Effect of a Novel Chemical Mixture on Senescence Processes and Plant−Fungus Interaction in Solanaceae Plants

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The effects of exogenous application of a chemical mixture consisting of adipic acid monoethyl ester, furfurylamine, and 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose (FGA) on various metabolic pathways and the plant−fungus interaction have been studied in Solanaceae plants. Tomato and pepper plants were sprayed with the FGA mixture, and different biochemical parameters such as gas exchange, chlorophyll concentration, protein, cell wall sugar and phenolics contents, and peroxidase and phenylalanine ammonia lyase (PAL) activities were measured. FGA-treated plants showed, in general, an increase in cell wall sugar content and decreases in the chlorophyll degrading rate and the peroxidase activity. These results suggest that FGA (a possible synthetic regulator) could act as a retardant−antisenescence agent in Solanaceae plants. The FGA mixture increased the PAL activity and promoted an overall rise in the concentration of flavonoids and phenolic compounds. Therefore, FGA induced the synthesis of compounds that could give protection to plants against pathogens or insects. To further verify this putative protection, several fungi were inoculated in intact plants. Exogenous FGA applications on intact plants delayed fungus-provoked lesion development. In addition, data also showed that applications of 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose inhibited fungal growth in vitro. These results confirm that FGA can activate protective mechanisms in plants upon contact with invaders such as fungi.

INTRODUCTION

Plants synthesize plant growth regulators, which influence essential physiological processes. Modification of plant development and metabolism through the use of exogenous applications of these plant growth regulators is becoming an increasingly important aspect of the modern agriculture (*1*). The availability of synthetic regulators that mimic the effect of plant hormones has greatly expanded this practice. Monocarboxylic acids and related compounds, amines, polyamines, and sugar analogues, could be constituents of natural or synthetic plant growth regulators. It is already known that these compounds have different effects on plants and pathogens (*2, 3*). Some of these effects are described below.

Several monoesters of carboxylic acids retard plant growth and delay senescence processes by inhibition of chorophyllase and peroxidase activities (*4−7*). The monoethyl ester carboxylic acid induces the synthesis of polyols that act as precursors of neutral sugars and uronic acids (*8*). In addition, treatments with polyhydroxycarboxylic acids result in an increase root growth (*9*). A higher root mass area will induce greater synthesis of cytokinins, which coupled with auxins leads to the formation of secondary roots and absorption hairs, resulting in a final enhancement of the root activity.

The ability of polyhydroxycarboxylic acids to increase nutrient uptake (P, K^+, N, and Ca^{2+}), resulting therefore in an improvement of yields, has been also described in cotton plants (*10*). Furthermore, a positive effect of the carboxylic acid on carbohydrate synthesis was observed (*11*).

With regard to the amines, it is known that they influence crucial physiological processes in plants such as seed germination, vegetative growth, flowering, and senescence (*12*). The polyamines putrescine, spermidine, and spermine prevent loss of chlorophyll associated with senescence of excised leaf tissues (*13*). Moreover, 1,3-diaminopropane plays an important role in retarding the senescence process by inhibiting ethylene biosynthesis (*14*).

Recently, it has been found that several polyamine-like compounds, such as ketoputrescine (*15*), aliphatic putrescine analogues (*16, 17*), and novel cyclic diamines (*18*), show antifungal properties.

Sugar analogues have been known to affect metabolic processes in fungal and plant cells (*19*). Janisiewicz (*20*) and El Ghaouth et al. (*21*) have shown that 2-deoxy-D-glucose is an efficient treatment to prevent postharvest diseases. However, the physiological effects these sugar analogues could cause on intact plants remain unknown.

The accumulation of phenolic compounds, isoflavonoids, and their precursors is a common plant response to fungal elicitors or pathogenic injuries (*22−25*). Little is known about the induction of phenolic

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2570 *J. Agric. Food Chem.,* Vol. 49, No. 5, 2001

Flors et al.

compounds when plants are not infected despite the effort of a few groups that have studied the process of synthesis and/or accumulation of some flavonones in response to different phytoregulators such as benzylaminopurine (*26*), ethylene (*27*), and brotomax (a phytosanitary product that contains nitrogen, sugar, and an organic component) (*28*).

The aim of this research is to find a novel plant regulator mixture that could delay senescence processes and induce inherent plant defenses against phytopathogens. A mixture containing adipic acid monoethyl ester, furfurylamine, and 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (FGA) has been exogenously applied to intact plants. The effects of this mixture on various metabolic pathways and plant–pathogen interactions have been evaluated.

MATERIALS AND METHODS

Plant Material. Tomato (*Lycopersicon esculentum* L.) and pepper (*Capsicum annuum* L.) seeds were germinated under glasshouse conditions. Seedlings were then transplanted and grown under the same conditions in 5l pots filled with perlite. Temperatures ranged between 16 and 18 °C (night) and 25–27 °C (day). Relative humidity was maintained to ~80%. Plants were irrigated daily with modified Hoagland's nutrient solution (*29*).

Chemicals and Treatments. For the biochemical assays, different solutions were applied to plants, repeating the treatment five times (every 15 days) along the vegetative development cycle as described below. One hundred days after plant transplanting, excised leaves from control and treated plants were washed with distilled water and stored at −80 °C until posterior analysis.

FGA Treatments. A stock solution containing 10 mM adipic acid monoethyl ester, 7.5 mM 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose, and 2.5 mM furfurylamine was prepared. This mixture was termed FGA. Four different doses of FGA (0.1, 0.2, 0.3. and 0.4%) were sprayed to different groups of five plants. Each group of five plants was treated with 50 mL/plant of these solutions. Dripping was avoided when the leaves were soaked. Gas exchange was measured during this experiment. The experiment was carried out on tomato and pepper plants.

Furfurylamine Treatments. Furfurylamine was applied at three different concentrations of 0.004, 0.006, and 0.008 mM. Groups of five plants were sprayed with 50 mL/plant of these solutions. Gas exchange was measured during this experiment. This experiment was carried out using the same conditions described above.

Carboxylic Acid Treatments. Adipic acid monoethyl ester was applied at two different concentrations: 0.01 mM for pepper and 0.02 mM for tomato. Groups of five plants were sprayed with 50 mL/plant of these solutions. Plant growth and fruit features were measured during this experiment. This experiment was carried out using the same conditions described above.

For the fungus assays the following solution was used:

1,2,3,4-Tetra-O-acetyl-β-D-glucopyranose Treatment. A 0.5 cm diameter dish of a 10-day-old fungal culture grown in potato dextrose agar (PDA) was placed on three Petri dishes containing 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose at concentrations of 0.01, 0.1, 0.5, and 1% (w/v) in PDA. Growth of fungi *Botrytis cinerea*, *Alternaria solani*, and *Phytophthora citrophthora* was tested.

Study of Plant—Fungus Interactions. Tomato and pepper plants were sprayed three times on days 15, 30, and 45 after transplanting with 0.2% FGA (tomato) or 0.1% FGA (pepper). Control plants were treated similarly but with sterile water. After the FGA treatments, inoculations with different fungi were carried out as described below.

Inoculation Procedures. Cultures of *Phytophthora capsici* and *P. citrophthora* were maintained on PDA.

FGA-treated plants and control plants were inoculated by introducing one plug (5 mm in diameter) of an actively growing mycelium of the fungus *P. citrophthora* (tomato) or *P. capsici* (pepper) as close as possible to the apex.

Analytical Procedures. *Growth and Fruit Quality Measurements.* The height was measured with a graduated rule and the sugar content with a refractometer.

Gas Exchange. Net photosynthetic rate, transpiration rate, and water use efficiency (WUE) were measured 56, 86, and 100 days after treatment with FGA. A closed gas-exchange Li-Cor (LCA4) portable photosynthesis system was used for the measurements. Laminae of leaves were totally enclosed within a fan-stirred cuvette and maintained under natural conditions (leaf temperature between 25 and 34 °C and irradiance between 800 and 1300 μmol m^{-2} s^{-1}). Ten consecutive measurements were taken at 3–5 min intervals.

Chlorophyll Content. Chlorophyll was extracted with 80% acetone. Poly(ethylene glycol) (PEG) was added to precipitate the protein, followed by centrifugation (8000g, 10 min). This process was repeated twice. Chlorophyll content was determined in the supernatant by measurement of the optical density at 645 and 663 nm.

Total Peroxidase Activity. Total peroxidase (TPOx) (EC 1.1.1.1.7) activity was determined according to the Boyarkin method (*30*). Approximately 0.5 g (fresh material) was homogenized with 5 mL of 100 mM borate buffer (pH 8.5). The crude enzyme extract was obtained after centrifugation (8000g, 30 min). All operations were carried out at 4 °C. The assay medium contained 0.5 mL of 0.2 M acetate buffer (pH 4.7), 0.7 mL of 5 mM benzidine dihydrochloride, 0.3 mL of 40 mM H_2O_2, and 0.1 mL of crude enzyme extract. The reaction was initiated immediately by adding H_2O_2 at room temperature. A cuvette containing all components except H_2O_2 was used as a control. Enzyme activity is expressed as catalytic units per milligram of protein; one catalytic unit is the amount of enzyme extract producing a ΔA of 0.01 in 1 min.

Protein Determination. Fresh material (2.5 g) was homogenized with 25 mL of 100 mM borate buffer (pH 8.5). Then, 0.5 g of polyvinylpyrrolidone, 0.025 mL of dithiothreitol, and 0.035 mL of β-mercaptoethanol were added at 4 °C. The crude extract was obtained by centrifugation at 8000g for 30 min at 4 °C.

After dilution, the total soluble protein content was estimated in the crude extract using the Bradford reagent as described by Bradford (*31*). The content was expressed as milligrams of total protein oer gram of fresh weight (fw).

Cell Wall Extraction and Determination of Cellulosic Sugar, Noncellulosic Sugar, and Uronic Acid Contents. Cell wall material was extracted from tomato and pepper leaves and fractionated into cellulosic sugar fraction and noncellulosic sugar fraction, where the uronic acid concentration and the total noncellulosic sugar content were measured.

Fresh material (2 g) was homogenized for 15 min with 25 mL of MeOH and sequentially rinsed with MeOH, acetone (80%), and MeOH/CHCl$_3$ (1:1). The homogenate was filtered. The filtrate was collected and dried at room temperature.

Cell wall material was first hydrolyzed with 2 N TFA and then fractionated. The pellet obtained after centrifugation (3500g, 10 min) was considered to be the cellulosic sugar fraction. Cellulosic sugars were identified following the Dubois method (*32*). The supernatant was considered to be the noncellulosic sugar fraction. Two aliquots were used for uronic acids (*33*) and total noncellulosic sugar identification (*32*).

Extraction and Determination of Phenolic Contents. Leaf phenolics were extracted with MeOH/H$_2$O (80:20) and fractionated in cell wall phenolics and soluble phenolics (*34*). The soluble phenolic fraction was dried and redissolved in MeOH/H$_2$O (30:70) for HPLC determination. After extraction of soluble phenolics, the filtered residual was washed twice with MeOH/H$_2$O (50:50). The resulting filtrate was hydrolyzed with 1 N NaOH and centrifuged, and the supernatant was acidified and extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness and redissolved in MeOH/H$_2$O (30:70). This preparation was used to determine the cell wall-bound

Senescence Processes and Plant–Fungus Interaction in Solanaceae Plants

J. Agric. Food Chem., Vol. 49, No. 5, 2001 **2571**

Table 1. Effect of FGA on Cellulosic Sugars, Uronic Acids, and Total Noncellulosic Sugars of Tomato and Pepper Plants

	treatment	cellulosic sugars (mg/g of cell wall)	uronic acids (mg/g of cell wall)	total noncellulosic sugars (mg/g of cell wall)
tomato	control	26.0 ± 1.8	60.0 ± 7.3	51.8 ± 5.2
	0.2%	24.3 ± 3.1	114.6 ± 8.5	110.1 ± 9.7
pepper	control	54.0 ± 2.0	165.0 ± 2.0	390.0 ± 10.0
	0.1%	77.0 ± 5.0	180.0 ± 2.0	400.0 ± 10.0

phenolics by HPLC. For HPLC analysis of phenolics, a 14×4 mm, 5 μm i.d., Hypersil C_{18} reverse phase column (Hewlett-Packard) and a solvent containing methanol/TFA 0.5 M in water were used. The gradient conditions were as follows: 0–5 min in 20:80 MeOH/TFA; 5–7min in 35:65 MeOH/TFA; 7–7.5 min in 80:20 MeOH/TFA; 7.5–9 min 90:10 in MeOH/TFA; 9 min in 20:80 MeOH/TFA. Compounds were photometrically detected (maxplot between 260 and 550 nm) by a photodiode array detector (model 1100 Hewlett-Packard). The amounts of ferulic acid, chlorogenic acid, caffeic acid, quercetin, and quercitine were determined using standards (Sigma Spain). Total free phenolics (excluding chlorogenic acid and caffeic acid) and total flavonoids were determined as equivalents of ferulic acid and quercitine, respectively (*34*).

Phenylalanine Ammonia Lyase (PAL) Activity. The PAL enzyme was extracted and assayed following the Zucker method (*35*). Enzyme activity is expressed as catalytic units per gram of dry extract. One catalytic unit is defined as the amount of extract producing an ΔA (290) of 0.01 in 1 min.

Statistical Analysis. Statistical analysis was carried out using the Statgraphics software support. Means were expressed with their standard error (SE). They were compared by least significant difference (LSD) test. Differences were taken into account only when they were significant at the 5% level. All experiments were repeated, at least, three times.

RESULTS

Exogenous Application of FGA Mixture. Plants were sprayed with different FGA solutions (0.1, 0.2, 0.3, and 0.4%), and different biochemical parameters such as gas exchange, chlorophyll concentration, protein, cell wall sugar and phenolic contents, and peroxidase and PAL activities were measured.

Plants responded differently to the FGA applications. Thus, depending on the biochemical parameter analyzed, the optimal FGA dose varied (data not shown). However, to make this work short and concise, only the best FGA dose from a global point of view has been chosen. This dose was 0.2% for tomato plants and 0.1% for pepper plants.

Effects of FGA on Photosynthetic Rate, Transpiration Rate, and WUE. First, the effects of the FGA treatment on photosynthetic and transpiration rates and WUE were tested. No statistical differences between doses in these parameters were found after FGA applications in both cultures. However, an increase in photosynthetic rate and WUE was observed during vegetative growth (data not shown).

Effect of FGA on Cell Wall Sugar Content. The effects of the FGA treatment on cellulosic sugars, uronic acids, and total noncellulosic sugars were also studied in tomato and pepper plants. Data shown in Table 1 indicate that FGA promoted a clear increase in uronic acids in both species (2-fold in tomato and 1.2-fold in pepper). In general, a rise in cell wall sugar content was also detected after the treatment, although in tomato the increase was in noncellulosic sugars (2-fold), whereas in pepper the increase was in cellulosic ones (~1.5-fold).

Table 2. Effect of FGA on Chlorophyll and Protein Contents and Peroxidase Activity in Tomato and Pepper Plants

	treatment	[chlorophyll] (mg/g of fw[a])	[protein] (mg/g of fw)	[peroxidase activity] (cat. units[b]/ mg of protein)
tomato	control	0.18 ± 0.02	1.76 ± 0.24	210 ± 48
	0.2%	0.22 ± 0.02	1.97 ± 0.11	140 ± 21
pepper	control	0.59 ± 0.02	2.08 ± 0.10	75 ± 2
	0.1%	0.62 ± 0.06	2.40 ± 0.22	43 ± 2

[a] fw, fresh weight. [b] cat. units, catalytic units.

Table 3. Effect of FGA on PAL Activity in Tomato and Pepper Plants

	treatment	PAL activity (cat. units[a]/g of dry extract)
tomato	control	15.6 ± 5.4
	0.2%	24.0 ± 0.0
pepper	control	11.6 ± 0.7
	0.1%	24.0 ± 1.4

[a] cat. units, catalytic units.

Table 4. Effect of FGA on Phenolic Contents in Tomato and Pepper Plants

	treatment	free phenolics	chlorogenic acid	flavonoids
Cell Wall Phenolics (Micrograms of Phenol per Gram of Cell Wall)				
tomato	control	178.0 ± 14.0	-	1.3 ± 0.1
	0.2%	430.0 ± 20.0	-	3.1 ± 0.1
pepper	control	10.0 ± 3.0	-	248.0 ± 34.0
	0.1%	10.0 ± 2.0	-	221.0 ± 38.0
Soluble Phenolics (Micrograms of Phenol per Gram of Fresh Weight)				
tomato	control	0.0 ± 0.0	9.4 ± 0.7	119.0 ± 8.0
	0.2%	112.4 ± 7.0	45.7 ± 4.0	212.0 ± 7.0
pepper	control	48.0 ± 6.0	80.0 ± 10.0	338.0 ± 56.0
	0.1%		180.0 ± 48.0	818.0 ± 106.0

Differences in the parameters studied between control and FGA-treated plants were statistically significant when an LSD test with $p \leq 0.05$ was used.

Effects of FGA on Chlorophyll Concentration, Protein Content, and Total Peroxidase Activity. Pepper and tomato plants showed very similar responses to FGA in terms of chlorophyll concentration (Table 2). At the end of the experiment FGA-treated plants showed chlorophyll contents 120 and 105% higher than control ones for tomato and pepper, respectively (differences were significant by LSD analysis; $p \leq 0.05$). With regard to the protein content, a trend of increase was found in response to the FGA treatment, being statistically significant in tomato (Table 2). In addition, the FGA treatment induced a reduction in the peroxidase activity. However, due to the high SE in the data obtained for tomato control plants, only the pepper data were statistically significant (LSD $p \leq 0.05$) (Table 2).

Effect of FGA on PAL Activity and Phenolics Content. PAL is a characteristic enzyme of the phenylpropanoid pathway. Table 3 shows that FGA applications increased the PAL activity by more than 50 and 100% in tomato and pepper plants, respectively.

Table 4 shows the concentration of phenolic derivatives in tomato and pepper plants. Exogenous applications of FGA induced an increase in soluble antipathogenic compounds (free phenolics, chlorogenic acid, and flavonoids) in both species. However, the response to the FGA treatment, in terms of cell wall phenolics content, was different for each of the species studied.
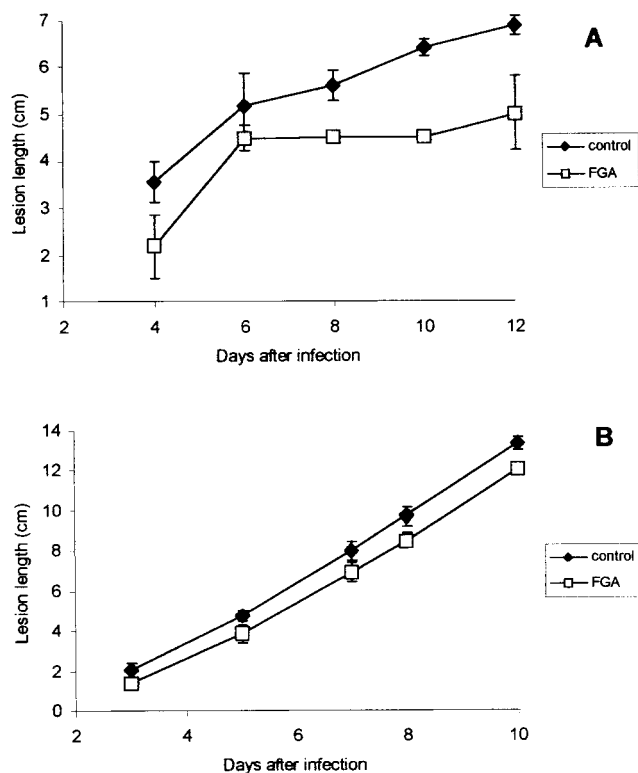
2572 *J. Agric. Food Chem.,* Vol. 49, No. 5, 2001

Flors et al.

Figure 1. Effect of FGA on fungal growth in intact plants: (A) lesion length along the stem (cm) in intact tomato plants infected with *P. citrophthora*; (B) lesion length along the stem (cm) in intact pepper plants infected with *P. capsici*.

Whereas in tomato plants free phenolics increased by >100%, in pepper plants no differences were found (Table 4).

This fact inversely correlates with levels of flavonoids found in the same plants. In both cases statistical analysis (LSD analysis; $p \leq 0.05$) showed significant differences (Table 4).

Determination of Fungicidal Effects of FGA Treatments on Intact Plants. Control and FGA-treated plants of tomato were inoculated with *P. citrophthora*. Development of fungal lesions progressed more rapidly in control intact plants than in the FGA-treated ones (Figure 1A). After the plants had been kept in a greenhouse for 12 days, significant differences (LSD analysis; $p \leq 0.05$) between control and treated plants were observed. During this time, rates of lesion expansion were 0.79 and 0.56 cm/day for control and treated plants, respectively. No infection was observed in non-inoculated plants.

When pepper plants were inoculated with *P. capsici*, lesion development also progressed more rapidly in control plants than in FGA-treated ones (Figure 1B). Although data are statistically significant (LSD analysis; $p \leq 0.05$), the results were less marked in pepper than in tomato plants.

Exogenous Applications of Isolated Components of the FGA Mixture. *Effects of Adipic Acid Monoethyl Ester on Plant Growth and Fruit Features.* The application of 0.01 mM adipic acid monoethyl ester to pepper plants increased the length and weight of fruits by 16.6 and 12%, respectively, when compared to control plants. In addition, an increase in fruit sugar content was observed (13%). Tomato FGA-treated plants (0.02 mM adipic acid monoethyl ester) also showed an increase

Table 5. Effects of Furfurylamine on Photosynthetic and Transpiration Rates and Water Use Efficiency (WUE) in Tomato Plants

treatment (mM)	photosynthetic rate (μmol m^{-2} s^{-1})	transpiration rate (mol m^{-2} s^{-1})	WUE (μmol mol^{-1})
	After Two Treatments (56 Days)		
control	6.8 ± 0.9	2.7 ± 0.1	2.5 ± 0.5
0.008	9.5 ± 3.0	2.2 ± 0.8	4.1 ± 0.2
	After Four Treatments (86 Days)		
control	3.3 ± 0.1	4 ± 0.5	0.8 ± 0.1
0.008	1.6 ± 0.6	2.4 ± 0.6	0.7 ± 0.1
	After Five Treatments (100 Days)		
control	3.7 ± 0.5	2.6 ± 0.8	1.2 ± 0.5
0.008	1.8 ± 0.2	2.1 ± 0.1	0.9 ± 0.2

Table 6. Effect of Furfurylamine on Phenolic Contents in Tomato Plants

treatment (mM)	free phenolics	chlorogenic acid	flavonoids
	Cell Wall Phenolics (Micrograms of Phenol per Gram of Cell Wall)		
control	178.0 ± 14.0		1.3 ± 0.1
0.008	224.0 ± 34.0		
	Soluble Phenolics (Micrograms of Phenol per Gram of Fresh Weight)		
control	0.0 ± 0.0	9.4 ± 0.7	119.0 ± 8.0
0.008	6.9 ± 0.9	1.4 ± 0.04	54.0 ± 7.0

in the internode number (40%) when compared to control plants.

Effects of Furfurylamine on Photosynthesis, Transpiration, WUE, and Phenolic Content in Tomato Plants. As a result of two applications of the furfurylamine treatment (0.008 mM), an almost 40% increase in the photosynthetic rate was observed (Table 5). Together with this, a slight reduction of transpiration rate was obtained. These combined effects resulted in an increase of the WUE. However, associated with leaf aging, important decreases in photosynthetic rates were found (Table 5). The application of furfurylamine reduced even more the already low levels of photosynthetic rates (Table 5, 86 and 100 days). This effect confirmed that treated plants showed lower levels of WUE than control ones.

On the other hand, the furfurylamine treatment resulted in an increase of PAL activity, which was 1.6-fold that of control plants (data not shown). A rise in free phenolics of cell wall and soluble fractions was also observed. On the contrary, chlorogenic acid and flavonoid contents in the soluble fraction greatly decreased after the furfurylamine treatment (Table 6).

Effect of 1,2,3,4-Tetra-O-acetyl-β-D-glucopyranose on Fungal Growth. Addition of the sugar analogue 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose to fungal culture grown in PDA was effective and showed statistically significant differences (LSD analysis; $p \leq 0.05$) in delaying radial growth of different fungi (*B. cinerea*, *A. solani*, and *P. citrophthora*). In each case, the greatest effect occurred at the highest concentration of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose tested (Figure 2). 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranose (0.5 or 1%) completely inhibited *B. cinerea*, *A. solani*, and *P. citrophthora* growth during the first 3, 4, and 10 days after the treatment, respectively (Figure 2).

DISCUSSION

The aim of this research was to find a novel plant regulator mixture (synthetic regulator) that could delay
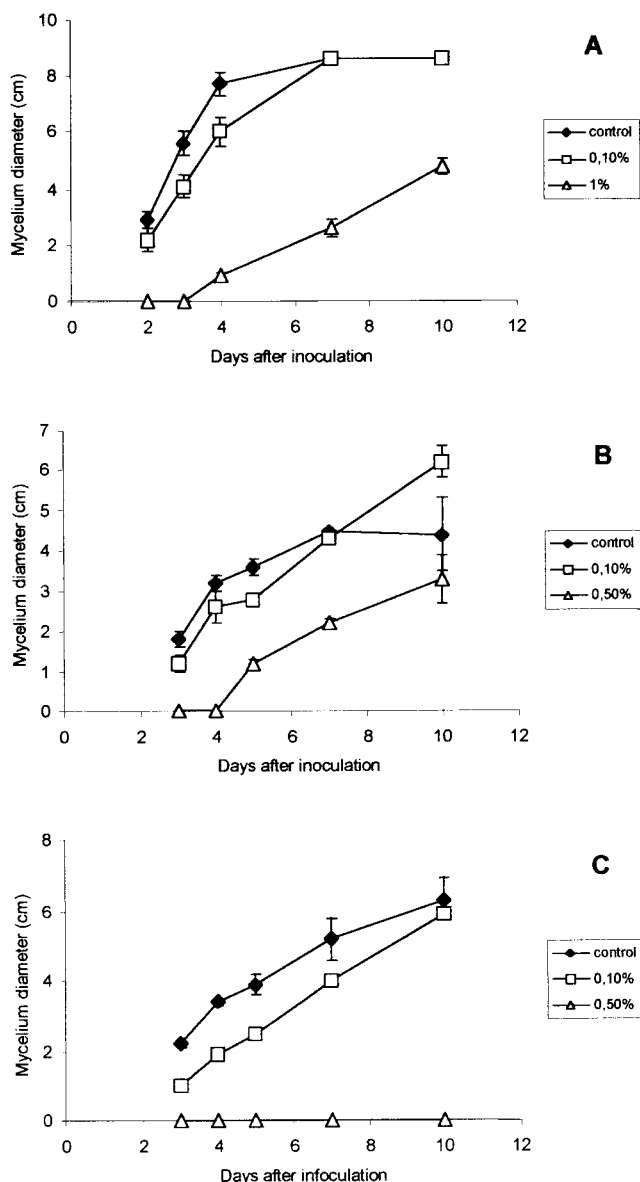
Figure 2. Effect of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose on fungal growth in fungi grown in vitro: (A) effect on *B. cinerea* growth; (B) effect on *A. solani* growth; (C) effect on *P. citrophthora* growth.

plant senescence and reinforce their natural defenses against phytopathogens.

There are several studies claiming different effects of monoesters of carboxylic acids and amines on plant metabolism and pathogen infections (*7, 10*). Sugar analogues have also been described as potential fungicides (*21*).

On the basis of the available data, a synthetic mixture was prepared consisting of adipic acid monoethyl ester, furfurylamine, and 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (FGA). This mixture was exogenously applied to intact plants, and its effect on metabolic pathways and plant–pathogen interaction was evaluated. In addition, the effect of the individual components was studied.

Effect on Senescence. FGA application increased chlorophyll content in pepper and tomato plants. These data confirm previous results showing that carboxylic acids (and its derivatives) delayed chlorophyll catabolism during plant aging (*5, 6*). The observed senescence-retarding effect could be due to a reduction of chloro-

phyll degradation, an increase of chlorophyll synthesis, or a combination of both (*7*). Additionally, it is known that polyamine (spermidine, spermine, and putresceine) treatment prevented chlorophyll loss and preserved thylakoid membrane structure under certain light conditions (*13*). These findings suggest that adipic acid monoethyl ester in addition to furfurylamine can act by the same mechanism when they are applied as part of the FGA mixture.

FGA treatment also reduced peroxidase activity. This effect could be explained by a nonenzymatic reduction of free radicals (peroxide and superoxide), which are substrates for peroxidase enzymes, by effective scavengers such as flavonoids and their aglycons (*25, 36, 37*). This hypothesis is consistent with the increment of flavonoid concentration produced by FGA treatment.

FGA application also increased protein concentration in both cultures, being statistically significant in tomato.

All of these results suggest an antisenescence effect for the new synthetic regulator FGA on Solanaceae species. This is in agreement with previous papers showing antisenescence properties for dicarboxylic acids and amines (*2, 7, 12, 14*).

Effect on Plant Defenses. Phytoalexin production appears to be a common mechanism of resistance to pathogenic microbes in a wide range of plants (*22, 34*). These compounds are synthesized by the phenylpropanoid pathway, PAL being one of the best characterized activities (*38–40*).

FGA application increased PAL activity and promoted an overall rise in the concentration of flavonoids and phenolic compounds. However, significant differences in the level of several phenolics were observed between both species, which is consistent with previous studies showing variations of these components among Solanaceae species (*41*). Despite this variation, FGA produced a clear increase of chlorogenic acid and flavonoids in the soluble fraction of both species. These results indicate that FGA induced the synthesis of compounds involved in plant defense mechanisms against pathogens.

Furfurylamine exogenously applied increased the PAL activity and produced a rise in free phenolics of cell wall and soluble fractions. However, furfurylamine treatment did not result in an increase of flavonoids or chlorogenic acid, as was observed after FGA treatment. On the contrary a strong reduction of both components was observed. This result suggests a possible synergistic effect among the different components of the FGA mixture. In this sense the possible role of adipic acid monoethyl ester is supported by previous data showing that carboxylic acids alter the phenylpropanoid pathway to produce more lignin and a constant biosynthesis of phytoalexin-like compounds (*10*).

Another plant defense mechanism against exogen damaging agents are physical barriers such as the cell wall (*42*). The increase in cell wall sugars, especially uronic acids (main pectin components), produced in tomato and pepper plants after FGA treatment supports the possible effect of the mixture improving plant defenses.

To establish whether the concomitant increase in PAL activity, flavonoid and phenolic compounds, and cell wall sugars really conferred resistance to plants against the attack of pathogens, a plant–fungus interaction study was developed. These experiments showed that FGA exogenously applied delayed fungal growth in intact

plants. These results confirm the potential use of the FGA mixture as an inducer of plant defense mechanisms against invaders such as fungi.

Antifungal Effect of 1,2,3,4-Tetra-*O*-acetyl-*β*-D-glucopyranose. Little is known about the effect of sugar analogues on plant metabolism. Some authors have shown that sugar analogues have antifungical properties, reducing fungal growth and producing morphological changes (*21*). The suggested mechanism of action is based on the fact that sugar analogues can form phosphate esters that cannot be further metabolized, which interferes with metabolic processes such as ATP synthesis and cell wall biosynthesis in yeasts and fungi. Our results indicate that 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose strongly inhibits in vitro growth of several fungi. These results confirm the potential antifungal effect of this sugar analogue and its possible effect as a fungicide in intact plants as a part of the FGA mixture.

In conclusion, we have shown that FGA, a new mixture consisting of adipic acid monoethyl ester, furfurylamine, and 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose, delays senescence processes and induces inherent mechanisms of resistance. Adipic acid and furfurylamine could act in a synergistic way by increasing PAL activity and the content of phenolic compounds. 1,2,3,4-Tetra-*O*-acetyl-*β*-D-glucopyranose could retard fungal infection in plants by means of its antifungical properties.

This research opens an interesting field in the study of new synthetic plant growth regulators. FGA treatment enhances metabolic activity and reinforces plant defenses without the negative effects shown by some phytoregulators (*2*). In addition, FGA shows promise as a treatment for fungal diseases. A possible agronomic use of FGA could result in a reduction of fungicidal treatments, which are very expensive and potentially dangerous to the environment. Control of plant pathogens still relies mainly on the use of synthetic fungicides, but the development of fungicide-resistant pathogens and the public demand to reduce pesticide use have increased the search for alternative control strategies. Additional studies are necessary to establish the mechanism of action of FGA and its possible application in other stress situations. The study of the effect of FGA on fungal cell wall and spore development is also underway. Obviously, effective formulations that are safe to humans must be developed before this technology can be used commercially.

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Senescence Processes and Plant–Fungus Interaction in Solanaceae Plants

J. Agric. Food Chem., Vol. 49, No. 5, 2001 **2575**

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