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PHYSIOLOGY

Effect of carbenicillin on ethylene inhibition and antibacterial properties of silver nitrate

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Abstract Color variations observed in tissue culture media containing silver nitrate prompted a series of experiments aimed at determining what ingredients were involved and whether their interaction impacted tissue culture systems. The classical formation of a precipitate between silver and the halides iodine and chloride was noted. In addition, an interaction was found between silver nitrate and carbenicillin, a common antibiotic used to control bacterial growth in culture after *Agrobacterium tumefaciens*-mediated transformation. Silver nitrate is used in tissue culture to block the ethylene response of plant tissue and to inhibit bacterial growth in culture. The silver in silver nitrate and carbenicillin appeared to react less in the dark but strongly interacted when exposed to light. At concentrations higher than 132.1 μM, carbenicillin reacted with silver to eliminate the antimicrobial activity of the silver. Depending on the plant species tested and the culture system used, the interaction of silver and carbenicillin in the light had variable effects on the ethylene-blocking activity of silver.

Keywords Silver nitrate · Carbenicillin · Antibiotics · Silver halides

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Introduction

The potential for components of culture media and blood sera, such as sugars and salts, to interact with antibiotics has been known since the 1940s (Donovick *et al.* 1948). Invariably, these studies focused on how media components impacted the antimicrobial activity of the antibiotics. These components, particularly the ionic metals, influenced antimicrobial activity by altering media or sera ionic strength resulting in a reduced binding of the antibiotic to the bacterial cell (Berkman *et al.* 1947; Beggs and Andrews 1975, 1976). Antibiotics and metals can also form complexes (Fazakerley and Jackson 1975; Anacona 2001), much like metal chelators (Kassanis *et al.* 1975; Light and Riggs 1978). In some cases, these complexes increased the antimicrobial activity of antibiotics (Anacona 2001). In other cases, these complexes inhibited antimicrobial activity (Kassanis *et al.* 1975; Light and Riggs 1978).

Plant tissue culture media contain large numbers of compounds, many of which can potentially interact to form new components. For instance, in an MS medium (Murashige and Skoog 1962), there is the possibility of interactions of magnesium and calcium with phosphate, or iron with oxygen (Weast 1971). Most of these interactions are detected only as slight precipitates in liquid media, or go unnoticed in gelled media. On the other hand, if silver nitrate is added to MS medium, a visible chemical reaction occurs as the medium darkens after exposure to white light. Surprisingly, MS medium containing silver nitrate did not darken when exposed to light if carbenicillin was also present in the culture medium (Fig. 1).

Silver nitrate is often added to tissue culture media to block the action of ethylene (Beyer 1976). The addition of silver nitrate improved embryogenic tissue formation in maize (Vain *et al.* 1989; Songstad *et al.* 1991); embryo production in Brussel sprout anther culture (Biddington *et al.* 1988); and plant regeneration in maize (Songstad *et al.* 1988), cucumber (Mohiuddin *et al.* 1997), *Brassica oleracea* (Williams *et al.*

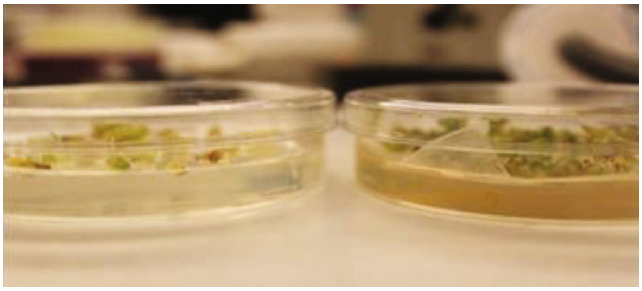

Figure 1. Culture medium after a light exposure that typically induces a reaction between Ag^+ and medium components to darken the medium. The *left* Petri dish contains MS medium containing 1321.3 μM carbenicillin and 20 μM silver nitrate. The *right* Petri dish contains MS medium containing 20 μM silver nitrate.

1990), and *Brassica campestris* (Palmer 1992). Additionally, silver as the metal or in ionic form has been shown to be an effective antibiotic (reviewed by Roy *et al.* 2007).

Carbenicillin is a common antibiotic added to plant culture media for the control of *Agrobacterium* sp. during *Agrobacterium*-mediated genetic transformation (for example Teixeira da Silva and Fukai 2001).

Considering the utility of silver nitrate and carbenicillin in plant tissue culture media, it is disconcerting to observe that media containing silver nitrate change color in light and do not change color with carbenicillin present. The present study was undertaken to determine if this lack of color change indicates chemical changes that compromise the effectiveness of these compounds.

Materials and Methods

Medium component testing. MS medium (Murashige and Skoog 1962) was used to analyze what components in plant tissue culture medium reacted with silver nitrate. Components were first tested against silver nitrate as groups of macronutrients, micronutrients, and vitamins (commercial formulations were purchased from PhytoTechnology Laboratories, Shawnee Mission, KS). Subsequently, individual components or combinations of individual components from groups were tested against silver nitrate. All components were tested at their final concentration in MS medium. Silver nitrate was added to a final concentration of 560 μM to 5 ml of each medium in a 17×100 mm test tube. The tubes were exposed to 14 μmol photons m^{-2} s^{-1} fluorescent light (Philips ALTO F32T8/TL635 32 W, 4100 K cool white universal hi-vision fluorescent bulb, Philips North America Corp, Andover, MA, USA) at room temperature for at least 1 h. Visual observations of color changes of the reaction mixture and the formation of precipitates were recorded. All culture media components were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Maize (Zea mays L.) callus culture. MSW57 callus induction and maintenance medium was used for all treatments (Sidorov and Duncan 2009). Treatment media contained either 1321.3 μM carbenicillin (Amresco LLC, Solon, OH), 20 μM silver nitrate (Sigma-Aldrich Chemical Co.), or both. Control medium contained no amendments. The media were adjusted to pH 5.8 with KOH, sterilized *via* passage through a 0.2-μm filter, and then exposed for 16 h to either 102 μmol photons m^{-2} s^{-1} fluorescent light (Sylvania Octron Eco 4100 K 17 W, Osram Sylvania, Danvers, MA) at 28°C, darkness at 28°C, or darkness at 4°C. Petri dishes (100 × 25 mm) were filled with three sterile, 40-cm^2, 100% polyester felt pieces and 30 ml of medium (Duncan *et al.* unpublished). For callus induction, approximately 1.8–2.0-mm-long immature embryos were isolated 9–10 d post-pollination from proprietary inbred lines A and B and placed on the media scutellum side up. All plates were incubated in darkness at 28°C for 3 wk. Immediately prior to the addition of maize tissue to the medium filter-sterilized 1-aminocyclopropane-1-carboxylic acid (ACC; Sigma-Aldrich Chemical Co.) was added to plates individually to achieve final concentrations of either 50 μM or 200 μM to drive ethylene biosynthesis. ACC was not added to a set of treatment plates. These plates plus their associated immature embryos were placed in a different growth chamber from the ACC-treated plates to avoid any ethylene generated in other treatments from affecting these plates. Observations were made on callus morphology.

Maize immature embryo germination. MSW57 without plant growth regulators but amended with 3.5 g L^{-1} Phytagel™ (Sigma-Aldrich Chemical Co.) was autoclaved at 121°C and 1.406 kg cm^{-2} for 22 min. Prior to medium solidification, ACC, carbenicillin, and silver nitrate were added to achieve final concentrations of 200 μM of ACC and either 1321.3 μM carbenicillin, 20 μM silver nitrate, or both. The control medium contained no amendments. These media were then dispensed in 5-ml aliquots into scintillation bottles. The bottles were closed with serum caps, and once the media solidified, the bottles were exposed for 16 h to either 102 μmol photons m^{-2} s^{-1} fluorescent light (Sylvania Octron Eco 4100 K 17 W, Osram Sylvania) at 28°C or darkness at 28°C. A set of bottles was also made without the addition of ACC.

Approximately 1.8–2.0-mm-long immature embryos were isolated 9–10 d post-pollination from proprietary inbred lines A and B and placed scutellum side down on the media. All bottles were exposed to 102 μmol photons m^{-2} s^{-1} fluorescent light (Sylvania Octron Eco 4100 K 17 W, Osram Sylvania,) at 28°C with a 16-h photoperiod for 7 d to induce embryo germination. The growing embryos were observed for morphological changes, anthocyanin production, and shoot and root size. Ethylene in the head space above the 7-d-old embryos

was measured by removing 1.0 ml of gas through the serum cap of each bottle using a gastight syringe. The gas was manually injected into an Agilent HP6890 gas chromatograph (GC) (Agilent Company, Santa Clara, CA) fitted with a Supelco, Carboxen 1010 PLOT, 30 m × 0.53 mm column, and an FID detector. Helium was used as a carrier gas at 10 ml min^{-1}. The injector ran in splitless mode at 200°C isothermally for 4 min. Acetylene eluted at 2.011 min and ethylene at 2.787 min.

Assessment of potato shoot growth. Single node cuttings of potato (*Solanum tuberosum* var. Atlantic) were placed in 150 × 25 mm culture tubes containing Sorobod® cellulose reservoirs (Ilacon Limited, Kent, UK) with 8 ml MS medium without plant growth regulators and amended with nothing or with either 1321.3 μM carbenicillin, 20 μM silver nitrate, or both, with or without 100 μM ACC. The media were exposed to 14 μmol photons m^{-2} s^{-1} fluorescent light (Philips ALTO F32T8/TL635 32 W, 4100 K cool white universal hi-vision fluorescent bulb, Philips North America Corp) at room temperature while being made and for 1 h while cuttings were added to the culture tubes. All bottles were exposed to 102 μmol photons m^{-2} s^{-1} fluorescent light (Sylvania Octron Eco 4100 K 17 W, Osram Sylvania) at 23°C with a 16-h photoperiod for 10 d prior to observations of height, rooting, and general development.

Assessment of bacterial growth Escherichia coli (*E. coli*) strain DH5α (Lucigen Corporation, Middleton, WI) containing pUC18, which confers resistance to carbenicillin *via* a gene for β-lactamase (GenBank/EMBL accession number L09136, Thermo Fisher Scientific, Pittsburgh, PA) was used to test the antibacterial properties of silver nitrate alone and in combination with carbenicillin. One bacterial colony was placed into 2 ml LB medium (Bertani 1951) and placed on a shaker at 28°C in darkness overnight (~16 h).

Table 1. The reaction color of different components from MS medium with 560 μM silver nitrate ($AgNO_3$). Reagents were at the final concentration in MS medium

Reagent combinations	Colorless	White ppt	Yellow ppt	Brown ppt
Macronutrients		• Some white ppt		
Cl, $AgNO_3$		•		
vitamins	•			
Micronutrients				•
Micronutrients, 280 μM $AgNO_3$	•			
Micronutrients, 560 μM $AgNO_3$				• Appeared more purple next to 0.5 ml Ag
Micronutrients, 1.1 mM $AgNO_3$				•
I, Zn, Mn, Mo, Co, Cu, $AgNO_3$				•
Zn, Mn, Mo, Co, Cu, $AgNO_3$	•			
I, Zn, Mn, Co, Cu, $AgNO_3$				•
I, Zn, Mn, Mo, Cu, $AgNO_3$				•
I, Mn, Mo, Co, Cu, $AgNO_3$				•
I, Zn, Mn, Mo, Co, $AgNO_3$				•
I, Zn, Mn, Mo, $AgNO_3$				•
I, Mo, Cu, $AgNO_3$			•	
I, Mo, Co, $AgNO_3$			•	
I, Zn, Mo, $AgNO_3$			•	
I, Mo, $AgNO_3$			•	
I, Mn, $AgNO_3$				•
I, $AgNO_3$			•	
Mn, $AgNO_3$	•			
Mn, I	•			
165.2 μM carbenicillin added first, Mn, I, $AgNO_3$	•			
Mn, I, $AgNO_3$, 165.2 μM carbenicillin added last				• Brown forming from yellow very slowly
165.2 μM carbenicillin, $AgNO_3$	•			
0.25 ml carbenicillin, I	•			
0.25 ml carbenicillin, Mn	•			

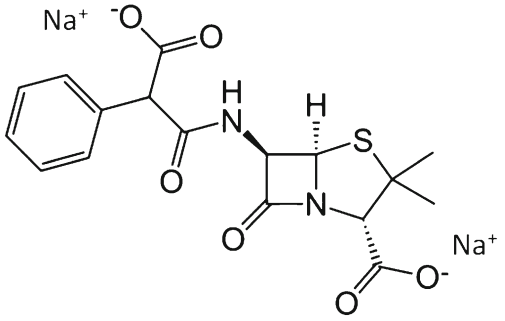

Figure 2. Structure of carbenicillin adapted from Wikimedia Commons http://upload.wikimedia.org/wikipedia/commons/0/09/Carbenicillin.svg.

MSW57 medium was prepared without plant growth regulators and amended with either nothing or 23.6 μM silver nitrate, or 1321.3 μM carbenicillin followed by 23.6 μM silver nitrate, or 23.6 μM silver nitrate followed by 132.1 or 1321.3 μM carbenicillin. Culture tubes (5-ml capacity) were filled with 2 ml medium and exposed to 0, 10, 20, 30, 40, 50, or 60 min of 14 μmol photons s^{-1} m^{-2} fluorescent light (Philips ALTO F32T8/TL635 32 W, 4100 K cool white universal hi-vision fluorescent bulb, Philips North America Corp) at room temperature. All tubes were maintained at 4°C in the dark until the desired light exposure time. All tubes were inoculated with 20 μl bacterial solution, resulting in an initial OD of approximately 0.01 for each inoculated tube, and incubated on a rotary shaker in darkness at 30°C for 3 h. A 1-ml aliquot was then removed from each treatment and measured for optical density at 660 nm on a Spectronic 20 Genesys spectrophotometer (Thermo Fisher Scientific). For each experiment, there was a minimum of three replicates per treatment.

Results and Discussion

MS medium containing silver nitrate darkened (Fig. 1) when exposed to light, so it was of interest to determine what components of the medium were reacting with silver nitrate to produce the coloration. When medium components, either in groups or separately, were exposed to silver nitrate, the only components associated with visible coloration were $CaCl_2$ (white cloudy solution) and KI (yellow to brown solution, Table 1). This was anticipated, as these are typical silver–halide reactions (Swift and Schaefer 1962). Although there is $CoCl_2$ in MS medium, no visible color change or precipitate could be detected as a result of exposure to silver nitrate, which may have been due to the low concentration of $CoCl_2$ in the medium (0.1 μM). The reaction of KI and silver nitrate was the easiest to observe because of the dark end product. When carbenicillin was added to a reaction mixture containing KI and silver nitrate, a brown coloration formed only if carbenicillin was added as the last reagent (Table 1). This observation suggests that carbenicillin interferes with the silver–iodine reaction as opposed to solubilizing the already-formed reaction product. These simple reactions, however, do not indicate which components of the reaction interact with the antibiotic.

Carbenicillin has two acid groups in its structure (Fig. 2) that could attract Ag^+, making an interaction with the metal possible. Silver has antibiotic activity (Roy *et al.* 2007) and this property was used to observe the interaction of silver with carbenicillin by examining the growth of carbenicillin-resistant bacteria in the presence of silver and carbenicillin. When a carbenicillin-resistant *E. coli* strain was grown in the presence of 23.6 μM silver nitrate, little growth was observed

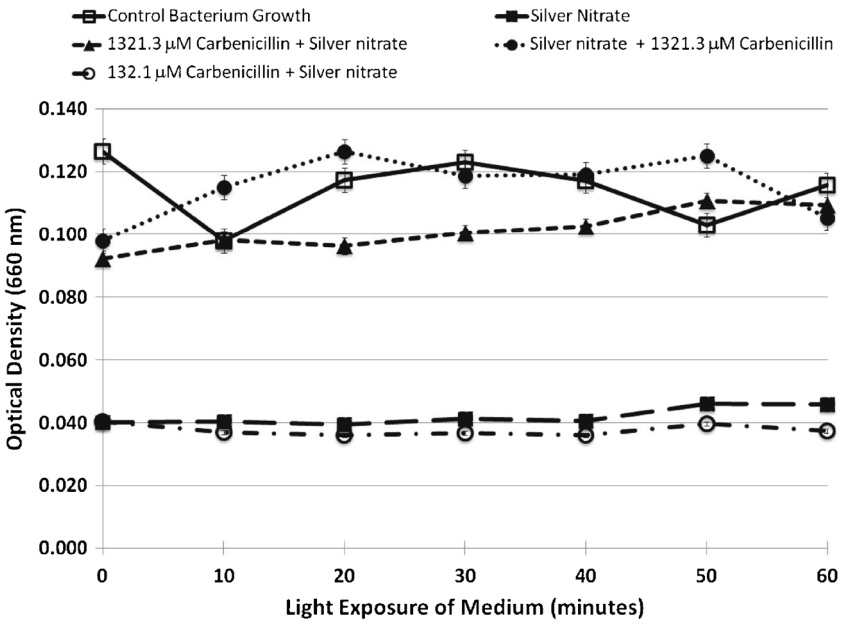

Figure 3. The growth of carbenicillin-resistant *E. coli* in MSW57 medium containing 132.1 μM carbenicillin, or 1321.3 μM carbenicillin, 23.6 μM silver nitrate, or a combination of these compounds. The *treatment labels in the legend* indicate the order in which the reagents were mixed together.

(Fig. 3). However, if 1321.3 μM carbenicillin was also present in the bacterial culture medium, the bacterial growth was comparable to that of bacteria not exposed to silver nitrate (Fig. 3). A level of 132.1 μM carbenicillin was insufficient to inhibit the antimicrobial activity of silver (Fig. 3).

Progressively longer times of exposure of culture media to light resulted in *E. coli* growth rates that increased by 28% when both carbenicillin and silver nitrate were present. These results appear to be the first report of an interaction between an antibiotic and monovalent silver. What has not been determined is if the silver degrades the antibiotic activity of the carbenicillin.

The effect of the interaction between silver and carbenicillin on plant tissues was initially examined by inducing embryogenic callus from immature maize embryos. To assure adequate ethylene was being produced by the tissues, some treatments had ACC added to a final concentration of 200 μM. Ethylene production was verified by gas chromatography (Fig. 4).

Callus development from the two proprietary lines tested was quite different on media with vs without silver nitrate. Inbred line A grew larger, had less embryogenic callus, and showed tissue browning in the presence of ethylene. Callus of inbred line B appeared overall to be less embryogenic, grew less than callus of line A, and showed an even greater growth reduction but very little tissue browning in the presence of ethylene (Fig. 5). Tissues on media containing carbenicillin without silver nitrate looked very much like the control in terms of the browning and growth reduction that was attributed to ethylene. The most embryogenic-looking tissues were found on media with only silver nitrate or media with carbenicillin and silver nitrate that were not exposed to light (Fig. 5). Tissues on these media did not appear as viable and vigorously growing as tissues on silver nitrate-containing medium, but they looked much more viable than tissues on media with and without ACC and carbenicillin (Fig. 5).

Silver was able to inhibit the negative effects of ethylene and facilitate embryogenic callus induction for inbred lines A and B (Fig. 5), which is consistent with the literature on maize tissue culture (Vain *et al.* 1989; Songstad *et al.* 1991). Only when carbenicillin was added to a silver nitrate-containing medium was a decrease of callus production and tissue browning detected for Line A (Fig. 5). The impact of the carbenicillin–silver interaction became more severe when

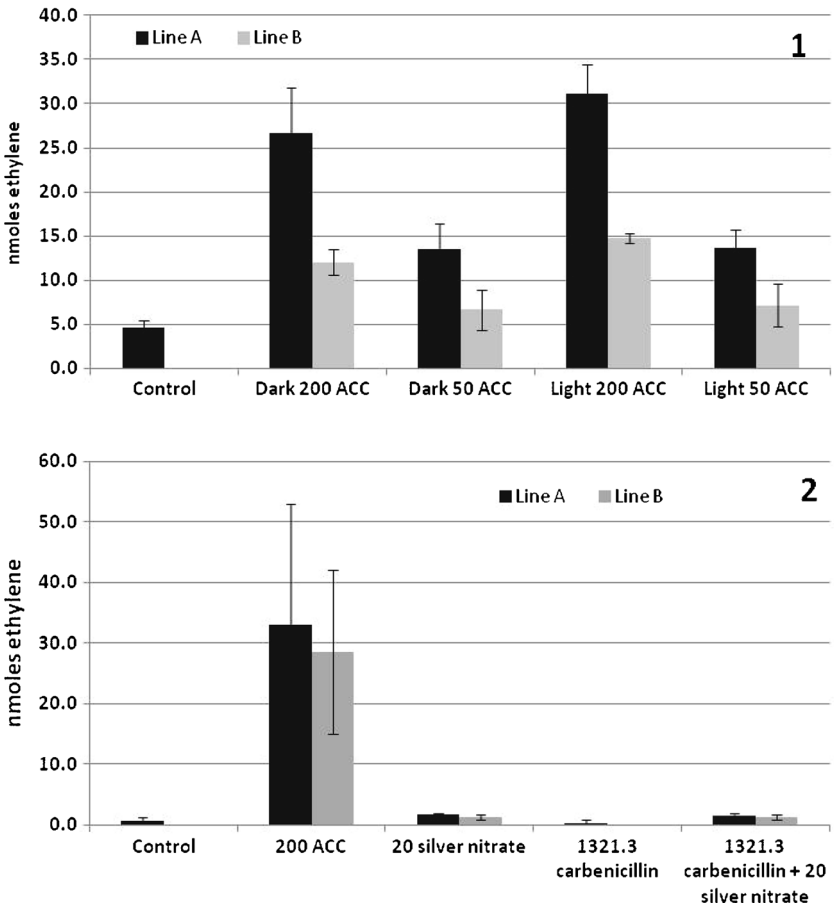

Figure 4. The production of ethylene from 2-wk-old callus (*1*) or 1-wk-old germinating immature embryos (*2*) of two proprietary maize inbred lines. The callus grew for 2 wk exposed to either 50 or 200 μM 1-aminocyclopropane-1-carboxylic acid (ACC), 1321.3 μM carbenicillin (carb), 20 μM silver nitrate (Ag), or a combination of these compounds. The media were exposed to either 102 μmol photons s^{-1} m^{-2} fluorescent light or darkness at 28°C for 16 h. The germinating immature embryos were grown in the 102 μmol photons m^{-2} s^{-1} fluorescent light at 28°C on MS medium containing 200 μM 1-aminocyclopropane-1-carboxylic acid (ACC), 1321.3 μM carbenicillin, 20 μM silver nitrate or a combination 1321.3 μM carbenicillin and 20 μM silver nitrate.

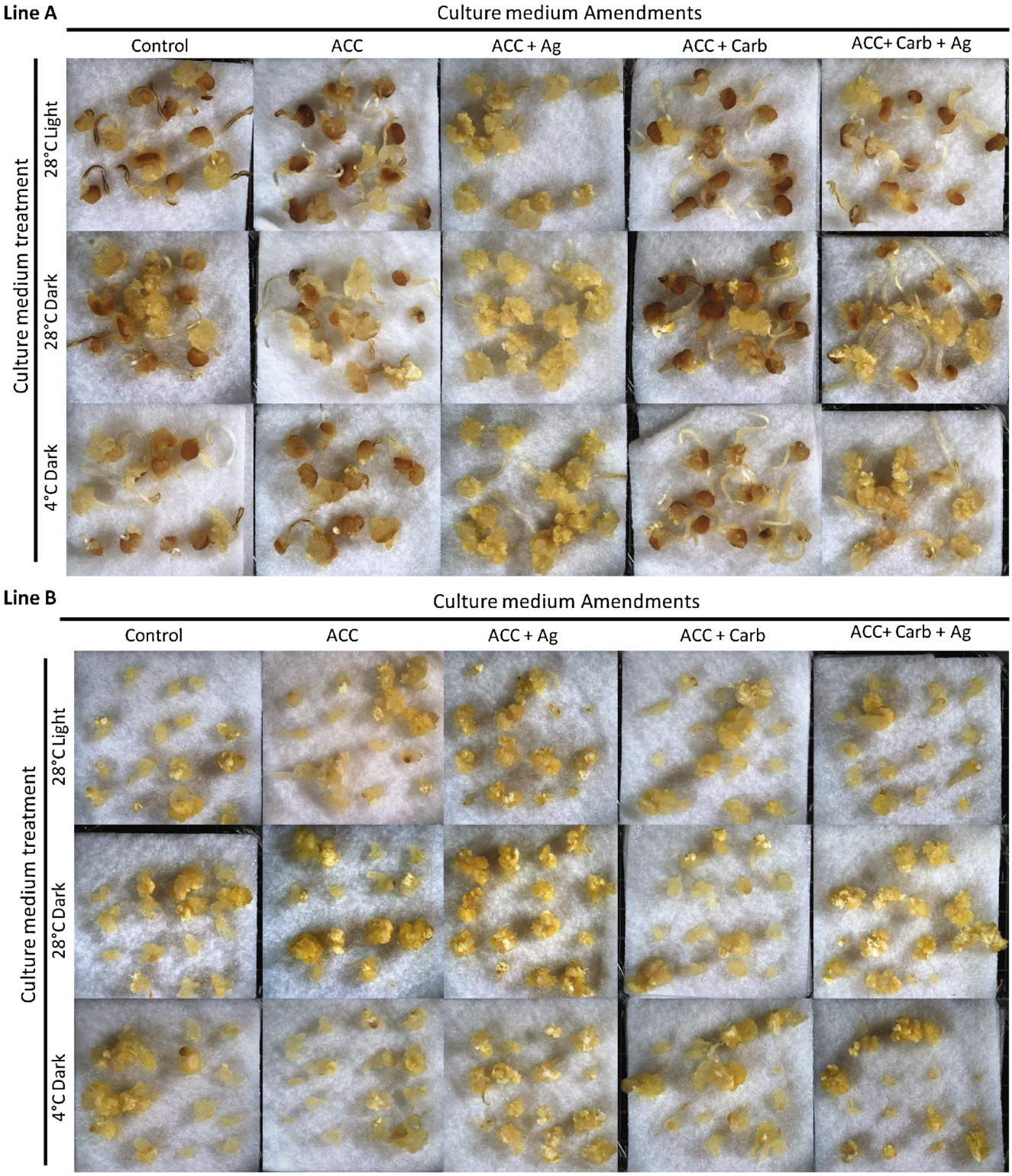

Figure 5. The callus induction response of two proprietary maize lines (*A* and *B*) exposed to MS medium containing 200 μM 1-aminocyclopropane-1-carboxylic acid (ACC), 1321.3 μM carbenicillin, 20 μM silver nitrate, or a combination of these compounds.

media were exposed to light prior to use. These results are consistent with those observed for *E. coli* growth in the presence of carbenicillin and silver nitrate (Fig. 3). Since silver nitrate is used in many tissue culture systems (for instance, Biddington *et al.* 1988; Williams *et al.* 1990; Palmer 1992; Mohiuddin *et al.* 1997), the interaction between silver and carbenicillin may have similar negative effects on cultured plant species other than maize.

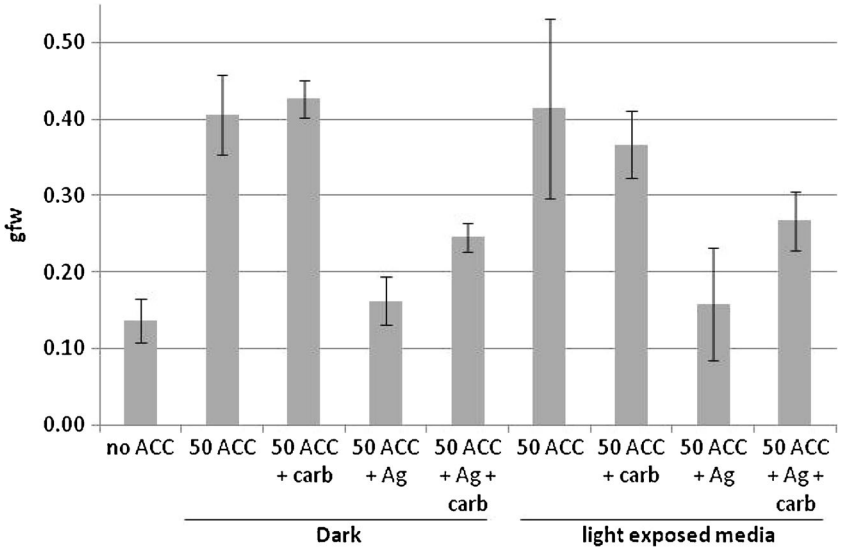

Figure 6. The fresh weight gain of callus developing from immature maize embryos (*Zea mays* L.) of proprietary inbred line A exposed to picloram- and 2,4-D-supplemented MS medium containing no additional additives except 50 μM 1-aminocyclopropane-1-carboxylic acid (*ACC*), 660.7 μM carbenicillin (*carb*), 20 μM silver nitrate (*Ag*), or a combination of these compounds.

The browning associated with 200 μM ACC prompted additional experiments with 50 μM ACC, which might be a less damaging treatment. These tests used proprietary inbred A. Ethylene stimulated non-embryogenic callus production (as determined by fresh weight gain compared to embryos not exposed to ACC) and this effect could be reversed by silver (Fig. 6). As previously observed for embryogenic callus production in the presence of 200 μM ACC and for *E. coli* growth, the effect of silver was less when carbenicillin was present, especially when media were exposed to light (Fig. 6).

To further examine the interaction of carbenicillin and silver, the growth of germinating maize embryos was evaluated. Immature embryos placed on medium lacking growth regulators began to germinate and radicle and coleoptile elongation was observed. No anthocyanin production was seen and the scutella were relatively large (Fig. 7), indicating that some level of ethylene was produced by the germinating embryos (Craker and Wetherbee 1973; Kang and Burg 1973; Zdenko and Kordan 1987). If ACC was present in the culture medium (Fig. 4), no anthocyanin production was seen, the scutella were larger, and little or no root elongation was seen (Fig. 7). If silver was present, the roots and parts of the scutella produced anthocyanins, the scutella were reduced in size, and normal coleoptile and root development were seen, indicating that silver blocked the activity of the ethylene (Beyer 1976; Fig. 7). Surprisingly, when carbenicillin and silver were present, the roots were red, the scutella were small, and normal coleoptile and root growth were seen indicating that silver was still blocking the activity of ethylene (Fig. 7).

To further elucidate the effects of silver and carbenicillin on whole plants, the *in vitro* growth of potato cuttings was examined. The noticeable effects of ethylene exposure on potato were a reduction in leaf size and root number, and to some degree, increased shoot length (Fig. 8). Silver seemed to remediate the effect of ethylene on leaf size and shoot length, but not root growth, suggesting that roots may be very sensitive to ethylene (Fig. 8). As compared to plants exposed to carbenicillin and silver without ACC, carbenicillin slightly reduced the effectiveness of silver in blocking the effect ethylene had on leaf size and plant height (Fig. 8).

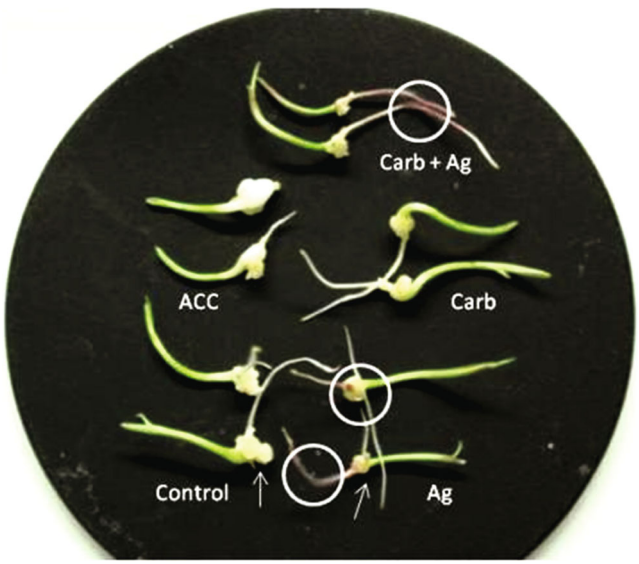

Figure 7. The development of roots, anthocyanin production, and scutella of germinating immature maize embryos (*Zea mays* L.) exposed to hormone-free MS medium containing 200 μM 1-aminocyclopropane-1-carboxylic acid (*ACC*), 660.7 μM carbenicillin (*carb*), 20 μM silver nitrate (*Ag*) or a combination of these compounds. The *arrows* indicate differences in scutellum size and the *circles* highlight tissues expressing anthocyanins.

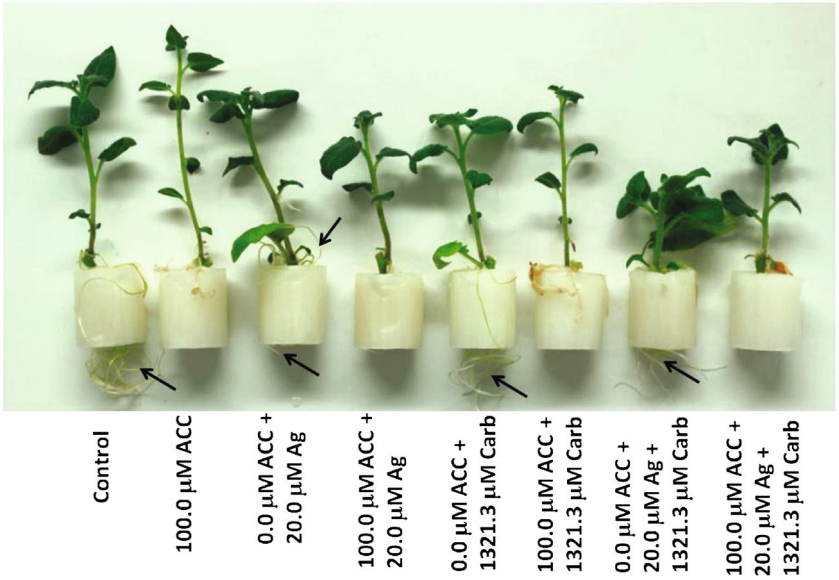

Figure 8. Potato (*Solanum tuberosum* var. Atlantic) development when exposed to hormone-free MS medium containing 100 µM 1-aminocyclopropane-1-carboxylic acid (*ACC*), 1321.3 µM carbenicillin (*carb*), 20 µM silver nitrate (*Ag*), or a combination of these compounds. *Arrows* highlight location of roots.

It must be presumed that silver and carbenicillin interacted in a similar manner in the maize germinating embryo and potato experiments as in the maize callus and *E. coli* experiments. The whole plant results suggest that not all silver had interacted with the carbenicillin and enough silver was available to reverse some or all the effects of ethylene. The results might further indicate that, compared to maize callus, there are fewer ethylene receptor sites in the germinating embryo and potato shoots and thus less silver was needed to reverse the effect of the ethylene.

The differences between the callus and whole plant experiments might also reflect differences in culture media composition. For both the germinating maize embryos and potato cuttings, no growth regulators were added to the culture medium, whereas the synthetic auxins 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram) and 2,4-dichlorophenoyxacetic acid (2,4-D) were present in the maize callus culture medium. The presence of exogenous synthetic auxins may have exacerbated the level and effect of ethylene in the callus system (Gaspar *et al.* 1996) rendering the interaction between silver and carbenicillin more obvious and more detrimental to the callus than to the germinating embryo or potato cutting.

Furthermore, penicillin-related antibiotics like carbenicillin are sensitive to light degradation (Aiello and Moses 2012). The 16-h daily light exposure used to grow the potato and maize embryos may have been sufficient to degrade a portion of the carbenicillin in the medium. When a low level (132.1 µM) of carbenicillin was added to the *E. coli* growth assay, there was adequate silver still present in the MSW57 medium to inhibit bacterial growth (Fig. 3). Similarly, a lower carbenicillin level, because of light degradation, might have left enough silver in the medium to block ethylene action in the whole plant tissues.

Taken as a whole, the results presented here indicate that silver and carbenicillin interact in culture media. Furthermore, depending on the culture system and the desired end result of adding the two components to a culture medium, the interaction could be detrimental. Other antibiotics are used in plant tissue culture and they may also interact with silver or other metals in the culture medium. It is important to note that adding a component to plant culture medium may not yield the desired results and the results may be difficult to interpret due to such interactions as seen with silver nitrate and carbenicillin.

References

Aiello SE, Moses MA (2012) The Merck Veterinary Manual Online. Merck Sharp & Dohme Corp, Whitehouse Station, NJ,http://www.merckmanuals.com/vet/pharmacology/antibacterial_agents/penicillins.html. Cited 01 July 2014

Anacona JR (2001) Synthesis and antibacterial activity of some metal complexes of beta-lactamic antibiotics. J Coord Chem 54:355–365

Beggs WH, Andrews FA (1975) Role of ionic strength in salt antagonism of aminoglycoside action on *Escherichia coli* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 7:636–639

Beggs WH, Andrews FA (1976) Inhibition of dihydrostreptomycin action on *Mycobacterium smegmatis* by monovalent and divalent cation salts. J Infect Dis 134:500–504

Berkman S, Henry JR, Housewright RD (1947) Studies on streptomycin I factors influencing the activity of streptomycin. J Bacteriol 53:567–574

Bertani G (1951) Studies on lysogenesis I. The mode of phage liberation by lysogenic *Escherichia coli*. J Bacteriol 62:293–300

Beyer EM (1976) A potent inhibitor of ethylene action in plants. Plant Physiol 58:268–271

Biddington NL, Sutherland RA, Robinson HT (1988) Silver nitrate increases embryo production in anther culture of brussels sprouts. Ann Bot 62:181–185

Craker LE, Wetherbee PJ (1973) Ethylene, light and anthocyanin synthesis. Plant Physiol 51:436–8

Donovick R, Bayan AP, Canales P, Pansy F (1948) The influence of certain substances on the activity of streptomycin III. Differential effects of various electrolytes on the action of streptomycin. J Bacteriol 56:125–137

Fazakerley GV, Jackson GE (1975) Metal ion coordination by some penicillin and cephalosporin antibiotics. J Inorg Nucl Chem 37:2371–2375

Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA (1996) Plant hormones and plant growth regulators in plant tissue culture. In Vitro Cell Dev Biol - Plant 32:272–289

Kang BG, Burg SP (1973) Role of ethylene in phytochrome induced anthocyanin biosynthesis. Planta 110:227–35

Kassanis B, White RF, Woods RD (1975) Inhibition of multiplication of tobacco mosaic virus in protoplasts by antibiotics and its prevention by divalent metals. J Gen Virol 28:I85–I9I

Light B, Riggs HB Jr (1978) Effect of triethylenetetramine dihydrochloride on the antibiotic susceptibility of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 13:979–984

Mohiuddin AKM, Chowdhury MKU, Abdullah ZC, Napis S (1997) Influence of silver nitrate (ethylene inhibitor) on cucumber *in vitro* shoot regeneration. Plant Cell Tiss Org Cult 51:75–78

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco cultures. Physiol Plant 15:473–497

Palmer CE (1992) Enhanced shoot regeneration from *Brassica campestris* by silver nitrate. Plant Cell Rep 11:541–545

Roy R, Hoover MR, Bhalta AS, Slawecki T, Dey S, Cao W, Li J, Hhaskar S (2007) Ultradilute Ag-aquasols with extraordinary bactericidal properties: role of the system Ag-O-H_2O. Mater Res Innov 11:3–18

Sidorov V, Duncan DR (2009) *Agrobacterium*-mediated maize transformation: Immature embryos *versus* callus. In: Paul Scott M (ed) Methods in molecular biology. Humana, Totowa, pp 47–58

Songstad DD, Armstrong CL, Petersen WL (1991) $AgNO_3$ increases type II callus production from immature embryos of maize inbred B73 and its derivatives. Plant Cell Rep 9:699–702

Songstad DD, Duncan DR, Widholm JM (1988) Effect of 1-aminocyclopropane-1 carboxylic acid, silver nitrate, and norbornadiene on plant regeneration from maize callus cultures. Plant Cell Rep 7:262–265

Swift EH, Schaefer WP (1962) Qualitative elemental analysis. Freeman, San Francisco

Teixeira da Silva JA, Fukai S (2001) The impact of carbenicillin, cefotaxime and vancomycin on chrysanthemum and tobacco TCL morphogenesis and *Agrobacterium* growth. J Appl Hort 3:3–12

Vain P, Flament P, Soudain P (1989) Role of ethylene in embryogenic callus initiation and regeneration in *Zea mays* L. J Plant Physiol 135:537–540

Weast RC (1971) Handbook of chemistry and physics. Chemical Rubber Co, Cleveland

Williams J, Pink DAC, Biddington NL (1990) Effect of silver nitrate on long-term culture and regeneration of callus from *Brassica oleracea* var. *gemmifera*. Plant Cell Tiss Org Cult 21:61–66

Zdenko R, Kordan HA (1987) Effects of growth regulators on light-dependent anthocyanin production in *Zea mays* seedlings. Physiol Plant 69:511–516