Efficiency of green manures for Cercospora leaf spot management in coffee plants

Rogério Manuel de Lemos Cardoso, Júlio César Dias Chaves, Denilson Fantín & Valdir Lourenço Jr.

Instituto Agronômico do Paraná, Rod. Celso Garcia Cid km 375, Três Marcos, 86047-902, Londrina, PR, Brazil

Author for correspondence: Valdir Lourenço Jr., e-mail: valdir.lourenco@iapar.br

ABSTRACT

The use of Mucuna aterrima, Dolichos lab lab, Crustalaria mucronata, Arachis hypogaea, Mucuna deeringiana, C. spectabilis, and C. breviflora, intercropped with coffee plants, was evaluated as green manures in the management of Cercospora coffeicola under field conditions in Carlópolis and Jacarezinho counties in the state of Paraná in the crop seasons of 2008/2009 and 2009/2010. The main plot was comprised by treatments with green manures and urea, whereas subplots by the application or not of the poultry litter. There were two controls without green manure and urea: one with and another one without application of Beauveria bassiana, which was used to control Hypothenemus hampei. Similar founds were obtained at both municipalities. The percentage of diseased leaves (DL) was lower for most of the green manures and urea treatments. However, all the treatments reduced the DL in 2009/2010. Although A. hypogaea did not differ statistically from the controls in most of the experiments, the number of lesions per leaf was lower in the other treatments. Similarly, the percentage of healthy coffee berries was higher in the treatments than the controls in most of the experiments in both years. In general, there was no synergistic effect between green manures and urea with the poultry litter, and the efficiency of most green manures was similar to urea in the management of Cercospora leaf spot.

Key words: Cercospora coffeicola, Coffea arabica, cultural control, legumes, nitrogen nutrition, organic crop.

INTRODUCTION

Cercospora leaf spot, caused by Cercospora coffeicola Berk. & Cke., is one of the most important diseases of coffee trees in Brazil (Souza et al., 2011). The pathogen affects all the aerial parts of the plant (Godoy et al., 1997). The symptoms on leaves are characterized by round necrotic lesions with a brown outer ring and a graywhite center area. The lesions on fruits appear as black, dry, elliptical spots on the skin which cause damage to the cherries pulp (Godoy et al., 1997). Under severe attack, C. coffeicola causes defoliation, yield losses above 50%, and reduction of coffee quality (Fernandez-Borrero & Lopez-Duque, 1971).

The disease is favored by temperatures around 24°C, leaf wetness periods of six to eight hours, and plants suffering stress caused by water and nutrient deficiency (Fernandez-Borrero & Lopez-Duque, 1971). This condition is common in the most Brazilian regions of coffee (Pavan et al., 1999).

Paraná is one of the states with largest areas of coffee production in Brazil, with a high concentration in the “Norte Pioneiro” region (Balota & Chaves, 2010). The occurrence of Cercospora leaf spot has reduced the coffee yield. Furthermore, the soil fertility degradation is another important factor associated with the low yield of coffee trees in the state, mainly in the organic crop system due to restriction of use of chemical fertilizers and lack of options to replace these compounds; thus, several farmers have abandoned the organic coffee systems in this region (Pavan et al., 1999).

The main method to control Cercospora leaf spot is the use of fungicides in the conventional coffee plantings (Patricio & Braghini, 2011). Nevertheless, the intensive use of fungicides can be harmful to the human health as well as the environment (Cheatham et al., 2009). Therefore, the development of sustainable control methods of C. coffeicola is desirable in conventional and organic crop systems. As Cercospora leaf spot is associated commonly to nutritional deficiency in coffee plants (Fernandez-Borrero & Lopez-Duque, 1971; Pozza et al., 2001), the establishment of strategies to increase and maintain the soil fertility can reduce the disease intensity.

The application of adequate nitrogen fertilizer in soil reduces the Cercospora leaf spot disease in coffee plants (Pozza et al., 2001). In a study conducted in Minas Gerais state, the application of nitrogen (N) at 3, 7, 11, and 15 mmol L⁻¹ reduced the area under the curve of disease progress of lesion numbers per leaf and the defoliation on coffee seedlings maintained in a nursery (Pozza et al., 2001). However, the increasing costs of acquisition and application of chemical fertilizers have fostered the development of alternative management strategies of plant diseases. A viable alternative management strategy is the use of green manures and other organic matter amendments, which have been used to improve soil tilt and fertility since early...
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Green manures were sown manually intercropped with coffee plants on the 5th and 6th October of each year. The green manures were cultivated in two rows (0.5 m spacing row) with the exception of *M. aterrima* that was conducted in one row. These green manures were cut off during the flowering of early season (*M. deeringiana, C. breviflora, and C. spectabilis*) and late season plants (*M. aterrima, D. lab lab, C. mucronata,* and *A. hypogaea*), in February and May, respectively.

Twenty-four coffee leaves of the second and third pairs of the apical primary branches were collected and taken to the laboratory to quantify the number of leaves diseased and the number of lesions per leaf caused by *C. coffeicola*. The evaluations were conducted monthly in 2009 (January to April) and 2010 (January to May) and the average number of lesions per leaf and the percentage of diseased leaves were estimated in this period. After the coffee harvest, 100 coffee berries were selected randomly in each subplot and the number of healthy coffee berries (without *C. coffeicola* visible symptoms) was quantified.

The average number of lesions per leaf (NL) and the percentage of diseased leaves (DL) and healthy coffee berries (PHCB) were estimated for the treatments and controls in each subplot. Data were subjected to the analysis of variance and Fisher’s protected least significant difference (LSD) test to determine differences among means. The statistical analyses were conducted using the SAS software (SAS Institute, Cary, NC, version 9.1).

**RESULTS**

**Effect on the percentage of leaves infected by* C. coffeicola***

There was effect of the treatments with green manures and urea in the DL caused by *C. coffeicola* in 2008/2009 and 2009/2010 in Jacarezinho (*P*<0.0001). Nevertheless, there was no synergistic effect of the treatments with application of the poultry litter. Furthermore, the application of the poultry litter was significant only in 2009/2010 (*P*<0.05). With the exception of *A. hypogaea*, the DL was lower in the treatments with green manures and urea in 2008/2009 (Table 1). However, all the treatments reduced the DL in 2009/2010 in Jacarezinho in subplots with or without application of the poultry litter (Table 1).

In the experiments conducted in Carlópolis, there was effect of the treatments with green manures and urea on DL in 2008/2009 and 2009/2010 (*P*<0.0001). However, there was synergistic effect between the treatments with application of the poultry litter only in the control 2 in 2008/2009 (*P*<0.05).

All the treatments reduced the DL in the subplots with application of the poultry litter in 2008/2009 (Table 2). The most efficient treatments were *C. mucronata,* *C. spectabilis,* urea, and *D. lab lab*. The highest DL was recorded in the control 2 (Table 2). Some similar results in the reduction of the DL were found in the subplots without application of the

**MATERIAL AND METHODS**

Fields experiments were carried out in two commercial coffee plantings in Jacarezinho and Carlópolis counties during 2008/2009 and 2009/2010. Soil textural classes in Jacarezinho and Carlópolis were classified as sandy and clay soil, respectively. The coffee cultivar used in the trials was IPR–98 which is resistant to *Hemileia vastatrix* and susceptible to *C. coffeicola*.

The experimental design was a split-plot with three and four randomized blocks in Jacarezinho and Carlópolis, respectively. The main plot with 16 plants was comprised by the treatments with urea and the green manures: *M. aterrima, D. lab lab, C. mucronata, A. hypogaea, M. deeringiana, C. spectabilis,* and *C. breviflora*. The urea was applied at 31 g plant⁻¹ on October, December, February, and March of each year. Each treatment was comprised by two subplots (eight plants) with or without application of the poultry litter at 1.2 kg plant⁻¹. The N amount quantified in the poultry litter was around 2-3% of the total chemical composition. This organic matter was applied in the beginning of October in 2008 and 2009. There were two controls without green manure: one with and another without application of *H. bassiana*. This fungus was used to control *H. hampei* on coffee plants in all treatments. The coffee plant spacing in Jacarezinho and Carlópolis trials was 3.0 x 0.7 m and 2.3 x 0.8 m, respectively. In the beginning of October in 2008 and 2009, each plot received applications of N, P₂O₅, and K₂O at 300, 150, and 300 kg ha⁻¹, respectively.

Times (Ghalavand et al., 2009). Besides, there are reports that show the potential use of green manure to reduce the nutritional deficiency, which favors some soilborne plant pathogens (Ochiai et al., 2007).

In the state of Paraná, the increasing of N concentration in the soil (130 kg ha⁻¹) provided by *Leucaena leucocephala* reduced the mortality of shoots and damages on coffee leaves caused by *C. coffeicola*. In addition, this green manure provided biomass and other nutrients as potassium and calcium (Chaves, 2001). Although the use of *L. leucocephala* is promising in the management of Cercospora leaf spot, it is important to evaluate other species which can be used for this purpose.

Therefore, the objective of this study was to evaluate the use of velvet bean (*Mucuna aterrima*), labe-labe (*Dolichos lab lab* L.), *Crotalaria mucronata*, horse peanut (*Arachis hypogaea*), dwarf mucuna (*Mucuna deeringiana*), *C. spectabilis,* and *C. breviflora* as green manures, intercropped with coffee plants, in the management of Cercospora leaf spot in two commercial fields in Paraná during two years. To prevent the use of insecticides in these areas, *Beauveria bassiana* was used to control *Hypothenemus hampei*. We expect that these green manures can be used in organic and conventional coffee crop systems to improve the soil fertility and consequently the nutrition of coffee trees, contributing to reduce the damages caused by *C. coffeicola*.

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poultry litter (Table 2). The most efficient treatments were urea, *D. lab lab*, *C. mucronata*, *C. spectabilis*, *M. aterrima*, *M. deeringiana*, and *C. breviflora* (Table 2).

In 2009/2010, there was reduction of the DL in the treatments with *D. lab lab*, urea, *C. breviflora*, *C. mucronata*, *M. deeringiana*, *A. hypogaea*, and *C. spectabilis* in subplots with application of the poultry litter (Table 2). Although the highest DL was found in the controls 1 and 2, there was no difference between the control 2 with *M. aterrima*, *A. hypogaea*, and *C. spectabilis* (Table 2). In the subplots without application of the poultry litter, the most efficient treatments were urea, *D. lab lab*, *M. deeringiana*, and *C. spectabilis* (Table 2). The highest DL was detected in the controls 1 and 2 (Table 2).

### Effect on the number of Cercospora leaf spots

There was effect of the treatments with green manures and urea in the NL caused by *C. coffeicola* in 2008/2009 and 2009/2010 in Jacarezinho (P≤0.0001). Nevertheless, there was no synergistic effect of the treatments with application of the poultry litter in both years.

The reduction of the NL was significant in the treatments with *M. aterrima*, *D. lab lab*, *C. mucronata*, *M. deeringiana*, *C. spectabilis*, *C. breviflora*, and urea in
The effects of the treatments with green manures and urea in 2008/2009 were significant in the PHCB without the symptoms of *Cercospora coffeicola* in 2008/2009 and 2009/2010 in Jacarezinho (*P*<0.0001). In addition, there was synergistic effect between the treatments and the application of the poultry litter in 2008/2009 (*P*<0.05). Nevertheless, this synergistic effect was not significant in 2009/2010.

The highest PHCB was recorded in the treatments with *D. lab lab*, urea, and *C. mucronata* in the subplots with application of the poultry litter (Table 3). Although the lower PHCB was found in the control 2, there was no difference between this control with *M. aterrima*, *A. hypogaea*, and the control 1 (Table 3). Similar results were obtained in the subplots without application of the poultry litter in 2008/2009 (Table 3). The most efficient treatments were *D. lab lab*, urea, and *C. mucronata*. The lowest PHCB was obtained in the control 1 and 2 (Table 3). In the interaction between the treatments with the poultry litter, the highest PHCB was found in the treatments with urea and *C. mucronata* in the subplots with application of the poultry litter with the exception of *C. spectabilis*. The synergistic effect between the treatments was not significant in the other treatments (Table 3).

In the experiment conducted in 2009/2010, the application of the poultry litter did not affect the treatments (Table 3). Thus, the most efficient treatments were *D. lab lab*, urea, *C. breviflora*, *C. mucronata*, *C. spectabilis*, *M. deeringiana*, and *M. aterrima*. The lowest PHCB was obtained in the control 2 which was not different from the control 1, *C. mucronata*, urea, *C. spectabilis*, *A. hypogaea*, and *M. deeringiana* (Table 3).

There was significant effect of the treatments with green manures and urea in the PHCB in 2008/2009 and 2009/2010 in Carlópolis (*P*<0.001). In addition, the effect of these treatments was significant in the subplots with and without application of the poultry litter in both years (*P*<0.05). However, there was no synergistic effect among the treatments and the application of the poultry litter.

The most efficient treatments were *D. lab lab*, urea, and *C. mucronata* in the subplots with application of the

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**TABLE 3** - Effect of green manures, urea, poultry litter, and *Beauveria bassiana* on the percentage of healthy coffee berries without *Cercospora coffeicola* symptoms in Jacarezinho, PR, Brazil, in 2008/2009 and 2009/2010

| Treatments | 2008/2009 | 2009/2010 |
|------------|-----------|-----------|
|            | PL²       | Control   |
| *Mucuna aterrima* | 39.0 Acd¹ | 37.3 Acd  | 53.8 a   |
| *Mucuna deeringiana* | 45.0 Abc | 33.7 Acd  | 37.3 abc |
| *Arachis hypogaea* | 37.7 Acd | 30.7 Ad   | 32.3 bc  |
| *Crotalaria spectabilis* | 77.7 Aa | 68.7 Aa   | 55.3 a   |
| *Dolichos lab lab*  | 82.0 Aa  | 70.3 Ba   | 44.8 abc |
| *Crotalaria mucronata* | 50.3 Ab  | 47.7 Abc  | 50.8 ab  |
| Urea        | 79.7 Aa  | 67.3 Ba   | 46.8 abc |
| Control 1²  | 40.7 Abcd| 28.0 Ade  | 33.2 bc  |
| Control 2³  | 30.3 Ad  | 15.7 Ae   | 29.7 c   |

¹Means followed by the same letter in capital (rows) or small (columns) are not significantly different according to the Fisher’s protected least significant difference (LSD) (*P* ≤ 0.05).

²Subplots with (PL) or without (Control) the application of the poultry litter at 1.2 kg plant⁻¹.

³Control 1: without green manure and without application of *B. bassiana*; Control 2: without green manure and with application of *B. bassiana*. 

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poultry litter in 2008/2009 (Table 4). The lowest PHCB was recorded for *A. hypogaea* and the controls 1 and 2 which were not distinct from *C. breviflora*, *C. spectabilis*, *M. deeringiana*, and *A. terrima* (Table 4). Similarly, *D. lab lab*, urea, and *C. mucronata* were the most efficient treatments in the subplots without application of the poultry litter (Table 4). Although the lowest PHCB was detected in the controls 1 and 2, there was no difference among *C. breviflora*, *A. hypogaea*, and *M. deeringiana* with the control 2 (Table 4).

In the trial carried out in 2009/2010, the highest PHCB was detected in the treatment with *D. lab lab* in the subplots with application of the poultry litter (Table 4). The treatments with urea, *C. mucronata*, *A. hypogaea*, *C. breviflora*, and *M. deeringiana* were distinct from the controls 1 and 2 (Table 4). Although *D. lab lab* was the most efficient treatment in the subplot without application of the poultry litter, this treatment did not differ from *C. breviflora* and urea (Table 4). The least PHCB was recorded in the controls 1 and 2 (Table 4).

**DISCUSSION**

To our knowledge this was the first study to evaluate the use of *A. terrima*, *D. lab lab*, *C. mucronata*, *A. hypogaea*, *M. deeringiana*, *C. spectabilis*, and *C. breviflora* as green manures in the management of *Cercospora* leaf spot on coffee plants under field conditions. Most of the green manures resulted in the reduction of the DL, NL, and damages on coffee berries caused by *C. coffeicola*. Furthermore, the efficiency of these green manures was similar or higher than the urea in the experiments.

The legumes used as green manures in this study add large amount of N to the soil, which increase the supply of this nutrient to the coffee plants (Balota & Chaves, 2010). Furthermore, these plants have the ability to form symbioses with N-fixing bacteria, which contribute with expressive amount of N to the soil-plant system (Balota & Chaves, 2010). There are reports that the total amount of N obtained in the biomass of *D. lab lab*, *M. aterrima*, *C. mucronata*, *C. spectabilis*, *C. breviflora*, and *M. deeringiana* was around 246, 199, 216, 96, 68, and 91 kg ha⁻¹ (Andrade Neto et al., 2010; Cesar et al., 2011). In our experiments, the amount of this nutrient in legumes biomass was above 60 kg ha⁻¹ (data not shown).

The only green manure with low efficiency in the most experiments was *A. hypogaea*. A possible explanation is that the development of this legume was limited due to lower spacing of coffee rows. In another study, *A. hypogaea* produced high amount of biomass when intercropped with coffee plants in rows spaced at four meters (Chaves et al., 1997).

As N is the main nutrient required by coffee plants, this nutrient promotes the increasing of the number of branches, leaves, and coffee berries. It can also be directly involved with the defense response through the action of reactive N, such as nitric oxide, which has been shown to be an important aspect of many physiological plant processes including defense responses (Bolton, 2009). Nevertheless, further studies should be conducted to investigate the biochemical role of N in the interaction of coffee plants with *C. coffeicola*.

In general, there was no synergistic effect between the treatments and the application of the poultry litter. As N is the main nutrient found in the poultry litter (Zárate et al., 2010; Ramos et al., 2011), the hypothesis is that the amount of this nutrient provided by the green manures to the soil was enough to the development of coffee plants. Furthermore, all subplots received a base application of N at 300 kg ha⁻¹ in both years.

Nevertheless, the only synergistic effect between the legumes and urea with the poultry litter was detected among the treatments with urea, *C. mucronata*, and *C. spectabilis*. The higher PHCB was recorded in the treatments with urea.

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**TABLE 4** - Effect of green manures, urea, poultry litter, and *Beauveria bassiana* on the percentage of healthy coffee berries without *Cercospora coffeicola* symptoms in Carlópolis, PR, Brazil, in 2008/2009 and 2009/2010.

| Treatments                  | 2008/2009 | 2009/2010 |
|----------------------------|-----------|-----------|
|                            | PL²       | Control   | PL²       | Control   |
| *Mucuna aterrima*          | 49.5 bc¹  | 47.3 bc   | 24.8 de   | 24.8 cd   |
| *Mucuna deeringiana*       | 54.5 bc   | 39.8 cd   | 29.8 cd   | 24.5 cd   |
| *Arachis hypogaea*         | 40.0 c    | 53.3 bc   | 42.0 bc   | 37.8 bc   |
| *Crotalaria spectabilis*   | 83.8 a    | 74.3 a    | 66.3 a    | 53.8 a    |
| *Dolichos lab lab*         | 64.0 ab   | 65.3 ab   | 44.8 b    | 37.3 bc   |
| *Crotalaria mucronata*     | 53.5 bc   | 45.8 cd   | 35.3 bcd  | 38.0 abc  |
| Urea                      | 83.0 a    | 72.8 a    | 43.3 b    | 41.8 ab   |
| Control 1¹                 | 40.0 c    | 28.0 d    | 15.0 ef   | 8.5 e     |
| Control 2                 | 39.0 c    | 34.5 cd   | 11.3 f    | 13.5 de   |

¹Means followed by the same letter are not significantly different according to the Fisher’s protected least significant difference (LSD) (*P* ≤ 0.05)

²Subplots with (PL) or without (Control) application of the poultry litter at 1.2 kg plant⁻¹

³Control 1: without green manure and without application of *B. bassiana*; Control 2: without green manure and with application of *B. bassiana*.
and C. mucronata in the subplots with application of the poultry litter, with the exception of C. spectabilis in the experiment conducted in Jacarezinho in 2008/2009 (Table 3). This fact is explained due to lower accumulation of N in the biomass of C. mucronata in the trials conducted in 2008/2009. It was estimated that the accumulations of this nutrient in C. mucronata in 2008/2009 and 2009/2010 were around 70 and 120 kg ha⁻¹, respectively (data not shown). For C. spectabilis, the accumulation of N was lower in 2009/10 (60 kg ha⁻¹) than in 2008/09 (80 kg ha⁻¹) (data not shown).

The data obtained in this study are consistent with other reports that show that N influences the susceptibility of coffee plants to C. coffeicola. In a study conducted in Colombia, the infection index values in the treatments with and without application of urea (1.3 g planta⁻¹ applied at planting and sidedressing) in coffee seedlings were 1.85% and 13.37%, respectively (Fernandez-Borrero & Lopez-Duque, 1971). Furthermore, the defoliation was lower in the treatment with urea (1.18%) than the one without the fertilizer (3.75%) (Fernandez-Borrero & Lopez-Duque, 1971). Similar founds were recorded in Brazil when the effect of N to C. coffeicola was evaluated in coffee seedlings (Pozza et al., 2001). For example, the values of area under disease curve progress of lesion numbers per leaf on coffee seedlings were around 400 and 300 in the treatments with doses of N at 7 and 15 mmol L⁻¹, respectively (Pozza et al., 2001).

In summary, most of the legumes evaluated as green manures in this study can be used in the management of C. coffeicola. Other studies should be conducted to evaluate these green manures integrated with application of fungicides, natural products or biocontrol agents to control C. coffeicola in coffee producing regions.

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