Phytotoxic Effects of Cyanamide on Seed Germination and Seedling Growth of Weed and Crop Species

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We examined the response of plants of various crop and weed species to cyanamide in order to evaluate allelo-chemical-mediated interactions between the species. We studied germination and seedling growth in the common weeds *Galium aparine* L. and *Amaranthus retroflexus* L., and the crops *Zea mays* L., *Triticum aestivum* L., *Lactuca sativa* L., *Solanum lycopersicum* L. and *Sinapis alba* L. as acceptor plants. Concentration-dependent phytotoxic effects of cyanamide were noted during seed germination and in the root and shoot growth of the tested plants. The monocotyledonous plants generally were less sensitive to cyanamide treatment. Seed germination and seedling growth of the dicotyledonous plants were strongly inhibited by the allelochemical at both tested concentrations (1.2 mM, 3 mM). We conclude that cyanamide has potential for use as a natural herbicide only in specific field systems of cyanamide-tolerant monocotyledonous crops accompanied by cyanamide-sensitive dicotyledonous weeds.

Key words: Allelopathy, germination, phytotoxicity, Pieper's value, seedling growth, weeds, crop plants.

INTRODUCTION

Allelopathy consists in the effects, negative or positive, of chemicals produced by plants of a given species or from decomposition of plant tissue (Duke, 2010). Certain substances released into the soil may inhibit the development of other organisms in the immediate surroundings. Such interactions are called phytotoxic. Allelopathic effects on target plants often are evaluated in laboratory conditions. The discovery that allelopathy can be an important factor in agricultural communities and may influence the relationships between crops and weeds opens the possibility of using this phenomenon in weed management strategies.

Cyanamide (CA) is a natural compound produced by some members of the Fabaceae family. Kamo et al. (2003) found CA in all vegetative organs of hairy vetch (*Vicia villosa* subs. *varia* Roth) at concentrations of 369–498 μg g^{-1} FW. This allelochemical is also produced in bird vetch (*Vicia cracca* L.) (Kamo et al., 2006) and black locust (*Robinia*

pseudoacaccia L.) (Kamo et al., 2008). Hairy vetch is used as an annual cover crop in no-till cropping systems as an additional nitrogen source (Inderjit and Asakawa, 2001). An additional benefit of using it is its action as an inhibitor of the growth of some weed species (e.g., whitestar *Ipomoea lacunosa* L., barnyard grass *Echinochlora crus-galli* L., prickly sida *Sida spinosa* L., crabgrass *Digitaria sanguinalis* L.) (Hoffman et al., 1993; Reddy and Koger, 2004; Hill et al., 2007; Teasdale et al., 2007).

Seed germination tests and morphometric observations are the methods most commonly used to investigate the allelopathic potential of allelochemicals. Such experiments can help determine whether to use them as an alternative weed management tool to increase crop yield. In this study we examined the effects of water solutions of CA on seed germination and post-germination growth of young seedlings of various crop and weed species. In previous work we demonstrated that water solutions of CA strongly inhibit the root growth of onion (*Alium cepa* L.) in a dose-dependent manner (Soltys

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et al., 2011). Based on that experiment, in this work we used CA at 1.2 mM and 3.0 mM concentrations, since higher concentrations (6–10 mM) induced cell death (Soltys et al., 2011). We examined the allelopathic action of CA in two dicotyledonous annual weeds: goosegrass (*Galium aparine* L.), native to North America and Eurasia, and redroot pigweed (*Amaranthus retroflexus* L.), native to the tropical Americas but widespread as an introduced species on most continents in a great number of habitats. As crops we selected two monocotyledons species, maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.), and three dicotyledons, lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.) and mustard (*Sinapis alba* L.). Lettuce and tomato were chosen as typical acceptor plants sensitive to most allelochemicals. Mustard was chosen as a plant that shares traits of both crops and weeds. In our previous studies of the allelopathic potential of sunflower, we used mustard as test material because of its rapid and uniform seed germination and seedling establishment (Bogatek et al., 2006; Oracz et al., 2007).

MATERIALS AND METHODS

PLANT MATERIAL

Seeds and caryopses of cultivated species were obtained in 2010 from Polish suppliers: tomato (*S. lycopersicum* L. cv. Malinowy Ożarowski) and lettuce (*L. sativa* L. cv. Lodowa) seeds from PNOS Ożarów; maize (*Z. mays* L. cv. Cukrowa) caryopses from Torseed Toruń; wheat (*T. aestivum* L. cv. Jasna) caryopses from Nasiona Kobierzyce; mustard seeds (*S. alba* L. cv. Bamberka) from the Smolice Plant Breeding Co. Seeds of the weed species goosegrass (*G. aparine* L.) and redroot pigweed (*A. retroflexus* L.) were collected in 2010 from fields and meadows around Warsaw. Seeds of each weed species were mixed and formed the bulk of seeds.

SEED GERMINATION

Seeds and caryopses were germinated in Petri dishes (18 cm diam, 30 seeds per dish) filled with 10 ml distilled water or CA (Sigma) water solution (1.2 or 3 mM) in darkness at 20°C. Seeds were cultured continuously for 7 days. They were regarded as germinated when radicles reached 1 mm length. The germinated seeds or caryopses were counted every day. The counts are expressed as percentages of the seed/caryopsis pool on each day (germination dynamics) and as Pieper's value describing the average germination time of one seed, calculated according to the formula:

$$\frac{\Sigma\, n \times t}{\Sigma\, n}$$

where n is the number of germinated seeds/caryopses on each day of culture, and t is germination time.

THICKNESS OF SEED COAT/PERICARP

Dry seeds or caryopses were cut in half under a stereomicroscope with a razor blade. Thin slices showing seed coat/pericarp structure were taken from the seed halves. Seed coat thickness was observed with an Olympus light microscope equipped with a digital camera and measured with COOLview software (Precoptic Co., Poland).

POST-GERMINATION GROWTH

Seeds were germinated in distilled water. After the radicles protruded, seedlings of equal size were transferred to Petri dishes (18 cm diam, 15 seedlings per dish) filled with 10 ml distilled water (control) or CA aqueous solutions (1.2 or 3 mM). Culture was prolonged for 7 days in darkness at 20°C. Seedling root and shoot length was measured after 7 days of culture. For wheat roots the longest root was measured. Data are expressed as absolute values and percentage of control (untreated) plants.

STATISTICAL ANALYSIS

The data are means of three measurements from each of three to five sets of experiments. Data were analyzed using StatGraphics 5.1; Means ±SE were computed for each experiment and the significance of differences was assessed with Tukey's studentized range test or Student's t-test. Differences are considered significant at P < 0.05.

RESULTS AND DISCUSSION

Allelopathic interactions between the various plant species in natural ecosystems and agroecosystems are a form of competition involving inhibition of seed germination and/or seedling growth. Such interactions may alter plant communities. Most recently, allelopathic interactions have been considered a useful tool for eco-friendly weed management based on natural compounds – allelochemicals. Some isolated compounds have been successfully launched as natural herbicides (e.g., leptospermone, cineol) and some (e.g., sorgoleone, heliannuol) are being screened for use in plant protection (Bhowmik and Inderjit, 2003; Sekutowski, 2010). Cyanamide is an allelochemical from hairy vetch whose inhibitory effect on weeds is potentially of use in agriculture,

TABLE 1. Pieper's value for various plant species germinated continuously 7 days in water (control) or CA aqueous solutions (1.2 mM, 3.0 mM)

Species	Control	CA concentrations	
		1.2 mM	3.0 mM
Goosegrass	5.3 ± 0.1	5.3 ± 0.1^{ns}	5.5 ± 0.1^{ns}
Redroot pigweed	4.3 ± 0.1	6.0 ± 0.9	non germinated
Tomato	3.0 ± 0.1	4.9 ± 0.1	non germinated
Lettuce	1.6 ± 0.2	2.1 ± 0.4^{ns}	4.5 ± 0.3
Maize	2.2 ± 0.2	2.3 ± 0.3^{ns}	2.5 ± 0.3^{ns}
Wheat	2.0 ± 0.2	2.0 ± 0.2^{ns}	2.2 ± 0.2^{ns}
Mustard	1.4 ± 0.2	1.5 ± 0.4^{ns}	2.7 ± 0.0

Means $\pm$SE; ns – no significant difference versus control value at $P < 0.05$ (Student's t-test).

mainly due to its low cost. Unfortunately its allelopathic potential as an inhibitor of seed germination and/or seedling growth has not been well studied. There are no published data comparing the phytotoxicity of CA to weeds (target plants) and to crops (non-target plants).

Various phytotoxins are known to inhibit or retard seed germination. A derivative of coumarin, 4-methylumbelliferone, inhibited germination of *Arabidopsis thaliana* seeds (Li et al., 2011); 6-metoxy-benzoxazolin-2(3H)-one (MBOA, a benzoxazolinone) or myrigalone A inhibited germination of cress (*Lepidium sativum*) seeds (Kato-Naguchi and Macias, 2006; Oracz et al., 2012). Pieper's value is one of the most useful parameters for describing seed germination rate. It expresses germination speed: higher values indicate longer seed germination time. Here we demonstrated that CA affected the germination of most of the examined plants. The germination time of dicotyledonous plants, both crops and one weed, was prolonged. Tomato seemed to be the crop most sensitive to CA. Tomato seeds imbibed in 1.2 mM CA had inhibited germination (Tab. 1). The phytotoxic effect of CA on tomato was evident at 3 mM CA: seed germination was blocked completely (Tab. 1). Seed germination also was arrested in the weed species redroot pigweed at 3 mM CA. In lettuce and mustard, on the other hand, only the higher CA concentration (3 mM) significantly affected Pieper's value for seed germination (Tab. 1). Treatment of maize and wheat caryopses with CA at both concentrations had no effect on Piper's value (Tab. 1).

The percentage of germinated seeds of goosegrass, redroot pigweed, tomato and lettuce declined with the increase of CA concentration (Figs. 1, 2). Mustard and wheat imbibed in 1.2 mM CA showed no effect on the number of germinated seeds after 7 days of culture (Figs. 2, 3). Maize caryopses responded similarly to CA applied at both tested concentrations: after 7 days only 70% of them had germinated (Fig. 3).

Based on these data we distinguish species sensitive or tolerant to CA. For plants designated as tolerant the percentage of germinated seeds after treatment with 1.2 mM CA did not fall below 50%, of the control. Under these criteria the CA-tolerant group of species includes the weed goosegrass, and the crops maize, wheat, mustard (Figs. 1–3). Redroot pigweed and tomato are the CA-sensitive plants in this study (Figs. 1, 2). The seeds of both species showed only 5–10% germination after treatment with 1.2 mM CA, and no germination at 3 mM CA.

We found no explicit correlation between germination time and germination percentage in the test-
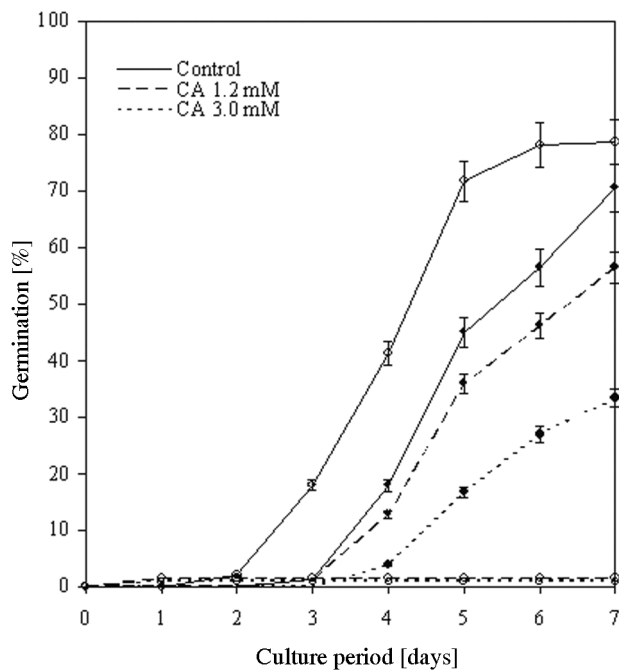

Fig. 1. Germination of goosegrass (black tags) and redroot pigweed (blank tags) during 7-day culture in water (control) or CA aqueous solution (1.2 mM, 3.0 mM). Means $\pm$SE.
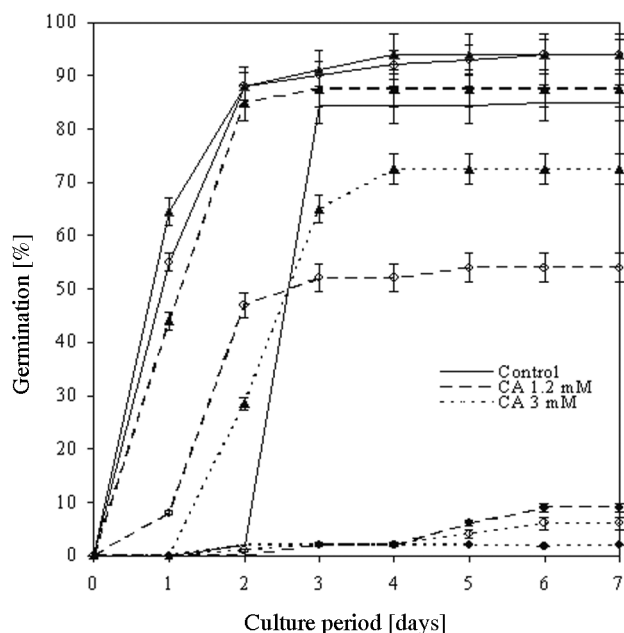
Fig. 2. Germination of mustard (triangle), tomato (black tags) and lettuce (blank tags) during 7-day culture in water (control) or CA aqueous solution (1.2 mM, 3.0 mM). Means ±SE.

Fig. 3. Germination of maize (black tags) and wheat (blank tags) during 7-day culture in water (control) or CA aqueous solutions (1.2 mM, 3.0 mM). Means ±SE.

ed plants (data not shown), except in mustard (Tab. 1, Figs. 1–3).

In this study the monocotyledonous crops were more tolerant to CA treatment during germination. The relatively low sensitivity of seeds of monocotyledon species to CA may be explained by their seed size and seed coat thickness (Tab. 2). The caryopses of the monocotyledonous plants we studied are much heavier than the seeds of the tested dicotyledons. Possibly CA more easily penetrates the seed coat of small seeds having relatively small endosperm (e.g., redroot pigweed, tomato and lettuce) (Tab. 2), quickly enters the embryo and finally disrupts germination. The caryopses of maize, wheat and mustard, with their high amount of starch, should be less permeable to CA. Such relations have been noted in *Arabidopsis* and lettuce seeds treated with allelochemicals (Pennacchio et al., 2005). Similarly, exposing bulk seed of hairy vetch to 10^{-3} M coumarin delayed and reduced germination, and the effect was more pronounced in the small seed class; as a percentage of the control, germination of large, medium and small seeds was 72%, 76% and 55%, respectively, and germination of small seeds was delayed by two days (Williams and Bartholomew, 2011). Such a high correlation as found between seed size and inhibition of germination may not be found for seed coat thickness, due to variation of the density, permeability and composition of this structure.
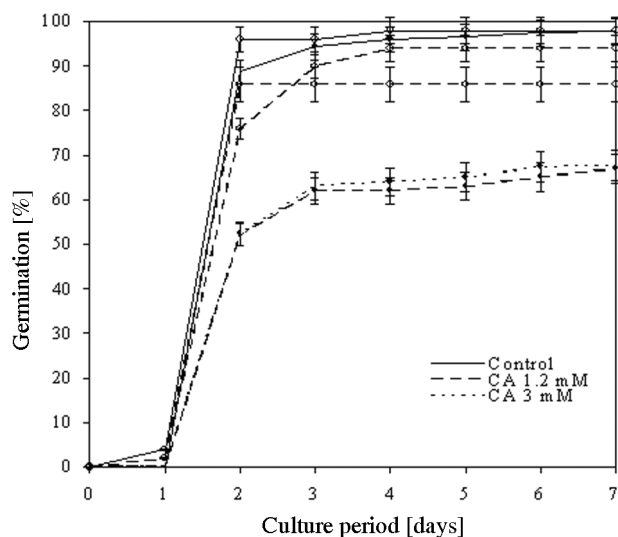
Post-germination growth of the tested plant species was also affected by CA toxicity (Fig. 4). As was observed for seed germination, increasing the CA dose increased the inhibition of seedling growth and development (Fig. 4). In the weeds redroot pigweed and goosegrass, CA showed its toxicity mostly in the effect on shoot growth (Fig. 4). Shoots of seedlings of both species were only 5% or 30% the length of control shoots after 7 days of exposure to 3 mM CA. The root growth of redroot pigweed was almost totally blocked by 3 mM CA, but CA at the lower concentration slightly stimulated its elongation (Fig. 4).

The phytotoxicity of cyanamide to root and shoot growth was highest in tomato and lettuce seedlings (Fig. 4), as was the case for seed germina-

TABLE 2. Weight of 1,000 dry seeds/caryopses and thickness of dry seed coat/pericarp

Species	Weight of 1,000 seeds/caryopses (g)	Thickness of seed coat/pericarp (µm)
Goosegrass	8.26 ± 0.05[a]	56 ± 4[a]
Redroot pigweed	0.44 ± 0.01[b]	18 ± 4[b]
Mustard	7.19 ± 0.01[g]	42 ± 4[c]
Tomato	2.10 ± 0.05[c]	13 ± 2[b]
Lettuce	1.12 ± 0.01[d]	15 ± 2[b]
Maize	233.20 ± 1.74[e]	35 ± 6[c]
Wheat	44.13 ± 0.47[f]	34 ± 6[c]

Means ±SE, a,b,c... – homogenous groups.

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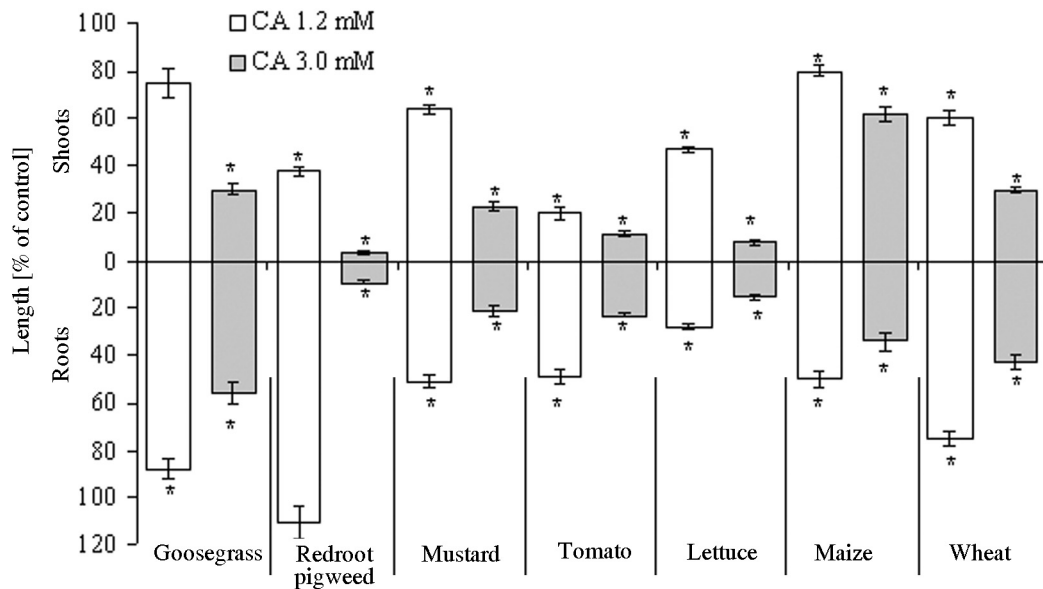*Cyanamide phytotoxicity to weeds and crops*   91

Fig. 4. Length of roots and shoots (% of control) grown continuously 7 days post-germination in 1.2 mM or 3.0 mM CA water solutions. Means ±SE, * – significant difference versus control value at P < 0.05 (Tukey's test).

tion. These data confirm the high allelopathic potential of CA against dicotyledonous crop species. Dose- and organ-specific reactions similar to the ones we found have been noted in *Arabidopsis* seedlings after 4-methylumbelliferone treatment (Li et al., 2011) and in soybean (*Glycine max*) exposed to juglone (Hejl and Koster, 2004).

CONCLUSIONS

Our data indicate that the monocotyledonous plants we studied are more CA-tolerant than the dicotyledonous ones, as they showed relatively uniform seed germination time after CA treatment. Their roots and shoots also are less sensitive to CA than those of the dicotyledonous plants. We cannot unequivocally state whether germination or seedling growth are more sensitive to CA treatment, since the observed responses depended rather on the plant species. Among the crops and weeds there were both CA-tolerant and CA-sensitive species. Mustard combined traits of the two groups for most of the tested parameters.

Our experiment suggests that CA holds potential as a natural herbicide only in specific field systems consisting of CA-tolerant crops accompanied by CA-sensitive weeds. The use of CA as a foliar herbicide against some weed species may also be indicated, as the growth of aerial parts of goosegrass or redroot pigweed was strongly inhibited by CA in water solution.

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