Weed Technology 2010 24:295–302

Surface-Applied Calcium Phosphate Stimulates Weed Emergence in Flooded Rice

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Weeds are the major biotic constraint to rice production. Field observations have suggested that certain fertilizer regimes could enhance infestations of particular weed species emerging with rice. The study objective was to determine the effect of surface-applied calcium phosphate on weed growth in flooded California rice systems. In field and pot studies, triple superphosphate (TSP) applied to the soil surface increased weed emergence. Surface-applied TSP increased the number of sedge and broadleaf weeds, including smallflower umbrella sedge, blue-flowered ducksalad, redstem, ricefield bulrush, waterhyssop, and California arrowhead. A laboratory study measured germination of smallflower umbrella sedge and ricefield bulrush in response to the application of phosphorus (P) and calcium (Ca), which comprise 20 and 15% of TSP, respectively. Calcium stimulated smallflower umbrella sedge germination and had no effect on ricefield bulrush germination. Phosphorus did not stimulate either smallflower umbrella sedge or ricefield bulrush germination. Results indicate that surface applications of calcium phosphate increase the growth of certain weed species and that Ca may stimulate germination of smallflower umbrella sedge. By incorporating preplant applications of calcium phosphate into the soil profile, growers can reduce weed pressure from certain species. Alternatively, surface applications of calcium phosphate may be useful to stimulate weed emergence in stale-seedbed management.

Nomenclature: Blue-flowered ducksalad, *Heteranthera rotundifolia* (Kunth) Griseb.; California arrowhead, *Sagittaria montevidensis* Cham. & Schlecht.; redstem, *Ammannia coccinea* Rottb.; ricefield bulrush, *Schoenoplectus mucronatus* (L.) Palla; waterhyssop, *Bacopa* spp. L.; smallflower umbrella sedge, *Cyperus difformis* L.; rice, *Oryza sativa* L.

Key words: Fertilizer application, phosphorus, weed, germination, aquatic weeds, sedges, integrated weed management, fertility management.

Las malezas son la principal restricción biótica para la producción de arroz. Las observaciones de campo sugirieron que ciertos regímenes de fertilizantes pueden provocar infestaciones de especies particulares de malezas que emergen con el arroz. El objetivo de este estudio fue determinar el efecto de la aplicación de fosfato de calcio a la superficie en el crecimiento de la maleza en sistema por inundación en las siembras de arroz en California. La aplicación de súper fosfato triple (TSP) a la superficie del suelo, incrementó la emergencia de las malezas tanto en el campo como en macetas. La aplicación de TSP incrementó el número de juncos y malezas de hoja ancha, incluyendo *Cyperus difformis* L., *Heteranthera rotundifolia* (Kunth) Griseb, *Ammannia coccinea* Rottb, *Schoenoplectus mucronatus* (L.) Palla, *Bacopa* spp. L y *Sagittaria montevidensis* Cham. & Schlecht. Un estudio de laboratorio midió la germinanción de *Cyperus difformis* L y *Schoenoplectus mucronatus* (L.) Palla como respuesta a la aplicación de fósforo (P) y Calcio (Ca), los cuales comprenden el 20 y 15% de TSP, respectivamente. El calcio estimuló la germinación de *Cyperus difformis* L., y no tuvo efecto alguno en la germinación de y *Schoenoplectus mucronatus* (L.) Palla. El fósforo no estimuló la germinación de ninguna de las dos malezas antes mencionadas. Los resultados indican que las aplicaciones de fosfato de calcio en la superficie incrementan el crecimiento de ciertas especies de maleza y que el calcio podría estimular la germinación de *Cyperus difformis* L. Por medio de la incorporación de aplicaciones pre-siembra de fosfato de calcio al perfil del suelo, los productores pueden reducir la presión de ciertas especies de malezas. Alternativamente, las aplicaciones de fosfato de calcio en la superficie podrían ser útiles para estimular la emergencia de malezas en el manejo de semilleros caducos.

Rice (*Oryza sativa* L.) feeds more people than any other crop (Pusadee et al. 2009) and is the staple food in the world's most impoverished regions (Maclean et al. 2002). Weed competition is cited as the major biological constraint to rice production (Ni et al. 2000). In California, where more than 200,000 ha of flooded rice are cultivated annually, there are more herbicide-resistant weeds than in any other crop or region in the United States (Fischer et al. 2000; Hill et al. 1994), and weed control represents 27% of the cost of purchased inputs (Mutters et al. 2007). Thus, management practices that reduce weed competition without escalating herbicide use can have significant biological and economic consequence.

Because weeds are often better able to exploit nutrients than agricultural crops, temporal and spatial manipulations of fertilizer rate and application can improve crop competitiveness (DiTomaso 1995). However, the success of such strategies depends on both the nutrient and weed in question. Weeds have shown a response to increasing phosphorus (P) levels (Andreasen et al. 2006; Hoveland et al. 1976), and the availability of P has been shown to influence crop–weed dynamics in a number of contexts. Blackshaw et al. (2004) reported that, when exposed to increasing P levels, 17 weed species increased shoot biomass more than spring wheat

DOI: 10.1614/WT-D-09-00082.1

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(*Triticum aestivum* L.) and 19 weed species increased shoot biomass more than canola (*Brassica napus* L.). Pandey et al. (1971) found that the demand for P by a population of weeds growing in the immediate vicinity of winter wheat (*Triticum vulgare* Vill.) exceeded that of the crop. Vengris et al. (1955) showed that, due to the reduced ability of pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album* L.) to establish themselves where P is limiting, maize (*Zea mays* L.) grown in competition with these weeds produced relatively higher yields where P was limiting compared to non–P-limited conditions. On the other hand, Santos et al. (2004) demonstrated that, relative to banded applications, broadcast applications of P increased common lambsquarters density and reduced lettuce (*Lactuca sativa* L.) yields by 11%, and Smith and Shaw (1966) reported that P applications stimulated weed growth in flooded rice. Taken together, these studies indicate that P is limiting to multiple weed species growing in competition with a variety of crops. Therefore, in situations where weeds respond to P application, the location, timing, and form of P made available to a crop can indirectly influence weed competitiveness.

In California, rice is grown under flooded conditions on heavy soils with acid to neutral pH. Phosphorus deficiency is rare in this system because growers routinely apply P fertilizer and, regardless of P fertilizer application, a sufficient fraction of iron-bound phosphate solubilizes under anaerobic conditions and becomes available to the developing roots (Shahandeh et al. 1994). Growers typically apply 20 to 30 kg ha^{-1} P to the soil surface prior to flooding and either incorporate it into the top 2 to 3 cm or leave it on the soil surface. Although surface applications of P fertilizer have been shown to increase P uptake and yield in rice (Diamond 1985), they may also increase weed growth.

Preliminary observations in 2006 indicated that weed growth was greater where tri-calcium di-hydrogen phosphate, $3Ca(H_2PO_4)_2$ (*aq*), (TSP) had been applied to the soil surface immediately prior to flooding as compared to where no TSP had been applied (B. A. Linquist, unpublished data). Based on these observations, we hypothesized that surface P applications stimulate weed growth. The objective for the following three studies was to determine whether surface-applied P fertilizer affects weed growth in flooded rice and whether the effects are specific to particular weed species or morphological groups (i.e., grasses, sedges, broadleaves).

Materials and Methods

Field Study. This study was conducted to quantify the percentage of weed cover at midtillering of rice (35 days after planting [DAP]) where TSP had either been applied or not to the soil surface prior to flooding. In 2007 and 2008 the percentage of weed cover at midtillering (35 DAP) was recorded visually based on the methods of Hamill et al. (1977) in 18 fields in Colusa, Glenn, Sutter, and Butte counties in California in continuous rice production. In 2007, nine fields were selected randomly from a larger P fertility study, which included 60 fields. In 2008, all nine fields were part of a P fertility study in which sites were selected to represent the major rice growing regions in the Sacramento Valley. All fields sampled in 2008 were different from the 2007 fields. With the exception of one entisol, all soils were vertisols and included the following soil series: Lofgren-Blavo Complex (very-fine, smectitic, thermic xeric epiaquerts); Subaco Clay (fine, smectitic, thermic xeric epiaquerts); East Biggs Complex (thermic duric xerarents); Clear Lake Clay (fine, smectitic, thermic xeric endoaquerts); Esquon-Neerdobe Complex (fine, smectitic, thermic xeric duraquerts); Esquon Clay (fine, smectitic, thermic xeric epiaquerts); and Alcapay Clay (fine, smectitic, thermic sodic haploxererts). The soils had the following properties (mean, range): pH (5.5, 4.8–6.9); initial Olsen P (10.8 mg P kg^{-1} soil, 2.2–23.3 mg P kg^{-1} soil); soil organic matter (2.5%, 1.4–3.8%); sand (18.5%, 7–34%); silt (35.0%, 25–47%); and clay (46.6%, 28–60%).

All fields were managed conventionally, which included preplant disking and land planing, preplant application of fertilizer, and water-seeding by airplane into a flooded field. Seasonal herbicide, N, and water management varied between fields. Prior to flooding and seeding, two treatments were applied to 5-m by 5-m plots within growers' fields: (1) TSP[1] (25 kg P ha^{-1}) applied to the soil surface immediately prior to planting and (2) no TSP. Nitrogen[2] and potassium (K)[3] were added at rates equivalent to what the farmer had applied. Treatments within fields were arranged in randomized complete blocks with three and four replications in 2007 and 2008, respectively.

To test the effect of surface-applied calcium phosphate on weed growth, we constructed a mixed model using the PROC MIXED procedure in SAS[4] with the variance from the P treatment partitioned as a fixed effect, and the variance from the field, the field by treatment interaction, and the blocks nested within the field partitioned as random effects. Assumptions of normality and homogeneity of variance were confirmed visually via inspection of plotted residuals. Total weed cover and all the individual weed species covers with the exception of ricefield bulrush and California arrowhead (*Sagittaria montevidensis* Cham. & Schlecht.) were arcsine square root transformed in order to improve homogeneity of variance. Significantly different least-squares means were identified using the Tukey-Kramer adjustment and are presented as back-transformed (where necessary) percentages. Random effects were tested using likelihood ratio tests. There was a significant field by treatment interaction, which, as stated in the Results section, can be explained by differences in weed seed bank and herbicide regimes between fields. Despite this interaction, the P treatment effect was highly significant ($P < 0.0001$). Therefore, means are presented across fields. For individual weed species, only fields where the species was observed were considered. Therefore, the number of observations (*n*) varied from species to species.

Pot Study. The objective was to quantify placement effects of calcium phosphate and determine whether the effects differed with time and among species and morphological weed groupings. In May 2007, soils were collected from rice fields in Butte County, CA, where large populations of broadleaf, sedge, and grass weeds common to rice fields had been observed in previous growing seasons. Soils were ground to < 1-cm aggregates and mixed thoroughly in a mixer. The soil was an Esquon Clay (fine, smectitic, thermic xeric epiaquerts) and had the following properties: 25% sand, 30% silt, 45% clay, 1.9% organic matter, pH 5.2, and an initial Olsen P (Olsen and Sommers 1982) value of 22 mg P kg^{-1} soil. Prior to the start of the experiment, some of the bulk soil was flooded, and weeds were allowed to germinate and grow until easily identifiable. The presence of the following species was confirmed in the bulk soil: smallflower umbrella sedge (*Cyperus difformis* L.) ricefield bulrush [*Schoenoplectus mucronatus* (L.) Palla], waterhyssop (*Bacopa* spp. L.), blue-flowered ducksalad [*Heteranthera rotundifolia* (Kunth) Griseb.], redstem (*Ammannia coccinea* Rottb.), common waterplantain (*Alisma plantago-aquatica* L.), pale smartweed (*Polygonum lapathifolium* L.), ladysthumb (*Polygonum persicaria* L.), bearded sprangletop [*Leptochloa fusca* (L.) Kunth var. *fascicularis* (Lam.) N. Snow], barnyardgrass (*Echinochloa crus-galli* L.), and early watergrass (*Echinochloa oryzoides* L.). Subsequently, a 9-cm depth of the bulk soil was placed in cylindrical 12.7-cm by 28-cm (height by diam) plastic pots with perforated bottoms. Pots were arranged into a randomized complete block design, replicated four times.

Three treatments were superimposed: (1) surface-applied TSP (0.94 g of P as TSP[1]); (2) buried TSP (0.94 g of P as TSP applied 2.5 cm below the surface); and (3) none (no P fertilizer applied). In addition, 0.87 g $(NH_4)_2SO_4$[2] and 0.35 g KCl[5] were also applied to the soil surface to ensure that these nutrients would not limit plant growth. All rates corresponded to rates used by growers when fertilizers are surface-applied. To allow for two sampling times, each pot was divided into two equal sections by a metal divider across its diameter, preventing root exploration between the two sides of the pot.

Pots were placed into a 0.20-m (depth) by 1.20-m by 4.57-m water-tight basin and flooded gradually over the course of 24 h so that the soil surface was inundated. For 7 d the water in the basin was held approximately 1 cm above the soil surface and below the height of each individual pot. On day 7 the basin was flooded to a height of 15 cm (6 cm above the soil surface). Before plant canopies were large enough to interfere with each other, between 21 and 24 DAP, plants were counted by species or by morphological groups in one-half of the pot. Seven days later, plants were counted in the remaining half of the pot. Dry weight biomass was also measured, but because of high variability, data are not reported here. The experiment was conducted outdoors in Davis, CA, between June 29 and July 29, 2007. Average daily (high/low) temperatures were 32.1/13.2 C.

Additional soil was collected from the same fields in November 2007 in order to repeat the experiment. The soil was processed as described earlier and thoroughly mixed with the corresponding remaining soil from the 2007 experiment. The soil was stored outdoors in order to mimic winter moisture and temperature regimes. In 2008, the experiment was repeated with the following modifications. Each 28-cm-diam pot was replaced with two 14-cm-diam pots so that each replication consisted of two pots, one for each weed infestation assessment. The resulting area of the two pots in 2008 was equal to half the area of the single, divided pot used in 2007. As a result, in 2008, each pot received 25% of the fertilizer rates reported for the 2007 study. Species were identified and counted in half of the block (one of the two pots per block) 23 DAP and the other half was counted 31 DAP. The experiment was conducted outdoors between August 11 and September 10, 2008 in the same location as the 2007 experiment. Average daily temperatures (high/low) were 33.9/13.9 C.

ANOVA using PROC GLM in SAS[4] was performed on the first and second weed counts separately using a randomized complete block model with block by treatment and year by treatment interactions included in the model. Because the weed counts from the 2008 study came from half the surface area of the 2007 study, 2008 weed counts were doubled so that they could be analyzed on an equivalent per-area basis. Because there were no significant block by treatment or year by treatment interactions, weed counts from 2007 and 2008 were combined with one exception: the redstem means for the 21 to 24 DAP include data from 2007 only because in 2008, there were no redstem plants in any of the treatments until the 31 DAP count. Normality and homogeneity of variance were confirmed prior to analysis. All mean separations were performed using Tukey's Honestly Significant Difference ($P < 0.05$).

Germination Study. TSP contains 20% elemental P and 15% elemental calcium (Ca). To ascertain the effect of these elements on the germination of two aquatic sedge weeds, smallflower umbrella sedge and ricefield bulrush seeds were germinated in a controlled, laboratory environment in the presence and absence of P and Ca.

In the fall of 2007 mature smallflower umbrella sedge and ricefield bulrush seeds were collected from rice fields in Yolo, Butte, and Glenn counties in California. To meet potential vernalization requirements for germination, seeds were placed on saturated paper towels inside sealed plastic bags and stored in the dark at 4 C for at least 12 mo. Seed germination was evaluated in five solutions: 8.1 mM $CaCl_2{\cdot}2H_2O$[6], 8.1 mM $Ca[H_2PO_4]_2{\cdot}H_2O$[7], 16.1 mM KCl[4], 16.1 mM KH_2PO_4[8], and 10.8 mM $CaCl_2{\cdot}2H_2O$[6] (Table 1). The 8.1 mM $Ca[H_2PO_4]_2{\cdot}H_2O$ and 16.1 mM KH_2PO_4 solutions had equivalent P concentrations. The 8.1 mM $CaCl_2{\cdot}2H_2O$ solution and the 16.1 mM KCl solution were used as controls to match the Ca and K concentrations in the 8.1 mM $Ca[H_2PO_4]_2{\cdot}H_2O$ and 16.1 mM KH_2PO_4 solutions, respectively. Because the solutions containing K had higher osmotic potential than the solutions containing Ca, and osmotic potential has been shown to affect seed germination (Uhvits 1946), the 10.8 mM $CaCl_2{\cdot}2H_2O$ solution was used to match the osmotic pressure of the K solutions and is hereafter referred to as the osmotic control. Osmotic pressure (Π) was calculated according to Mansoor and Sandmann (2002). The pH of each solution was determined analytically using a pH probe and meter.[9] Because the pH of the osmotic control matched that of the K solutions, the osmotic control also acted as a control for pH between the Ca and K solutions (see Table 1 for a complete summary of the treatment solutions).

An aerobic environment is ideal for smallflower umbrella sedge germination, whereas an anaerobic environment is ideal for ricefield bulrush (A. J. Fischer, personal communication). For smallflower umbrella sedge, 3.5-cm-diam filter papers[10] were placed in 3.5-cm-diam clear plastic wells[11] and saturated with 0.7 ml of the treatment solutions in a randomized

Table 1. Summary characteristics of the solutions employed in the germination study, including P, Ca, and K concentrations, and the osmotic potential[a] and pH of each solution.[b]

Treatment solution	Concentration P[b]	Ca	K	Osmotic potential[a]	pH
	ppmv	mM	mM	−kPa	
Calcium chloride	0	8.1	0	59.6	4.9
Calcium phosphate	500	8.1	0	59.6	4.5
Potassium chloride	0	0.0	16.1	79.1	5.1
Potassium phosphate	500	0.0	16.1	79.1	5.0
Calcium chloride	0	10.8	0	79.1	5.1

[a] Expressed as osmotic pressure (Π) with a negative sign, which was calculated according to Mansoor and Sandmann (2002).

[b] Abbreviations: P, phosphorus; Ca, calcium; K, potassium; ppmv, parts per million by volume.

complete block design replicated three times. Between 56 and 120 smallflower umbrella sedge seeds were placed in each well. The range of smallflower umbrella sedge seeds per replication was large because the seeds were too small to be manipulated easily. For ricefield bulrush, 25 seeds were placed into each well. Treatment solutions (3.5 ml) were added to the wells in a randomized complete block design replicated 5 times. The seeds were completely submerged. Wells for both species were placed in a 21.7 C growth chamber[12] with 14.5 h daylight at 500 μmol m^{-2} s^{-1} photosynthetic photon flux density delivered by a mixture of incandescent and fluorescent lights.

The number of germinated seeds was counted at the same time each day throughout the experiment, and treatment solutions were added to keep either the filter paper visibly saturated (in the case of smallflower umbrella sedge) or the seeds submerged (in the case of ricefield bulrush). Throughout the entirety of the experiment a total of 6.7 ml of treatment solution was added to each smallflower umbrella sedge well and 7.5 ml of treatment solution was added to each ricefield bulrush well. The experiment ended after four consecutive days with no observed increases in germination (25 d from the start of the experiment for smallflower umbrella sedge and 15 d from the start of the experiment for ricefield bulrush).

ANOVA was performed on smallflower umbrella sedge and ricefield bulrush separately using the PROC MIXED procedure in SAS[4] to test for the germination effect ($P < 0.05$) of the treatment solution, block, day, and their interactions. Homogeneity of variance was confirmed visually via inspection of plotted residuals. To correct for a significant block effect in smallflower umbrella sedge germination, data were rescaled relative to the maximum germination in each block. Subsequently, a three-parameter sigmoid function,

$$G = G_{max}/(1 + \exp[-(x - T_{50})/G_{rate}]) \quad [1]$$

(Chauhan and Johnson 2009), was fit to data using the PROC NLIN procedure in SAS[4], where G is the cumulative percentage of germination at time x, G_{max} is the maximum germination (%), T_{50} is the time (days) required to reach 50% of G_{max}, and G_{rate} is the slope at T_{50}. Modeled germination was compared between treatment solution combinations representing different P, Ca, K, and Π levels using a lack-of-fit F-test. Where significant differences in modeled germination existed, parameter estimates were compared using a Z-test.

Results and Discussion

Field Study. The field study confirmed our preliminary 2006 observations in calcium phosphate–treated field plots. By 35 DAP there was greater weed cover among several sedge and broadleaf species, including smallflower umbrella sedge, ricefield bulrush, waterhyssop, blue-flowered ducksalad, redstem, and California arrowhead when surface TSP was applied compared to no TSP application (Table 2). Across all observations, the total weed cover for surface-applied TSP was 61% greater than the control ($P < 0.05$). Importantly, rice in this experiment did not respond to P input (data not shown), ensuring that the weed response to surface-applied calcium phosphate was not simply due to a P-limited environment.

Because weed seed bank and control via herbicides were variable from field to field, the proportion of fields containing a given species and the absolute mean percentage of cover are confounded by these management factors. Nevertheless, the prevalence of smallflower umbrella sedge and ducksalad (Table 2) are notable because these species were also the most abundant in the more controlled pot study (discussed later). Of further note is that, with the exception of ricefield bulrush, all weeds that responded to surface-applied TSP are relatively small in seed size. Seibert and Pearce (1993) demonstrated that the small-seeded weeds redroot pigweed

Table 2. Percentage of weed cover at midtillering of rice for a field study comparing plots with or without surface applied TSP.[a]

	Cover at 35 DAP[a] Smallflower umbrella sedge	Ricefield bulrush	Waterhyssop	Blue-flowered ducksalad	Redstem	California arrowhead	Total
	%						
TSP placement							
Surface	14.0 a[b]	23.3 a	6.9 a	8.3 a	2.9 a	10.7 a	23.4 a
None	10.7 b	11.7 b	1.3 b	4.4 b	1.6 a	5.3 a	14.5 b
Proportion of fields	0.44	0.06	0.67	0.44	0.22	0.17	1

[a] Abbreviation: TSP, triple superphosphate; DAP, days after planting.

[b] Significantly different least square means ($P < 0.05$) were identified using the Tukey-Kramer adjustment and are indicated by different lowercase letters.

Table 3. Weed populations over time as influenced by TSP placement in a pot study. Data is averaged across 2 yr for each species or morphological grouping, except redstem 21 to 24 DAP.[a]

TSP placement	Weed count[b]							
	Smallflower umbrella sedge	Ricefield bulrush	Waterhyssop	Blue-flowered ducksalad	Redstem[c]	Other broadleaf[d]	Grass[e]	Total
	21 to 24 DAP							
Surface	79 a[f]	22 a	29 a	103 a	22 a	68 a	2 a	325 a
Buried	50 b	19 a	25 a	75 b	9 ab	54 a	2 a	234 b
None	51 b	17 a	22 a	68 b	5 b	52 a	1 a	216 b
	28 to 31 DAP							
Surface	40 a	18 a	49 a	92 a	28 a	73 a	2 a	302 a
Buried	21 b	8 b	34 b	71 a	12 b	54 a	2 a	202 b
None	25 ab	13 ab	29 b	61 a	13 b	51 a	1 a	193 b

[a] Abbreviations: TSP, triple superphosphate; DAP, days after planting.

[b] Plants 306 cm^{-2}.

[c] 2007 only for 21 to 24 DAP.

[d] Includes pale smartweed, ladysthumb, and waterplantain.

[e] Includes bearded sprangletop, barnyardgrass, and early watergrass.

[f] Significantly different means ($P < 0.05$) were identified using Tukey's HSD and are indicated by different lowercase letters.

and common lambsquarters have greater relative growth rates than the larger-seeded weeds velvetleaf (*Abutilon theophrasti* Medik.) and common cocklebur (*Xanthium strumarium* L.) and the crops sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* L.). They also showed that these small-seeded weeds develop relatively longer and smaller-diameter roots, which enable them to explore a greater soil volume early in their life cycle. In our experiment, the high concentrations of P and Ca at the soil-water interface created by surface applications of calcium phosphate would be advantageous to small-seeded weeds with adaptations similar to those species studied by Siebert and Pearce (1993). That the response to TSP was predominantly found among small-seeded aquatic weeds suggests that seed size may be a determining variable on the effect of calcium phosphate on seed germination, a hypothesis further supported by the results of our pot and germination studies (see below).

Pot Study. In the pot study, by 21 to 24 DAP, there were more smallflower umbrella sedge and blue-flowered ducksalad plants with surface TSP than with either buried TSP or the control (Table 3). There were also more redstem plants in surface TSP than in the control (Table 3). Furthermore, the total number of weeds in surface TSP was 39% higher than in buried TSP, and 51% greater than in the control ($P < 0.05$).

With surface-applied TSP, populations of smallflower umbrella sedge and redstem were still greater by 28 to 31 DAP, and by this time ricefield bulrush and waterhyssop plants also became more abundant with this treatment compared to either buried TSP or the control (Table 3). The total number of weeds with surface TSP was 50% larger than with the buried TSP, and 57% greater than the control ($P < 0.05$). Weed counts in buried TSP and the control were not statistically different at either 21 to 24 DAP or 28 to 31 DAP. That there was no greater emergence in the buried TSP than the control indicates that the stimulatory effect of calcium phosphate may be confined to weed seeds located near the soil surface or in the early stages of growth. Where P is not limiting to rice growth, a spike in Ca and P concentration at the soil surface would tend to benefit r-selected weed species whose growth requirements and reproductive strategies favor the early utilization of nutrients more so than rice (Pandey et al. 1971). This result supports similar findings by Santos et al. (2004), who suggested that burying P fertilizer below the soil surface (i.e., a banded application) reduces weed growth compared to surface (i.e., broadcast) applications.

Smallflower umbrella sedge and redstem were the only species with increased densities in response to surface-applied TSP at both 21 to 24 DAP and 28 to 31 DAP, although the smallflower umbrella sedge response was greater during the first count and redstem was greater during the second. Between the first and second counts, many smallflower umbrella sedge and blue-flowered ducksalad plants perished. This mortality is evident in the decreased plant counts for these species at 28 to 31 DAP and is likely due to the increasing inter- and intraspecies competition between the first and second counts. Weed densities and diversity were high in the pot experiments both because weeds were completely uncontrolled and because rice soils were taken from sites selected based on their unusually high historical weed populations.

Because our methods did not create an equivalent seed bank for each weed species, comparing the absolute number of weeds between species would be an overextension of the data. However, it is worth noting that smallflower umbrella sedge and blue-flowered ducksalad were between two and three times more abundant than the other species observed during the first count. Blue-flowered ducksalad was at least twice as abundant as any other species during the second count. These species were among the most prominently observed in the field study, and their strong, early response to surface-applied calcium phosphate supports the idea that P fertilizer placement has a relevant role to play in rice weed management in these areas.

Germination Study. Results from the pot study indicated that surface-applied calcium phosphate had a stimulatory effect upon the emergence densities of certain weed species.

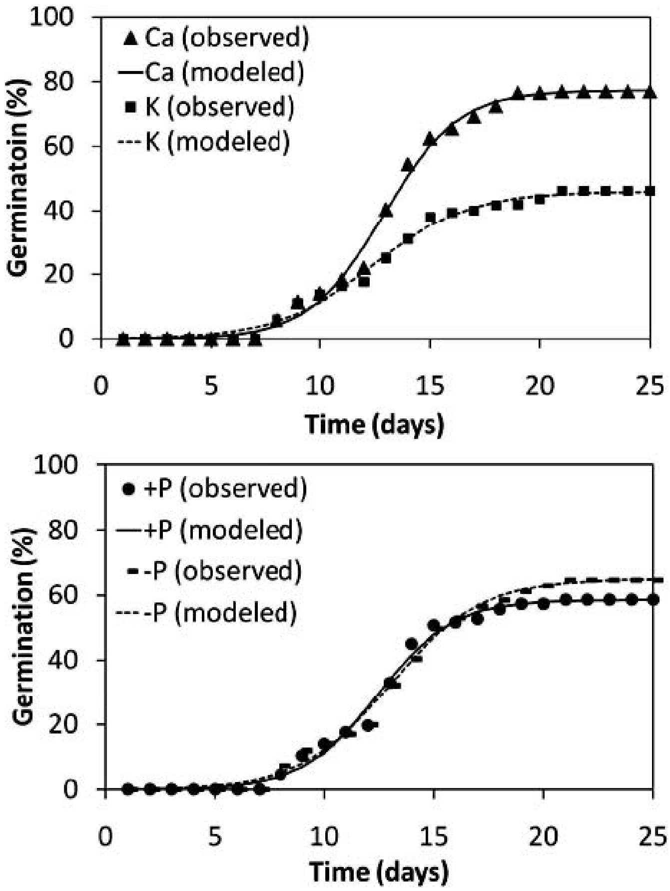

Figure 1. Effect of calcium (Ca), potassium (K), and phosphorus (P) on the germination of smallflower umbrella sedge. A three-parameter sigmoid model was fit to the data. See Table 4 for a presentation of the parameter estimates.

To explore if this effect was based on a direct stimulatory effect upon seed germination, we tested whether P or Ca affect germination, and whether this effect is species dependent. Smallflower umbrella sedge and ricefield bulrush are aquatic sedges with similar morphologies and environmental adaptations. However, they responded differently to surface-applied calcium phosphate in the pot studies. Smallflower umbrella sedge had a clear, early response to the surface TSP, whereas the differences in ricefield bulrush counts were only apparent at the later counting time and were probably attributable to higher mortality in the buried TSP and control treatments as compared to the surface TSP (Table 3). We thus chose to compare germination responses by smallflower umbrella sedge and ricefield bulrush in this study.

Calcium stimulated smallflower umbrella sedge germination relative to K ($P < 0.0001$) (Figure 1; Table 4). Additionally, there was no difference between smallflower umbrella sedge germination in the osmotic control and the Ca treatments whereas, the osmotic control and the K treatment were significantly different ($P < 0.0001$) (Table 4), which confirms that germination differences were due to the presence of Ca and not the result of osmotic pressure and pH differences between the Ca and K treatments (Table 1). Comparing parameter estimates between the Ca and K treatments, only the maximum germination was significantly different ($P < 0.0001$), indicating that Ca increased the overall germination of smallflower umbrella sedge, but not the rate of germination (Table 4). In contrast to smallflower umbrella sedge, Ca did not stimulate ricefield bulrush germination relative to K ($P = 0.09$) (Figure 2; Table 4). Finally, there was no effect of P on the germination of either smallflower umbrella sedge ($P = 0.55$) (Figure 1; Table 4) or ricefield bulrush ($P = 0.19$) (Figure 2; Table 4).

Plants exhibit differential responses to Ca availability, and the effect of Ca depends not only on the Ca content in a particular soil but also on the broader characteristics of the soil exchange complex and the distribution and associated adaptations (i.e., calcifuge, calcicole) of the plant itself (Rorison and Robinson 1984). Calcium has been shown to increase the germination of nonhalophytic plants (Tobe et al. 2003) and the early root growth of cotton (*Gossypium hirsutum* L.) (Kent and Lauchli 1986) by alleviating salt toxicity. Additionally, Hartley (2006) reported increases in ricefield bulrush germination in the presence of $CaCO_3$ and $CaSO_4$ as compared to $MgCO_3$ and $MgSO_4$, though these results more likely show an inhibitory effect resulting from the presence of Mg rather than a stimulatory effect of Ca. We are unaware of reports showing that Ca per se stimulates weed germination in the field. However, several studies support our experimental result. Calcium is an important secondary messenger (Klimecka and Muszynska 2007; Sanders et al.

Table 4. Results of nonlinear regression utilizing the three-parameter sigmoid model to describe seed germination of two weed species as influenced by selected solutions. G_{max} = percentage of maximum germination; T_{50} = days until 50% germination; and G_{rate} = slope at T_{50}. See Figures 1 and 2 for graphical presentations.

	Smallflower umbrella sedge				Ricefield bulrush			
	Parameter estimates (SE)				Parameter estimates (SE)			
Treatment	G_{rate}	T_{50}	G_{max}	R^2	G_{rate}	T_{50}	G_{max}	R^2
		d	%			d	%	
− P[a]	2.03 (0.42)	13.03 (0.50)	65.0 (3.2)	0.85	0.48 (0.05)	8.38 (0.06)	95.3 (1.5)	0.96
+ P	1.69 (0.44)	12.45 (0.52)	58.6 (3.1)	0.80	0.51 (0.06)	8.21 (0.07)	91.7 (1.7)	0.95
Ca	1.67 (0.26)	12.89 (0.30)	77.3 (2.5)	0.92	0.47 (0.06)	8.19 (0.06)	93.5 (1.7)	0.95
K	2.16 (0.55)	12.42 (0.66)	45.9 (2.9)	0.78	0.52 (0.05)	8.42 (0.06)	93.7 (1.5)	0.96
Π control[b]	2.04 (0.18)	13.12 (0.21)	79.2 (1.7)	0.98	0.40 (0.09)	8.18 (0.08)	92.5 (2.4)	0.95

[a] Abbreviations: P, phosphorus; Ca, calcium; K, potassium.

[b] Corrected for differences in osmotic potential between Ca and K treatments (see Table 1).

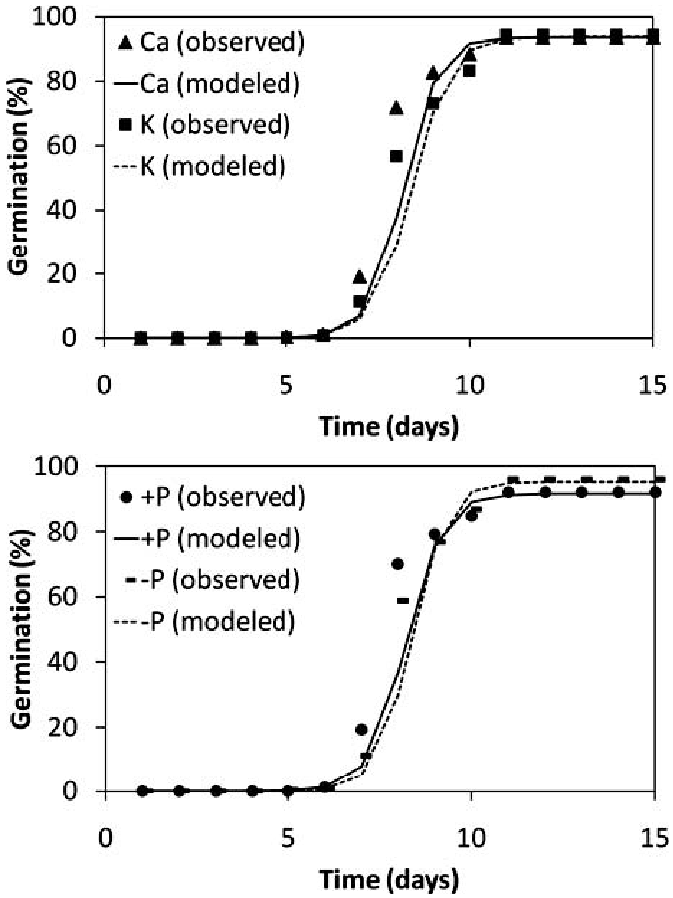

Figure 2. Effect of calcium (Ca), potassium (K), and phosphorus (P) on the germination of ricefield bulrush. A three-parameter sigmoid model was fit to the data. See Table 4 for a presentation of the parameter estimates.

2002), and elevated extracellular concentrations of Ca^{2+} have been shown to activate calcium-dependent protein kinases (Ludwig et al. 2004; Takahashi et al. 1997). Kinases are involved in a variety of signaling events including germination, embryogenesis, and phospholipid synthesis (Anil et al. 2000; Anil and Rao 2001; Kiselev et al. 2008). Thus, in flooded rice where surface applications of calcium phosphate elevate the Ca and P concentration at the soil–water interface, the increased extracellular Ca may help activate germination, embryogenesis, or both in certain weed species. Moreover, the abundance of P in the same location ensures that phospholipid synthesis essential for early growth is not P-limited.

Although we did not test the effect of seed size in our study, because smallflower umbrella sedge germination was affected by the presence of Ca, whereas germination by the larger-seeded ricefield bulrush was unaffected, our findings would agree with Seibert and Pearce's (1993) suggestion that small-seeded weeds have early growth adaptations relative to larger-seeded weeds and crops. It should be noted, however, that although ricefield bulrush germination was not affected by either P or Ca in the germination study, it nevertheless responded to surface applications of calcium phosphate in both the field and pot studies. This indicates that a Ca-induced germination effect may not be the only mechanism at play and could, in fact, work in combination with a growth-promoting effect by the added nutrients. Tamura et al. (2001) demonstrated that Ca stimulates arrowhead (*Sagittaria pygmaea* Miq.) shoot elongation under anaerobic conditions. Also, several studies have shown a plant growth effect on weeds as a result of P fertilization. Blackshaw et al. (2004) used KH_2PO_4 to demonstrate that P does increase growth in isolation of Ca. Finally, additions of calcium phosphate have been shown to increase cattail (*Typha domingensis* Pers.) germination (Miao et al. 2001) and corn spurry (*Spergula arvensis* L.) emergence (Freyman et al. 1989). Taken together, our results and previous studies demonstrate that the Ca and P present in calcium phosphate fertilizers stimulate both germination and growth of selected weed species. When concentrated at the soil–water interface of a flooded rice field, Ca and P increased sedge and broadleaf densities early in the season, which would exacerbate weed competition with the crop for light and nutrients as the season progresses (Gibson et al. 2002, 2003).

In summary, we hypothesized that surface applications of P fertilizers stimulate weed growth in flooded rice systems. Based on our results, a more accurate hypothesis would be that P fertilizers containing Ca stimulate aquatic sedge and broadleaf weeds by a combination of germination and growth effects. Indeed, although studies have shown that Ca and P stimulate weed growth in isolation from each other (Blackshaw et al. 2004; Tamura et al. 2001), most of the studies reporting weed germination and growth responses to P have used calcium phosphates as their source of P fertilization (Andreasen et al. 2005; Freyman et al. 1989; Hoveland et al. 1976; Miao et al. 2001; Vengris et al. 1955). The effect of Ca on seed germination and growth depends on a number of environmental variables, and Ca has been shown to suppress growth in some environments (Rorison and Robinson 1984). However, our results indicate that weed density responses to calcium phosphate may be due to a combined effect of Ca and P on the germination and growth of weeds and not just a P response as reported earlier.

Of all the weeds studied, smallflower umbrella sedge alone demonstrated a clear response to calcium phosphate in all three studies. In order to alleviate early-season weed pressure on water-seeded and flooded rice, efforts should be made to avoid surface applications of fertilizers containing calcium phosphate in fields heavily infested with smallflower umbrella sedge and other aquatic sedge and broadleaf weeds. Alternatively, rice systems using a stale-seedbed technique for weed control may be able to use surface-applied calcium phosphate to stimulate weed germination and growth prior to glyphosate application.

Sources of Materials

[1] Triple superphosphate (0–45–0), J.R. Simplot Company, 999 Main St., Suite 1300, Boise, ID 83702.

[2] Ammonium sulfate (21–0–0), Yara International ASA, P.O. Box 2464, Solli, Bygdøy allé 2, N-0202 Oslo, Norway.

[3] Potassium sulfate (0–0–50), Yara International ASA, P.O. Box 2464, Solli, Bygdøy allé 2, N-0202 Oslo, Norway.

[4] SAS System for Windows, Release 9.1, SAS Institute Inc., P.O. Box 8000, Cary, NC 27512.

[5] Potassium chloride, enzyme grade, Fisher Scientific, Fair Lawn, NJ 07410.

[6] Calcium chloride dihydrate, EMD Chemicals Inc., 480 S. Democrat Rd., Gibbstown, NJ 08027.

[7] Calcium phosphate monobasic, monohydrate, reagent grade, Mallinckrodt Baker Inc., Phillipsburg, NJ 08865.

[8] Potassium phosphate monobasic, laboratory grade, Fisher Scientific, Fair Lawn, NJ 07410.

[9] Orion Ross Sureflow pH probe, Orion 3 Star Benchtop pH meter, Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02454.

[10] Filter paper No. 161, 3.5-cm diam, VWR International, LLC, Bayshore Blvd., Brisbane, CA 94005.

[11] Falcon 6 well multiwall, Becton Dickinson Labware, Franklin Lakes, NJ 07417.

[12] Model PGW108, Number 3990.11, Percival Scientific, 505 Research Drive, Perry, IA 50220.

Acknowledgments

The authors would like to thank University of California Agriculture and Natural Resources and the California Rice Research Board for funding this research. Our gratitude as well to James Eckert, Louis Boddy, and Jennifer Williams for their assistance in weed identification, and to Cesar Abrenilla, Chisom Ofodire, Ravi Nakrani, Kirsten Sellheim, and Timothy Doane for their assistance in the laboratory and field.

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Received December 18, 2009, and approved April 7, 2010.