# Direct Action of the Biocide Carbendazim on Phenolic Metabolism in Tobacco Plants

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In view of the essential role of phenolic compounds in the development of pathogen resistance in plants, and given the influence that fungicides exert over phenolic metabolism, the aim of the present study was to determine the effect of the application of different rates of fungicide on the metabolism of phenolic compounds in tobacco plants (*Nicotiana tabacum* L. cv. Tennessee 86). The fungicide applied was carbendazim, with a purity of 100%, at three different rates: 1.3 mM ($carb_1$), 2.6 mM (this being the recommended concentration, $carb_2$), and 5.2 mM ($carb_3$). The control treatment was without carbendazim. The results in relation to control plants indicate that the application of $carb_1$ in tobacco plants not afflicted by damaging biotic and abiotic agents boosts phenolic accumulation. Therefore, in the case of carbendazim, the application of 50% less ($carb_1$, 1.3 mM) than the recommended dosage ($carb_2$, 2.6 mM) of this fungicide could be more effective, because the foliar accumulation of phenolics presented at $carb_1$ may imply an increased resistance of plants to pathogen infection. On the other hand, we found an inhibition of the phenolic oxidation by the application of carbendazim, principally at $carb_3$. These results suggest that the excessive application of carbendazim (5.2 mM) could be harmful for healthy plants, because, on inhibiting phenolic metabolism (biosynthesis and oxidation), such treatment would also sharply reduce the capacity of these plants to respond against pathogen attack.

**Keywords:** *Carbendazim; Nicotiana tabacum; pathogen resistance of plants; phenylalanine ammonia-lyase; peroxidase; polyphenol oxidase*

## INTRODUCTION

Plants react to pathogen attack through a variety of active and passive defense mechanisms. At the site of infection, a hypersensitive response is often initiated in resistant plants, which is frequently manifested as necrotic lesions resulting from host-cell death. In addition, the distal uninfected parts of the plant can develop systemic-acquired resistance, resulting in enhanced long-lasting defense against the same or even unrelated pathogens (*1, 2*). Both the hypersensitive response and systemic-acquired resistance are associated with increased expression of a large number of defense or defense-related genes (*3*). Examples of the defense reactions include the lignification and suberization of the plant cell wall (*4*), deposition of callose (*5*), de novo synthesis of pathogenesis-related proteins (*6*), production of active oxygen species (*7*), and biosynthesis of secondary metabolites (*8*). Much evidence suggests that the increases in salicylic acid levels are essential for the induction of systemic acquired resistance (*9*).

Phenolic compounds are among the most influential and widely distributed secondary products in the plant kingdom. Many of these play important physiological ecological roles, being involved in diverse processes such asrhizogenesis (*10*), vitrification (*11*), resistance to different types of stress (*12*), and participation in redox reactions (*13*). Nevertheless, the processes which have been most thoroughly studied and which most directly involve phenolic compounds are related to pest and disease resistance (*14, 15*). In relation to this, increased activity of polyphenol oxidase (PPO), peroxidase, and phenylalanine ammonia-lyase (PAL) has been reported in plants treated with various abiotic and biotic inducers of resistance (*16*). Given the scope of the processes in which phenolic compounds are involved, knowledge of the factors that regulate the metabolism of these compounds can enable the manipulation of their synthesis or degradation, depending on the conditions chosen or the results desired.

Prominent among the factors that directly influence phenolic metabolism are pesticides and herbicides (*17*), with the effects of herbicides having been most extensively studied (*18*). Some herbicides have been found to augment the activities of enzymes involved in the accumulation of hydroxyphenolic compounds in several plant species (*19*), whereas others diminished enzyme activity (*20*). On the other hand, in relation to the action of fungicides, Molina et al. (*17*) have demonstrated that the systemic-acquired resistance signal transduction pathway, a salicylic acid-dependent plant-defense mechanism, mediates fungicide action in the plant.

In the present study, we analyze the way in which different concentrations of the fungicide carbendazim influence the phenol metabolism in tobacco plants, given the recognized role of these compounds in this metabolic process. This fungicide was used in our experiment because (1) it is one of the most widely used fungicides in southeastern Spain, a zone of intensive agriculture, and (2) designed for a broad preventive spectrum, carbendazim is applied to large portions of the crop not

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even infected by the pathogen. The fungicide carbendazim is recommended for the control of diseases caused by *Botrytis* spp., *Cercospora* spp., *Coryneum* spp., *Erysiphe graminis*, *Fusarium* spp., *Fusicoccum* spp., *Gaeumannomyces graminis*, *Monilia* spp., *Piricularia oryzae*, *Rhizoctonia* spp., *Sclerotium* spp., *Septoria* spp., *Taphrina* spp., *Thielavia* spp., *Venturia* spp., etc. This fungicide is applied to a vast number of crops, including cereals, fruit (pome, stone, citrus, currants, strawberries, bananas, pineapples, mangos, avocados, pawpaws, etc.), vines, hops, vegetables, ornamentals, coffee, cotton, rice, flax, beet, sugar cane, peanuts, oilseed rape, cucurbits, rubber, tobacco, turf, mushrooms, and other crops (*21*). In addition, taking into account the function of phenols in plant−pathogen relationships, we examine the feasibility of reducing the application of fungicides without diminishing the resistance of the plants to pathogen attack.

## MATERIALS AND METHODS

**Crop Design and Plant Sampling.** The plant chosen for this experiment was tobacco, because prior research in our laboratory has revealed in these plants a substantial influence of B in the metabolism of phenolic compounds (*22, 23*). Seeds of *Nicotiana tabacum* L. cv. Tennesse 86 were sown in May 1999 in southern Spain (Granada). The seedlings were grown in individual pots of peat in an experimental greenhouse for 45 days, and then were transferred to individual pots (25 cm upper diameter, 17 cm lower diameter, 25 cm in height), filled with vermiculite. The plants were grown in a cultivation chamber under controlled environmental conditions with relative humidity of 60−80%, temperature of 30/20 °C (day/night), and a 16-h photoperiod at a photosynthetically photon flux density (PPFD) of 350 $\mu$mol $m^{-2}s^{-1}$ (measured at the top of the plants with a 190 SB quantum sensor, Li-cor Inc., Lincoln, NE). For one month (from day 45 until day 75 after sowing), before the experimental treatments, the plants received a nutrient solution of 6 mM $KNO_3$, 2 mM $NaH_2PO_4$, 1.5 mM $CaCl_2$, 1.5 mM $MgSO_4$, 5 $\mu$M Fe-EDTA, 2 $\mu$M $MnSO_4$, 1 $\mu$M $ZnSO_4$, 0.25 $\mu$M $CuSO_4$, 0.1 $\mu$M $(NH_4)_6Mo_7O_{24}$, and 2.5 $\mu$M $H_3BO_3$. The nutrient solution (pH 5.5 to 6.0) was renewed every 3 days.

At 75 days after sowing, we applied a foliar fungicide, in the form of an aqueous spray containing the surfactant Tween 20 (0.5% v/v), using a stainless steel sprayer. The fungicide, applied to runoff, was carbendazim (bencimidazol 2-il methyl carbamate; $C_9H_9N_3O_2$) with a purity of 100%, and it was applied at three different rates: 1.3 mM ($carb_1$), 2.6 mM (this being the recommended concentration, $carb_2$), and 5.2 mM ($carb_3$). The control treatment was without carbendazim.

The experimental design was a randomized complete block with 4 treatments, arranged in individual pots with 6 plants per treatment, each replicated 3 times. Each treatment was applied 3 times fortnightly. The plants were sampled just before the onset of flowering. From the same plants, leaves were sampled once, on day 135 after sowing. All the leaves sampled were in the mature state, with lengths of more than 10 cm. The leaf material was rinsed three times in distilled $H_2O$ after disinfecting with nonionic detergent at 1% (v/v) (Decon 90, Merk; *24*), then blotted on filter paper. A subsample of leaves was used fresh for the analysis of enzymatic activities of PAL, PPO, peroxidase, and total phenols, performing triplicate assays for each extraction, whereas the other subsample was dried in a forced air oven at 70 °C for 24 h. Dry weight was recorded and expressed as g dry weight (DW) $(leaf)^{-1}$.

**Plant Analysis.** *Extraction and Assay of PAL (EC 4.3.1.5).* The extraction was carried out following the method proposed by Lister et al. (*25*). Fresh plant material was ground at 4 °C in buffer (50 mM $Na_2HPO_4/KH_2PO_4$, pH 7.0, 5% poly(vinylpyrrolidone) (PVP) ($M_r$, 44 000), 50 mM sodium ascorbate, 18 mM mercaptoethanol, 0.1% (v/v) Triton X-100). The homogenate was filtered through four layers of cheesecloth and centrifuged at 20 000*g* for 10 min. $(NH_4)_2SO_4$ was added to the supernatant (to 35% saturation), which was then centrifuged for 20 min at 20 000*g* to remove the PVP. More $(NH_4)_2SO_4$ was added to this supernatant to reach a final saturation of 80%. This fraction was centrifuged at 20 000*g* for 20 min, and the pellet was resuspended in extraction buffer (without PVP and Triton). This solution was used for PAL assays. Protein was estimated by the method of Bradford (*26*) using BSA as a standard.

PAL activity was assayed by an adaptation of the methods of Zucker (*27*) and McCallum and Walker (*28*). The assay mixture consisted of 0.06 M sodium borate buffer, pH 8.8, and crude enzyme. The reaction was started by the addition of 11 mM L-phenylalanine. Tubes were incubated at 30 °C for 60 min, and the reaction was stopped by the addition of 35% (w/v) trifluoroacetic acid (TFA). Tubes were then centrifuged for 5 min at 5000*g* to pellet the denatured protein. PAL activity was determined from the yield of cinnamic acid, estimated from absorbance at 290 nm in the presence and absence of phenylalanine. A sample extract was boiled and assayed to determine whether the reaction was enzymatic.

*Extraction and Assay of PPO (EC 1.14.18.1).* The extraction method used was that proposed by Thypyapong et al. (*29*) with some modifications. Leaves were ground to a fine powder with a pestle and extracted at a ratio of 150 mg fresh weight to 1 mL of extraction buffer (100 mM Tris-HCl, pH 7.0, 100 mM KCl, 1 mM phenylmethanesulfonyl fluoride (PMSF) and 3% [w/v] PVP) containing SDS at 0, 0.5, 1, 2, or 4 (w/v) each. The homogenates were centrifuged at 12 000*g* for 15 min, and the supernatant was used to measure the protein concentration by the method of Bradford (*26*) using BSA as a standard. PPO was also assayed. All these procedures were carried out at 0−4 °C.

The PPO activity was assayed as described by Nicoli et al. (*30*) with some modifications. Optimum activity was reached using SDS at 2% (data not shown). The assay mixture consisted of 30 $\mu$M caffeic acid in 100 mM buffer ($Na_2HPO_4/KH_2PO_4$), pH 7.0, through which air was bubbled for 5 min. Catalase (420 units) from bovine liver (EC 1.11.1.6) (Fluka) was added in 0.1 mL of $H_2O$ to prevent peroxidation of the substrate (*31*). The assay was initiated by the addition of enzyme extract. PPO activity was measured by the change in 370 nm of the assay mixture (30 °C) based on the measurement of the disappearance of caffeic acid by enzymatic oxidation. The sample extract was boiled and assayed to determine whether the reaction was enzymatic.

*Extraction and Assay of Peroxidase (EC 1.11.1.7).* The method used was a modified version of that proposed by Kalir and Poljakoff-Mayber (*32*) and Badini et al. (*33*). Fresh plant material was ground with 50 mM Tris-acetate buffer, pH 7.5, 5 mM 2-mercaptoethanol, 2 mM 1,4-dithio-DL-threitol (DTT), 2 mM ethylenediamine tetraacetic acid (EDTA), 0.5 mM PMSF, and 1% (w/v) PVP. The homogenate was filtered through two layers of Miracloth filter fabric and centrifuged for 30 min at 37 000*g*. The pellet was discarded, and the supernatant was used for peroxidase assays and to measure the protein concentration by the Bradford method using BSA as a standard.

Peroxidase activity was determined by following the change of 485 nm due to guaiacol oxidation (*22, 32*). The reaction mixture contained 100 $\mu$M Tris-acetate buffer, pH 5.0, 1 $\mu$M guaiacol, and 0.003 $\mu$M $H_2O_2$. To test whether the reaction was due to peroxidase, control assays contained catalase from bovine liver (EC 1.11.1.6) (Fluka) (420 units in 0.1 mL of $H_2O$). The sample extract was boiled and assayed to determine whether the reaction was enzymatic.

*Extraction and Quantification of Total Phenols.* The phenolic compounds of the plant material were extracted with methanol. Total phenolic content was assayed quantitatively by 765 nm with Folin-Ciocalteau reagent (*34, 35*). The results obtained were expressed as $\mu$g of caffeic acid (g fresh weight [fw])$^{-1}$.

**Statistical Analysis.** The data shown are mean values ± SE. Differences between treatment means were compared using the LSD at the 0.05 probability level. Levels of signifi-

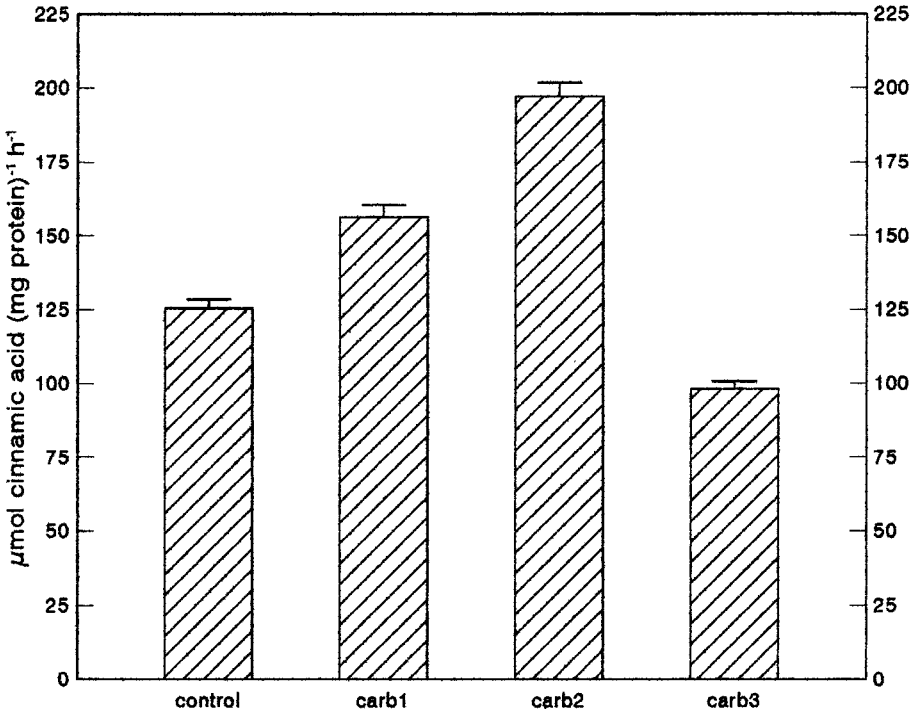


**Figure 1.** PAL activity in tobacco leaves in response to the application of carbendazim ($carb_1$ = 1.3 mM; $carb_2$ = 2.6 mM; $carb_3$ = 5.2 mM). Data are means ± SE ($n$ = 3).

cance are represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$, and NS represents not significant.

## RESULTS AND DISCUSSION

The metabolism of phenolic compounds is regulated by the activity of various enzymes. The first step necessary for biosynthesis of the phenylpropanoid skeleton in higher plants is the deamination of L-phenylalanine to yield transcinnamic acid and ammonia. This reaction is catalyzed by PAL and is commonly regarded as a key step in the biosynthesis of phenolic compounds (*36*). PAL activity is affected by a number of factors, including light, temperature, growth regulators, inhibitors of RNA and protein synthesis, wounding, and mineral nutrition (*22*, *23*, *37*−*39*).

Another factor that affects the metabolism of phenolic compounds, and more specifically PAL activity, is the application of herbicides. Some herbicides, by increasing or diminishing PAL activity, reportedly cause the accumulation or loss of foliar phenolic compounds (*19*, *40*). With respect to the action of the fungicides, Ruiz et al. (*23*) observed that the foliar application of carbendazim at 2.6 mM boosted PAL activity 2-fold over control values. In our experiment, we found similar results, as the highest activity of PAL appeared in $carb_2$, being 57% higher than in the control ($P < 0.001$, Figure 1). On the other hand, the foliar application of the highest carbendazim rate ($carb_3$, 5.2 mM) disminished PAL activity by 22% with respect to the control, and 50% in relation to values for $carb_2$ (Figure 1).

With respect to foliar concentration of total phenolics, these treatments did not follow the pattern of PAL activity, considering the highest phenolic concentrations were registered in $carb_1$ and the lowest were in the control ($P < 0.001$, Figure 2). The different behavior between PAL activity and foliar concentration of phenolics appears to explain the nonsignificant correlation between the two parameters ($r$ = 0.65 ns). Also, the absence of any relationship between these parameters could imply the action of oxidative enzymes.

The metabolism of the phenolic compounds also involves oxidative enzymes, such as peroxidase and PPO, the latter catalyzes the oxidation of phenolics to quinones (*29*). A large number of studies have demonstrated that these enzymes increase in response to biotic and abiotic stress (*22*, *23*, *29*, *39*). In addition, PPO has been identified as a pathogenesis-related protein and a proteinase inhibitor, and has been suggested to have a defensive role against herbivores and pathogens (*41*). In our experiment, the application of all treatments with carbendazim significantly decreased PPO ($P < 0.001$, Figure 3) and POD activities ($P < 0.001$, Figure 4) in relation to the highest activities presented in control plants. Notably, the greatest reduction of enzymatic activities PPO and POD corresponded to $carb_3$, with reductions of 74 and 38%, respectively.

Therefore, the application of carbendazim, and especially the $carb_3$ treatment, inhibited PPO and POD (Figures 3 and 4). This effect of the fungicide could be harmful to the resistance of these plants to pathogen infection. Oxidation of phenolic compounds by PPO and peroxidase in leaves leads to the production of quinones (*29*), which are known to be highly toxic and responsible for the production of active oxygen species (O, $OH^-$, and $H_2O_2$) (*42*). One of the principal events in the early phase of plant−pathogen interactions is the rapid and transient production of active oxygen species by the plant (*43*). This response has been reported in numerous plant−pathogen systems involving fungi (*44*), bacteria (*45*), viruses (*46*), or elicitors (*47*). Ample evidence indicates that active oxygen species perform multiple important functions in early defense responses of the plant (*7*), the increase in salicylic acid perhaps being the most important (*43*). Much evidence suggests that the increases in salicylic acid levels are essential to induce systemic acquired resistance (*9*).

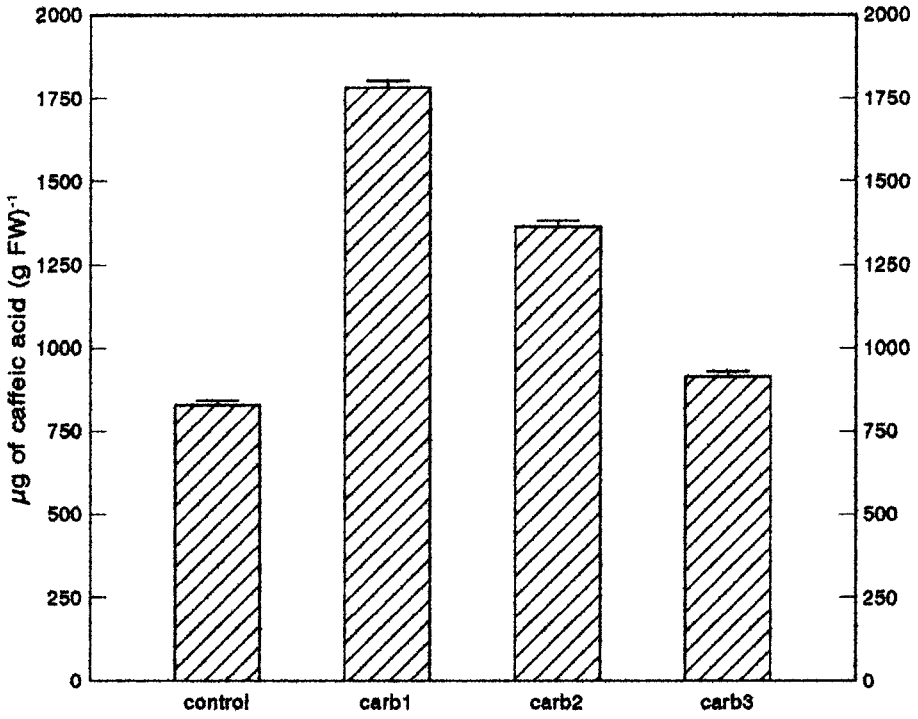


**Figure 2.** Leaf concentration of total phenols in response to the application of carbendazim ($carb_1$ = 1.3 mM; $carb_2$ = 2.6 mM; $carb_3$ = 5.2 mM) in tobacco plants. Data are means ± SE ($n$ = 3).

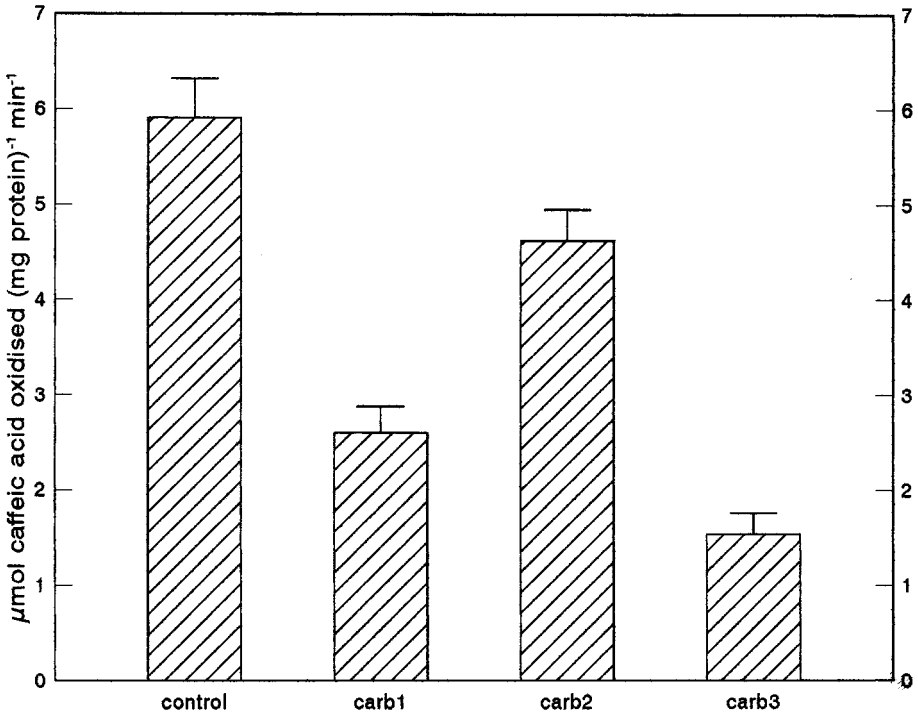


**Figure 3.** PPO activity in tobacco leaves in response to the application of carbendazim ($carb_1$ = 1.3 mM; $carb_2$ = 2.6 mM; $carb_3$ = 5.2 mM) in tobacco plants. Data are means ± SE ($n$ = 3).

The results of the PPO, POD, and PAL activities appear to account for the effects of the different treatments on the foliar phenolic concentrations. First, the control treatment registered the lowest concentrations of total phenolics (Figure 2), due to the high oxidation rate of these compounds (Figures 3 and 4). With respect to the plants subjected to the $carb_1$ and $carb_2$ treatments, the latter ($carb_2$) presented the lowest foliar phenolic concentration (Figure 2), because the oxidation rate of these compounds surpassed that of $carb_1$ plants (Figure 3 and Figure 4). Finally, the $carb_3$ plants, like the control, showed low foliar concentrations of phenolics (Figure 2), owing primarily to the lowest PAL activity in these plants (Figure 1).

On the other hand, Nemat Alla and Younis (*40*) and Ruiz et al. (*23*) related the changes and, more specifically, the decline in secondary metabolic processes, with the growth reduction of the plants treated with certain herbicides and fungicides. In our experiment, the greatest foliar biomass was found with the $carb_1$ treatment,

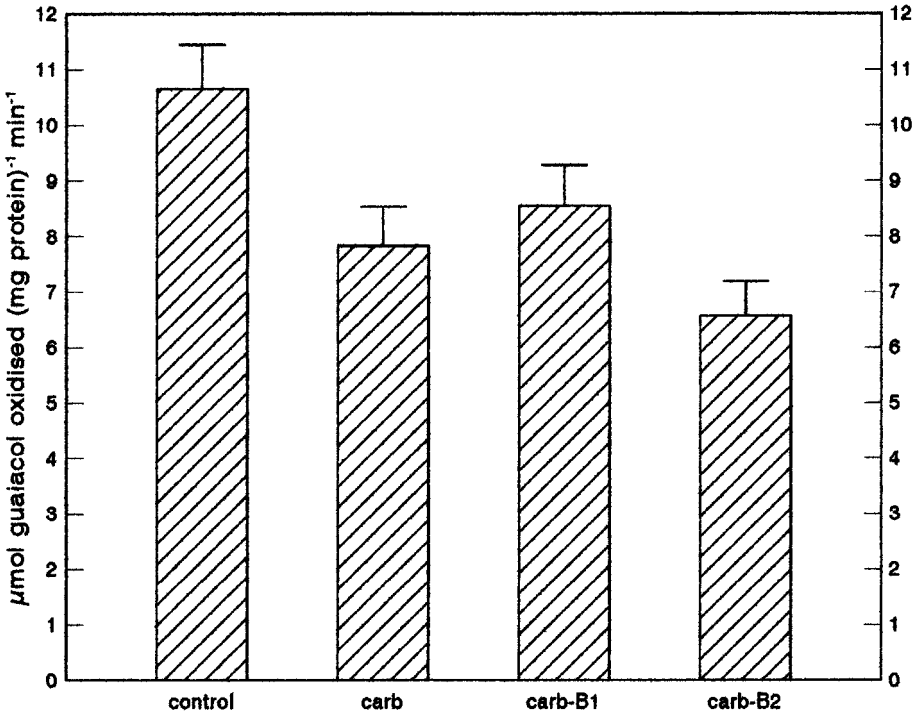


**Figure 4.** POD activity in tobacco leaves in response to the application of carbendazim ($carb_1$ = 1.3 mM; $carb_2$ = 2.6 mM; $carb_3$ = 5.2 mM) in tobacco plants. Data are means ± SE ($n$ = 3).

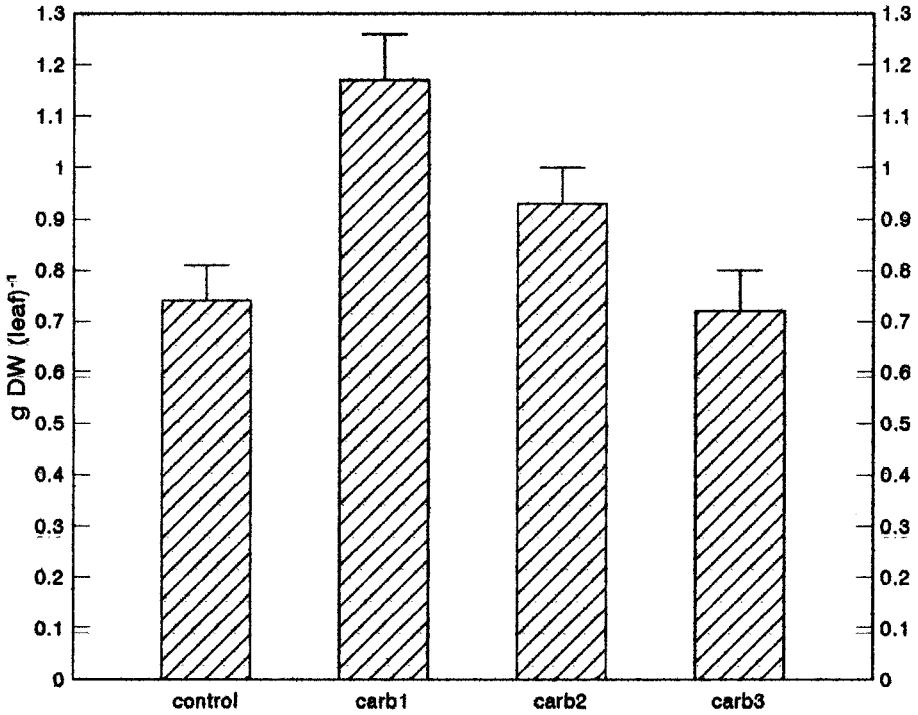


**Figure 5.** Leaf dry weight accumulation in response to the application of carbendazim ($carb_1$ = 1.3 mM; $carb_2$ = 2.6 mM; $carb_3$ = 5.2 mM) in tobacco plants. Data are means ± SE ($n$ = 3).

with an increase of 63% over the lowest values, registered by control and $carb_3$ plants ($P < 0.001$, Figure 5). Thus, our results support those of Nemat Alla and Younis (*40*) and Ruiz et al. (*23*), given that the increase in the foliar concentration of phenolics resulted in greater foliar biomass ($r = 0.97$ ***).

In summary, our results in relation to control plants indicate that the application of $carb_1$ in tobacco plants that are not afflicted by damaging biotic and abiotic agents boosts phenolic accumulation. Therefore, in the case of carbendazim, the application of 50% less ($carb_1$, 1.3 mM) than the recommended dosage ($carb_2$, 2.6 mM) of this fungicide could be more effective, because the foliar accumulation of phenolics presented at $carb_1$ may imply an increased resistance of plants to pathogen infection, given the essential role of phenolic compounds in lignification and suberization of the plant cell wall (*8, 14, 15*). On the other hand, as indicated above, in our experiment, we found an inhibition of the phenolic oxidation by the application of carbendazim, principally at $carb_3$. These results suggest that the excessive application of carbendazim (5.2 mM) could be harmful

for healthy plants, because, on inhibiting phenolic metabolism (biosynthesis and oxidation), such treatment would also sharply reduce the capacity of these plants to respond against pathogen attack.

## ABBREVIATIONS USED

Carb, carbendazim; DTT, 1,4-dithio-DL-threitol; EDTA, ethylenediamine tetraacetic acid; PAL, phenylalanine ammonia-lyase; PMSF, phenylmethanesulfonyl fluoride; PPO, polyphenol oxidase; PVP, poly(vinylpyrrolidone); SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid.

## LITERATURE CITED


(1) Fodor, J.; Gullner, G.; Ádám, A. L.; Barna, B.; Kömives, T.; Király, Z. Local and systemic responses of antioxidants to tobacco mosaic virus infection and to salicylic acid in tobacco. Role in systemic acquired resistance. *Plant Physiol.* **1997**, *114*, 1443−1451.

(2) Wendehenne, D.; Durner, J.; Chen, Z.; Klessig, D. F. Benzothiadiazole, an inducer of plant defenses, inhibits catalase and ascorbate peroxidase. *Phytochemistry* **1998**, *47*, 651−657.

(3) Klessig, D. F.; Malamy, J. The salicylic acid signal in plants. *Plant Mol. Biol.* **1994**, *26*, 1439−1458.

(4) Stein, B. D.; Klomparens, K. L.; Hammerschmidt, R. Histochemistry and ultrastructure of the induced resistance response of cucumber plants to *Colletotrichum lagenarium*. *J. Phytopathol.* **1993**, *137*, 177−188.

(5) Benhamou, N. Ultrastructural detection of $\beta$-1,3-glucans in tobacco root tissues infected by *Phytophthora infestans* var. *nicotianae* using a gold-complexed tobacco $\beta$-1,3-glucanase. *Physiol. Mol.Plant Pathol.* **1992**, *41*, 351−370.

(6) Siegrist, J.; Jeblick, W.; Kauss, H. Defense responses in infected and elicited cucumber (*Cucumis sativus* L.) hypocotyl segments exhibiting acquired resistance. *Plant Physiol.* **1994**, *105*, 1365−1374.

(7) Low, P. S.; Merida, J. R. The oxidative burst in plant defense: Function and signal transduction. *Physiol. Plant.* **1996**, *96*, 533−542.

(8) Daayf, F.; Schmitt, A.; Bélanger, R. R. Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of *Reynoutria sachalinensis*. *Plant Physiol.* **1997**, *113*, 719−727.

(9) Tenhaken, R.; Rübel, C. Salicylic acid is needed in hypersensitive cell death in soybean but does not act as a catalase inhibitor. *Plant Physiol.* **1997**, *115*, 291−298.

(10) Curir, P.; Van Sumere, C. F.; Termini, A.; Barthe, P.; Marchesini, A.; Dolci, M. Flavonoid accumulation is correlated with adventitious roots formation in *Eucalyptus gunnii* Hook micropropagated through axilary bud stimulation. *Plant Physiol.* **1990**, *92*, 1148−1153.

(11) Kevers, C.; Coumans, M.; Coumans-Gillés, M.; Gaspar, T. Physiological and biochemical events leading to vitrification of plants cultured in vitro. *Physiol. Plant.* **1984**, *61*, 69−74.

(12) Delalonde, M.; Barret, Y.; Coumans, M. P. Development of phenolic compound in maize anthers (*Zea mays*) during cold pretreatment prior to androgensis. *J. Plant Physiol.* **1996**, *149*, 612−616.

(13) Takahama, U.; Oniki, T. Regulation of peroxidase-dependent oxidation of phenols in the apoplast of spinach leaves by ascorbate. *Plant Cell Physiol.* **1992**, *33*, 379−387.

(14) Nagarathna, K. C.; Shetty, S. A.; Shetty, H. S. Phenylalanine ammonia lyase activity in pearl millet seedlings and its relation to downy mildew disease resistance. *J. Exp. Bot.* **1993**, *44*, 1291−1296.

(15) Dübeler, A.; Voltmer, G.; Gora, V.; Lundeerstadt, J.; Zeeck, A. Phenols from *Fagus sylvatica* and their role in defense against *Cryptococcus fagisuga*. *Phytochemistry* **1997**, *45*, 51−57.

(16) Smith-Becker, J.; Marois, E.; Huguet, E. J.; Midland, S. L.; Sims, J. J.; Keen, N. T. Accumulation of salicylic acid and 4-hydroxybenzoic acid in phloem fluids of cucumber during systemic acquired resistance is preceded by a transiet increase in phenylalanine ammonia-lyase activity in petioles and stems. *Plant Physiol.* **1998**, *116*, 231−238.

(17) Molina, A.; Hunt, M. D.; Ryals, J. A. Impaired fungicide activity in plants blocked in disease resistance signal transduction. *Plant Cell* **1998**, *10*, 1903−1914.

(18) Lydon, J.; Duke, S. O. Pesticide effects on secondary metabolism of higher plants. *Pest. Sci.* **1989**, *25*, 361−373.

(19) Scarponi, L.; Nemat Allah, M.; Martinetti, L. Metolachlor in corn (*Zea mays*) and soybean (*Glycine max*): persistence and biochemical signs of stress during its detoxification. *J. Agric. Food Chem.* **1992**, *40*, 884−889.

(20) Hoagland, R. E. Interaction of indole acetic acid and glyphosate on phenolic metabolism in soybean. *Pestic. Biochem. Physiol.* **1990**, *36*, 68−75.

(21) Tomlin, C., Ed. *The Pesticide Manual*, 10th ed.; The British Crop Protection Council and The Royal Society of Chemistry: Cambridge, England, 1994; ISBN: 0−948-404−79−5.

(22) Ruiz, J. M.; Bretones, G.; Baghour, M.; Belakbir, A.; Romero, L. Relationship between boron and phenolic metabolism in tobacco leaves. *Phytochemistry* **1998**, *48*, 269−272.

(23) Ruiz, J. M.; Garcia, P. C.; Rivero, R. M.; Romero, L. Response of phenolic metabolism to the application of carbendazim plus boron in tobacco. *Physiol. Plant.* **1999**, *106*, 151−157.

(24) Wolf, B. A comprehensive system of leaf analysis and its use for diagnosing crop nutrients status. *Commun. Soil Sci. Plant Anal.* **1982**, *13*, 1035−1059.

(25) Lister, C. E.; Lancaster, J. E.; Walker, J. R. L. Phenylalanine ammonia-lyase activity and its relationship to anthocyanin and flavonoid levels in New Zealand-grown apple cultivars. *J. Am. Soc. Hortic. Sci.* **1996**, *121*, 281−285.

(26) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(27) Zucker, M. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. *Plant Physiol.* **1965**, *40*, 779−784.

(28) McCallum, J. A.; Walker, J. R. L. Phenolic biosynthesis during grain development in wheat: changes in phenylalanine ammonia-lyase activity and soluble phenolic content. *J. Cereal Sci.* **1990**, *11*, 35−49.

(29) Thypyapong, P.; Hunt, M. D.; Steffens, J. C. Systemic wound induction of potato (*Solanum tuberosum*) polyphenol oxidase. *Phytochemistry* **1995**, *40*, 673−676.

(30) Nicoli, M. C.; Elizalde, B. E.; Pitotti, A.; Lerici, C. R. Effect of sugars and maillard reaction products on polyphenol oxidase and peroxidase activity in food. *J. Food Biochem.* **1991**, *15*, 169−184.

(31) Vaughn, K. C.; Duke, S. O. Tentoxin-induced loss of plastidic polyphenol oxidase. *Physiol. Plant.* **1981**, *53*, 421−428.

(32) Kalir, A.; Omri, G.; Poljakoff-Mayber, A. Peroxidase and catalase activity in leaves of *Halimione portulacoides* exposed to salinity. *Physiol. Plant.* **1984**, *62*, 238−244.

(33) Badani, M.; De Biasi, M. G.; Felici, M. Soluble peroxidase from winter wheat seedlings with phenoloxidase-like activity. *Plant Physiol.* **1990**, *92*, 489−494.

(34) Singleton, V. L.; Rossi, J. A., Jr. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(35) Singleton, V. L.; Salgues, M.; Zaya, J.; Trousdale, E. Caftaric acid disappearance and conversion to products of enzymatic oxidation in grape must and wine. *Am. J. Enol. Vitic.* **1985**, *36*, 50−56.
(36) Rösler, J.; Krekel, F.; Amrhein, N.; Schmid, J. Maize phenylalanine ammonia-lyase has tyrosine ammonia-lyase activity. *Plant Physiol.* **1997**, *113*, 175−179.
(37) Jones, D. H. Phenylalanine ammonia-lyase: regulation of its induction and its role in plant development. *Phytochemistry* **1984**, *23*, 1349−1359.
(38) Dixon, R. A.; Paiva, N. L. Stress induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085−1097.
(39) Sánchez, E.; Soto, J. M.; García, P. C.; López-Lefebre, L. R.; Rivero, R. M.; Ruiz, J. M.; Romero, L. Phenolic and oxidative metabolism as bioindicators of nitrogen deficiency in french bean plants (*Phaseolus vulgaris* L. cv. Strike). *Plant Biol.* **2000**, *2*, 272−277.
(40) Nemat Alla, M. M.; Younis, M. E. Herbicide effects on phenolic metabolism in maize (*Zea mays* L.) and soybean (*Glycine max* L.) seedlings. *J. Exp. Bot.* **1995**, *46*, 1731−1736.
(41) Lamb, C. J.; Lawton, M. A.; Dron, M.; Dixon, R. A. Signals and transduction mechanisms for activation of plant defenses against microbial attack. *Cell* **1989**, *56*, 215−224.
(42) Pillinger, J. M.; Coope, J. A.; Ridge, I. Role of phenolic compounds in the antialgal activity of barley straw. *J. Chem. Ecol.* **1994**, *20*, 1557−1569.
(43) Wu, G.; Shortt, B. J.; Lawrence, E. B.; León, J.; Fitzsimmons, K. C.; Levine, E. B.; Raskin, I.; Shah, D. Activation of host defense mechanisms by elevated production of $H_2O_2$ in transgenic plants. *Plant Physiol.* **1997**, *115*, 427−435.
(44) Vera-Estrella, R.; Blumwald, E.; Higgins, V. J. Effect of specific elicitors of *Cladosporium fulvum* on tomato suspension cells. *Plant Physiol.* **1992**, *99*, 1208−1215.
(45) Keppler, L. D.; Baker, C. J.; Atkinson, M. M. Active oxygen production during a bacteria induced hypersensitive reaction in tobacco suspension cells. *Phytopathology* **1989**, *79*, 974−978.
(46) Doke, N.; Ohashi, Y. Involvement of an O generating system in the induction of necrotic lesions on tobacco leaves infected with tobacco mosaic virus. *Physiol. Mol. Plant Pathol.* **1988**, *32*, 163−175.
(47) Baker, C. J.; Orlandi, E. W.; Mock, N. M. Harpin, an elicitor of the hypersensitive response in tobacco caused by *Erwinia amylovora*, elicits anctive oxygen production in suspension cells. *Plant Physiol.* **1993**, *102*, 1341−1344.

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