | a | 7.7 | op | 1.6 | efghi |
| Switch 62.5WG 14 oz + Actigard 0.33 oz alerts, alt Thiram SC 2.5 qt + Actigard 0.33 oz other weeks | 46128.8 | ab | 9.0 | nop | 1.4 | fghi |
| Thiram SC 2.5 qt, weekly | 40200.1 | bc | 17.6 | k | 2.2 | abcdefghi |
| ProBlad Verde 45.7 fl oz alerts, Thiram SC 2.5 qt other weeks | 39031.2 | bcd | 15.6 | kl | 2.0 | cdefghi |
| ProBlad Verde 32 fl oz alerts, Thiram SC 2.5 qt other weeks | 38086.9 | bcd | 11.7 | lmn | 2.0 | bcdefghi |
| Bravo Weather Stik 1.5 pt, weekly | 36592.6 | bcd | 11.7 | lmn | 1.3 | ghi |
| Switch 62.5WG 14 oz alerts, alt Thiram SC 2.5 qt other weeks | 36466.1 | bcd | 6.7 | p | 1.1 | i |
| Yarden (YA2104) 26.6 oz, weekly | 33562.1 | cde | 13.1 | lm | 2.0 | bcdefghi |
| Switch 62.5WG 14 oz alerts, ProBlad Verde 32 fl oz other weeks | 31104.5 | cdef | 30.7 | j | 2.3 | abcdefgh |
| Control, non-inoculated | 30010.4 | def | 10.9 | mno | 1.9 | defghi |
| Aprovia Top 13.5 fl oz + Actigard 0.33 oz, weekly | 29419.1 | def | 32.5 | j | 2.7 | abcd |
| Aprovia Top 13.5 fl oz, weekly | 26249.4 | efg | 31.2 | j | 2.0 | bcdefghi |
| Regev (RE5928) 8.5 oz, weekly | 23951.0 | efgh | 35.2 | ij | 2.6 | abcde |
| Switch 62.5WG 14 oz alerts, alt Oxidate 5.0 0.39% v/v/+OxiPhos 2.5 qt, alt PerCarb 3 lb other weeks | 22306.9 | fghi | 40.8 | hi | 3.0 | ab |
| Serenade Opti 1 lb, weekly | 21993.6 | fghij | 45.0 | fgh | 2.3 | abcdefg |
| Switch 62.5WG 14 oz alerts, ProBlad Verde 45.7 fl oz other weeks | 21934.9 | fghij | 40.5 | hi | 2.1 | abcdefghi |
| ProBlad Verde 32 fl oz, weekly | 18015.9 | ghijk | 53.1 | abcde | 2.8 | abcd |
| Actigard 0.33 oz, weekly | 17384.5 | ghijk | 52.1 | bcde | 2.8 | abcd |
| Kitogreen 120 g, weekly | 16148.6 | hijk | 43.6 | gh | 2.9 | abc |
| LPC 5860-211 100 g + LPC 5860-212 120 g, weekly (1st appl: 24h prior inoculation)* | 15627.9 | hijk | 55.6 | abc | 2.8 | abcd |
| AVIV(AV8000) 28 oz (first week) then QAM 2.7 lb, weekly | 15467.3 | hijk | 51.3 | cdef | 2.3 | abcdef |
| LPC 5860-211 100 g, weekly (1st appl: 24h prior inoculation)* | 15296.0 | hijk | 48.2 | defg | 2.5 | abcde |
| ProBlad Verde 45.7 fl oz, weekly | 13729.4 | ijk | 58.1 | ab | 2.6 | abcd |
| Timorex ACT (TA1040) 35 oz, weekly | 13410.9 | ijk | 55.9 | abc | 3.1 | abc |
| ProBlad Verde 45.7 fl oz alerts, Serenade Opti 1 lb other weeks | 13180.9 | ijk | 56.2 | abc | 2.7 | abcd |
| Howler 2.5 lb + Theia 1.5 lb + Induce 2pt, weekly | 12665.3 | jkl | 48.0 | efg | 2.7 | abcd |
| Control, inoculated | 12281.3 | jk | 55.1 | abcd | 2.7 | abcde |
| ProBlad Verde 32 fl oz alerts, Serenade Opti 1 lb other weeks | 11243.8 | k | 53.3 | abcde | 3.3 | a |
| Theia 3 lb + Induce 2 pt, weekly | 9116.7 | k | 59.2 | a | 3.0 | abc |
| Howler 5 lb + Induce 2 pt, weekly | 8216.1 | k | 59.1 | a | 2.75 | abcd |

[w] Weekly applications over 14 weeks from 29 Oct 2021 to 27 Jan 2022.

[x] Yield and fruit rot incidence based on harvest data and fruit grading from 9 Dec 2021 to 10 Feb 2022 (16 harvests).

[y] Leaf spot severity evaluated on 2 Feb 2022 using an ordinal severity scale with 7 score levels (0 – no symptoms; 1 – slight infection, a few leaf spots; 2 – moderate infection, a few large spots on the leaf; 3 – moderate infection, leaf spots and wilting of leaves; 4 – moderate to severe infection, some dead leaves, and all leaves with at least one *Neopestalotiopsi*s sp. spot; 5 – severe infection, several dead leaves and all leaves have spots; 6 – plant dead).