

# Fludioxonil + Mefenoxam Seed Treatment Improves Edamame Seedling Emergence

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ADDITIONAL INDEX WORDS. *Glycine max*, vegetable soybean, fungicide, soilborne pathogens

**SUMMARY.** Poor crop establishment is a major problem in edamame (*Glycine max*), a specialty type of soybean produced in locations throughout the United States. The objective of this research was to quantify the extent to which seed treatment with fludioxonil + mefenoxam improves seedling emergence of edamame. Emergence characteristics of fludioxonil + mefenoxam-treated and nontreated seed of 30 cultivars were characterized in field conditions over 2 years. Edamame cultivars used in the study exhibited poor field emergence in a previous study despite high germinability. Seed treatment with fludioxonil + mefenoxam at 2.5 and 3.75 g/100 kg seed, respectively, improved crop emergence 33% to 47% more than the nontreated control. The emergence rate (days to 50% emergence) was improved the most by the seed treatment for several cultivars that were generally slow to emerge. Crop establishment is essential for further development of domestic edamame production. Seed treatment with fludioxonil + mefenoxam, at the rate currently registered for use on grain-type soybean, offers one approach to help vegetable growers improve edamame seedling emergence.

Edamame, also known as vegetable soybean, is a specialty type of soybean harvested for human consumption at an immature seed stage. Edamame produces large seeds (>20 g/100 seeds) that have a sweet, nutty flavor. Traditionally a crop produced and consumed in East Asia, edamame has grown in popularity among American consumers (Sams et al., 2012). Certain barriers to domestic edamame production have been removed recently, such as the registration of several herbicides for preemergence and post-emergence weed control (Williams and Nelson, 2014). Nonetheless, research and development to improve edamame production pales in comparison with grain-type soybean, for which the United States cultivates on some 83 million acres [U.S.

Department of Agriculture (USDA), 2016].

Poor crop establishment is a major problem in edamame. Poor seedling emergence and uneven crop stands appear to be the norm in field trials, from North Dakota (Duppong and Hatterman-Valenti, 2005) to Georgia (Rao et al., 2002), in Illinois (Williams, 2015b) and Pennsylvania (Sánchez et al., 2005). Using 136 edamame entries over a 3-year period, high germination of seed lots failed to account for poor seedling emergence (Williams, 2015b). Because of limited research, edamame is often grown using agronomic practices employed in grain-type soybean production, with limited knowledge of the appropriateness of such approaches (Williams, 2015a; Zhang et al., 2013).

Seedling diseases of soybean caused by soilborne fungal and oomycete pathogens, such as *Fusarium* species, *Phytophthora sojae*, *Pythium* species, and *Rhizoctonia solani*, are not uncommon and caused estimated

annual yield losses averaging 11,749,000 Mg in North America grain-type soybean between 2010 and 2014 (Allen et al., 2017). Seedling diseases can lead to poor crop establishment in soybean and can be managed with fungicide seed treatments (Bradley, 2008; Dorrance and McClure, 2001; Dorrance et al., 2003). Fungicides with broad-spectrum activity on fungi and oomycetes provide the best level of seedling disease management. A fungicide that has been commonly used as a seed treatment on soybean with activity against *Fusarium* species and *R. solani* is fludioxonil (Broders et al., 2007b; Dorrance et al., 2003; Munkvold and O'Mara, 2002). Mefenoxam or the related compound metalaxyl has often been used as a seed treatment on soybean to manage seedling diseases caused by oomycete pathogens *P. sojae* and *Pythium* species (Broders et al., 2007a; Dorrance and McClure, 2001; Dorrance et al., 2004). A mixture of fludioxonil + mefenoxam is currently registered for use on grain-type soybean as well as several other legume crops.

The objective of this research was to quantify the extent to which seed treatment with fludioxonil + mefenoxam improves seedling emergence of edamame.

## Materials and methods

**GERMPLASM.** Edamame cultivars were obtained from two public and four private sources (Table 1). Cultivars were selected from a list of 136 entries tested in a previous research (Williams, 2015b), which showed high seed germination (up to 99%) in controlled conditions, yet inadequate (<50%) seedling emergence in the field. In addition, a widely used grain-type cultivar, Asgrow AG-3253 (Monsanto, St. Louis, MO), was included as a control.

**EXPERIMENTAL APPROACH.** Field experiments were conducted at the

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## Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
1.1209	lb/acre	kg·ha <sup>-1</sup>	0.8922
28.3495	oz	g	0.0353
62.5000	oz/100 lb	g/100 kg	0.0160
0.9072	ton(s)	mg	1.1023
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

**Table 1. Soybean cultivar, type, 100-seed weight, germination, and seed source of germplasm used in field emergence experiments.**

Cultivar	Type	100-seed wt (g) <sup>z</sup>	Germination (%)	Source
Asgrow AG-3253	Grain	18.6	99.5	Monsanto
Agate	Edamame	25.2	65.0	USDA Soybean Germplasm Collection
AGS292	Edamame	23.4	71.5	Washington State University, Pullman
Asmara	Edamame	16.9	91.5	USDA Soybean Germplasm Collection
Bellesoy	Edamame	8.9	86.5	Wannamaker Seeds, Saluda, NC
BeSweet 2015	Edamame	30.9	95.0	Rupp Seeds, Wauseon, OH
BeSweet 292	Edamame	31.7	93.5	Rupp Seeds
Bukers Favorite	Edamame	26.7	86.5	Rupp Seeds
Butterbean	Edamame	17.4	95.5	Johnny's Selected Seeds, Winslow, ME
Early Hakucho	Edamame	22.1	97.5	USDA Soybean Germplasm Collection
Eda Mame Uase Chaurame	Edamame	21.0	98.5	USDA Soybean Germplasm Collection
Fiskeby III	Edamame	19.2	95.5	USDA Soybean Germplasm Collection
Fuji	Edamame	14.2	65.5	USDA Soybean Germplasm Collection
Gardensoy 01	Edamame	23.6	95.0	University of Illinois Urbana-Champaign
Gardensoy 21	Edamame	26.8	97.5	University of Illinois Urbana-Champaign
Gardensoy 23	Edamame	19.4	92.0	University of Illinois Urbana-Champaign
Gardensoy 32	Edamame	26.0	95.0	University of Illinois Urbana-Champaign
Gardensoy 41	Edamame	32.7	95.5	University of Illinois Urbana-Champaign
Gardensoy 43	Edamame	19.8	93.0	University of Illinois Urbana-Champaign
Kaohsiung 1	Edamame	27.8	88.5	USDA Soybean Germplasm Collection
Kegon	Edamame	25.0	95.0	USDA Soybean Germplasm Collection
Komagi dadacha	Edamame	20.4	84.5	USDA Soybean Germplasm Collection
Miyagi shirome	Edamame	17.1	96.5	USDA Soybean Germplasm Collection
Okuhara daizu	Edamame	22.2	95.0	USDA Soybean Germplasm Collection
Randolph	Edamame	20.2	86.0	USDA Soybean Germplasm Collection
Taiwame	Edamame	31.2	87.0	Evergreen Seed
Tambagura	Edamame	22.9	77.5	USDA Soybean Germplasm Collection
Ware	Edamame	17.2	95.5	USDA Soybean Germplasm Collection
Wase shiroyama dadacha (A)	Edamame	22.7	94.0	USDA Soybean Germplasm Collection
Yi wo liang	Edamame	23.7	91.0	USDA Soybean Germplasm Collection

<sup>z</sup>1 g = 0.0353 oz.

University of Illinois Vegetable Crop Farm near Urbana, IL, in 2014 and 2015. The soil was a Flanagan silt loam (fine, smectitic, and mesic Aquic Argiudolls) averaging 3.1% organic matter and a pH of 6.3. Experiments followed the soybean year of a sweet corn (*Zea mays*)-soybean rotation. Before planting, fields were prepared using two passes of a field cultivator. Planting dates were 21 May 2014 and 27 May 2015. Planting depth was 1.2 inches. Within 1 d of planting, S-metolachlor was applied at a rate of 1.6 lb/acre across the experimental site for pre-emergence weed control.

The treatment design was a factorial arrangement of cultivars and seed treatments. The seed treatment factor had two levels: none and treatment with fludioxonil + mepanipyrim at the rate currently registered for use on grain-type soybean. Seeds were treated with fludioxonil + mepanipyrim (ApronMaxx RTA; Syngenta Crop

**Table 2. The main effect of seed treatment (fludioxonil + mepanipyrim at 2.5 and 3.75 g/100 kg seed, respectively) on soybean emergence at 1, 2, and 3 weeks after planting (WAP) across 29 edamame cultivars and one grain-type cultivar.<sup>z</sup>**

Seed treatment	1 WAP	2 WAP	3 WAP
	Emergence (%)		
None	9 b <sup>y</sup>	38 b	42 b
Fludioxonil + mepanipyrim	13 a	51 a	57 a

<sup>z</sup>1 g/100 kg = 0.0160 oz/100 lb.

<sup>y</sup>Means within a column followed by a different letter are different based on the type 3 test of fixed effects ( $P < 0.05$ ).

Protection, Greensboro, NC) at a rate of 5.0 fl oz/100 lb seed (fludioxonil + mepanipyrim at 2.5 and 3.75 g/100 kg seed, respectively).

The experimental design was a split plot with three replications. Cultivars were assigned to main plots consisting of two rows planted with 50 seeds/row over a length of 8.0 ft. The levels of seed treatment were applied to single-row subplots, such that treated and nontreated seed of each cultivar were adjacent to one another in each replicate.

**DATA COLLECTION.** Weight of 100 seeds of each cultivar and germinability were determined before planting. A total of 100 seeds per cultivar were incubated on a distilled water-moistened germination paper at 70 °F and 90% relative humidity. Germinated seedlings were counted and removed daily until germination ceased within 10 d. In field trials, a seedling was considered emerged when its cotyledons were above-ground and completely free of soil surface contact. Emergence counts were

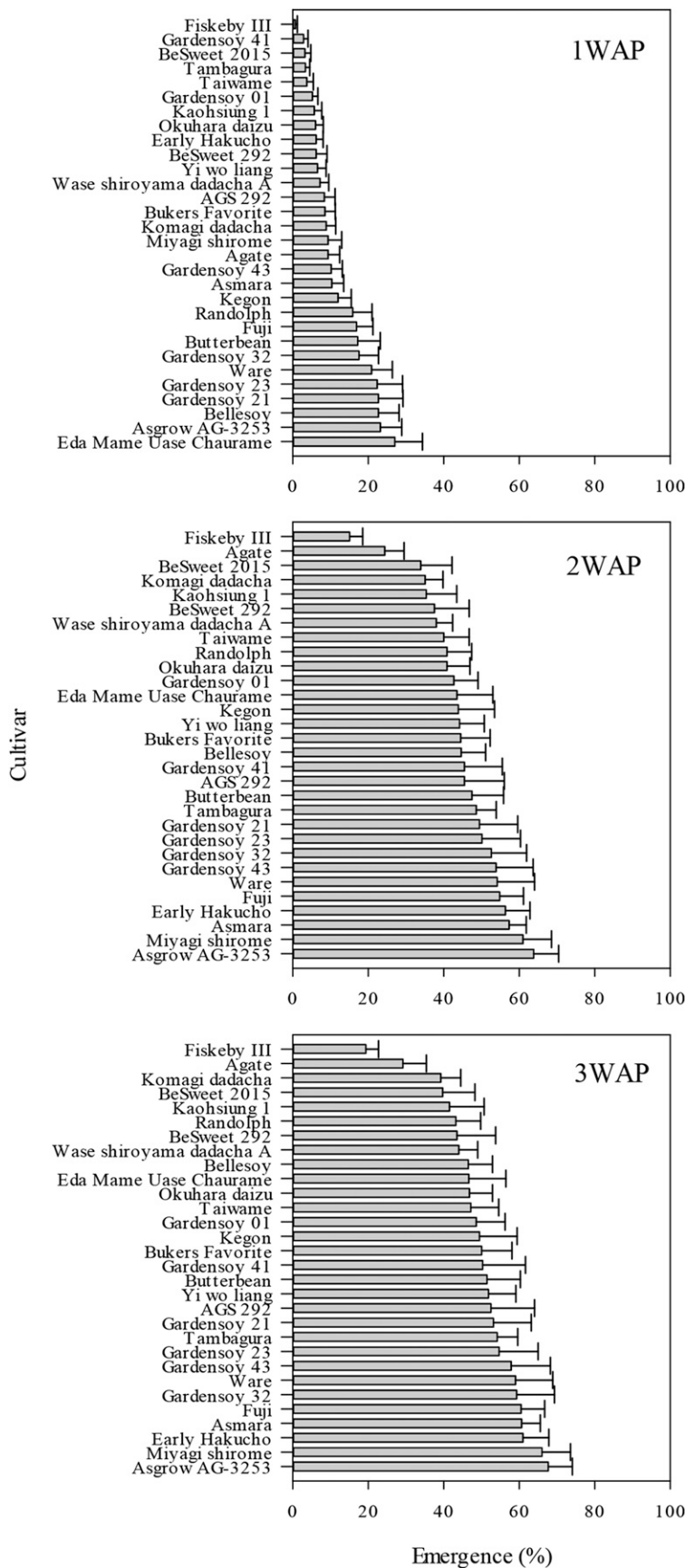


Fig. 1. The main effect of soybean cultivar (29 edamame cultivars and one grain-type cultivar) on emergence at 1, 2, and 3 weeks after planting (WAP). Error bars are SE.

made daily until one-half of planted seeds emerged. In addition, emerged seedlings were counted 1, 2, and 3 weeks after planting (WAP). No seedlings emerged 3 WAP. These data were used to determine time from planting to 50% emergence.

**DATA ANALYSIS.** The visual examination of residuals indicated crop emergence 2 and 3 WAP met assumptions of homoscedasticity and normality after arc-sine transformation, whereas other response variables met analysis of variance assumptions without transformation. To determine the effect of cultivar, seed treatment, and their interaction on crop emergence, data were analyzed using the mixed procedure of SAS (version 9.3; SAS Institute, Cary, NC). Fixed effects included cultivar, seed treatment, and their interaction. Random effects included year and replicate nested within year. Nontransformed means and standard errors are presented for ease of interpretation. In addition, linear associations between crop response variables were quantified using Bonferroni-corrected correlation coefficients. Analyses were declared significant at  $P < 0.05$ .

**Results**

**ENVIRONMENTAL CONDITIONS.** Postplanting weather was a characteristic of the variable growing conditions in the midwestern U.S. The 2014 trial experienced suboptimal edaphic conditions during crop emergence. Soil crusting was observed, likely the result of 1.0 inch of rainfall occurring in a short period immediately after planting, followed by conditions favoring rapid evapotranspiration with no additional rainfall for 1 week (data not shown). By contrast, the 2015 trial experienced favorable conditions for several weeks after planting. After planting into a moist seedbed, rainfall occurred 4 d after planting and on a regular basis for 3 weeks. As a result, the soil surface largely remained moist and loose during the study.

**CROP EMERGENCE.** Soil conditions in 2014 likely delayed crop emergence compared with 2015. For instance, average emergence 2 WAP was 24% and 66% in 2014 and 2015, respectively. Nonetheless, rank order of cultivar emergence was relatively consistent, with the grain-type cultivar (Asgrow AG-3253) and edamame cultivar Miyagi shirome

(USDA Soybean Germplasm Collection, Urbana, IL) among the highest emerging cultivars, whereas edamame cultivars Agate and Fiskeby III (USDA Soybean Germplasm Collection) were among the lowest emerging cultivars (data not shown).

Seed treatment affected crop emergence both years at all sampling times ( $P < 0.001$ ). Seed treatment with fludioxonil + mefenoxam improved crop emergence 33% to 47% more than the nontreated control. For instance at 3 WAP, the nontreated control averaged 42% crop emergence compared with 57% emergence in the fludioxonil + mefenoxam treatment (Table 2).

The main effect of cultivar also affected crop emergence at all sampling times ( $P < 0.001$ ). The grain-type

cultivar Asgrow AG-3253 was among the highest emerging cultivars tested, averaging 23%, 64%, and 68% emergence at 1, 2, and 3 WAP (Fig. 1). By contrast, 'Fiskeby III' was the lowest emerging cultivar, averaging 1%, 15%, and 19% emergence at 1, 2, and 3 WAP.

Cultivar and seed treatment had an interactive effect only on emergence rate ( $P = 0.004$ ). Most early-emerging cultivars (e.g., less than 9 d to 50% emergence) showed similar emergence rates between treated and nontreated seed (Fig. 2). However, several later-emerging cultivars benefited from seed treatment with fludioxonil + mefenoxam. For example, time to reach 50% emergence was 17 d for nontreated seed of edamame cultivar Taiwame (Evergreen Seed,

Anaheim, CA), whereas only 10 d were needed for treated Taiwame seed.

Most response variables were associated. Weight of 100 seeds was negatively correlated with emergence 1 WAP [correlation coefficient ( $\rho$ ) =  $-0.23$ ] and 2 WAP ( $\rho = -0.52$ ), yet positively correlated ( $\rho = 0.40$ ) with time to 50% emergence (Table 3). Germination was positively correlated with emergence 2 ( $\rho = 0.32$ ) and 3 WAP ( $\rho = 0.30$ ). Time to 50% emergence was negatively correlated with germination and emergence at all three sampling times ( $\rho \leq -0.32$ ). Weight of 100 seeds was not associated with germination.

## Discussion

Emergence of most edamame cultivars was poorer than the grain-type control. Williams (2015b) reported similar findings in a survey of soybean germplasm, whereby mean emergence of 136 edamame lines was 11% to 24% lower than 14 grain-type entries. The grain-type control used in this study, 'Asgrow AG-3253', is adapted for use in Illinois and has genes conferring resistance to soybean cyst nematode [*Heterodera glycines* (gene *MR3*)] and phytophthora root and stem rot [caused by *P. sojae* (gene *Rps1c*)] (Monsanto, 2015). By contrast, the extent to which these edamame cultivars are resistant to soil pathogens or adapted to the location is unknown.

Larger-seeded cultivars generally took longer to emerge and had lower emergence than smaller-seeded cultivars. Factors driving such a response have not been elucidated. Generally, grain-type cultivars have seed weight  $<20$  g/100 seeds, and under these conditions, "large" seed have exhibited greater seedling vigor than "small" seed (Burris et al., 1971; Place et al., 2011), even under simulated soil compaction (Longer et al., 1986).

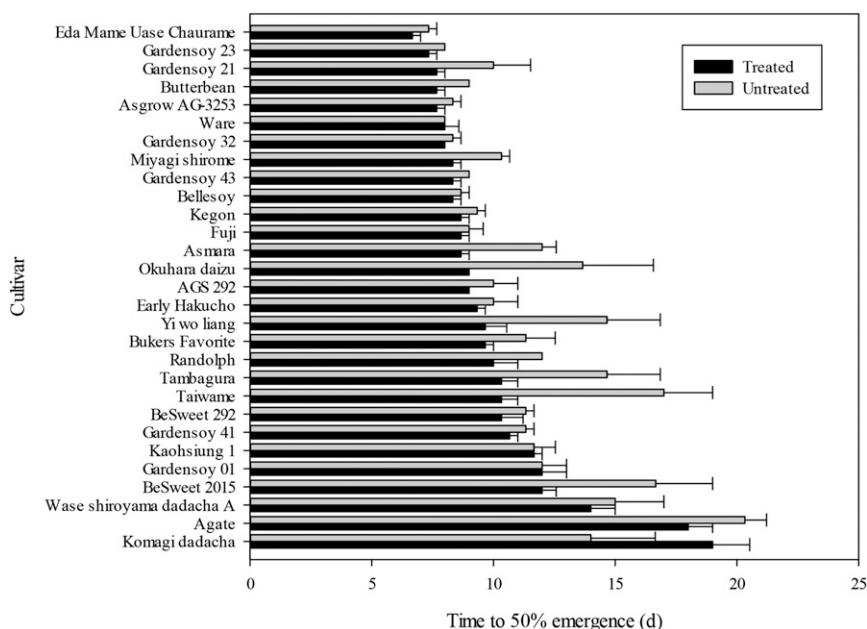


Fig. 2. The interactive effect of soybean cultivar (29 edamame cultivars and one grain-type cultivar) and seed treatment [fludioxonil + mefenoxam (2.5 and 3.75 g/100 kg seed, respectively) or nontreated] on time to 50% emergence (d). Cultivar Fiskeby III was not included because of  $<50\%$  emergence. Error bars are SE; 1 g/100 kg = 0.0160 oz/100 lb.

Table 3. Bonferroni-corrected correlation coefficients ( $\rho$ ) between soybean seed and emergence traits at 1, 2, and 3 weeks after planting (WAP) and time to 50% emergence across 29 edamame cultivars and one grain-type cultivar.

	Germination	Emergence 1 WAP	Emergence 2 WAP	Emergence 3 WAP	Time to 50% emergence
	Correlation coefficient				
100-seed weight	0.11	$-0.52^*$	$-0.23^*$	$-0.12$	$0.40^*$
Germination	—	0.01	$0.32^*$	$0.30^*$	$-0.32^*$
Emergence 1 WAP	—	—	$0.44^*$	$0.35^*$	$-0.56^*$
Emergence 2 WAP	—	—	—	$0.94^*$	$-0.82^*$
Emergence 3 WAP	—	—	—	—	$-0.77^*$

\* $P < 0.05$ .

Although edamame cultivars used in this work varied in seed size, the vegetable industry prefers seed sizes >20 g/100 seeds (Rao et al., 2002). Considering industry preference for larger seed, it is interesting that seedling emergence declined for the largest seed size class of exceptionally large lines (>22 g/100 seeds), presumably because of greater soil resistance on the large seed (Burris et al., 1973).

Seed treatment with fludioxonil + mefenoxam improved edamame seedling emergence. In a rolled towel assay, seed treatment with fludioxonil + mefenoxam at 3.75 + 2.5 g/100 kg seed, respectively, failed to reduce symptoms caused by *Fusarium graminearum* infection of soybean seedlings (Ellis et al., 2011). In a potted greenhouse experiment inoculated with *P. sojae*, seed treatment with mefenoxam at 3.75 g/100 kg of seed improved emergence and seedling growth of a soybean cultivar with partial resistance to the pathogen (Dorrance and McClure, 2001). In field trials conducted in North Dakota, fludioxonil + mefenoxam significantly improved soybean stand establishment and yields at locations in which soybean planting was followed by cool, wet conditions (Bradley, 2008). By comparison, field experiments in New York showed seed treatment with fludioxonil + insecticide combinations did not affect soybean establishment compared with the non-treated control (Cox et al., 2008). Uneven inoculum distribution and variable environmental conditions complicate field-testing of fungicide seed treatment efficacy (Dorrance and McClure, 2001). That an emergence-mediated improvement from fludioxonil + mefenoxam in the present work was detected across two variable field environments reflects the robust nature of the seed treatment contribution to seedling emergence. Further research is needed to identify exactly which pathogens are involved in seedling blight of edamame. Conceivably, seedling disease pathogens of edamame may be the same pathogens of grain-type soybean, including *Fusarium* species, *P. sojae*, *Pythium* species, and *R. solani* (Koening and Wrather, 2010).

Rapid, uniform crop emergence after planting is desirable for maximizing soybean productivity. Improvements in edamame emergence are likely to come from multiple tactics. Recent work by

Crawford (2017) showed edamame is more susceptible to planting depth than grain-type soybean. Effort among the U.S. edamame breeding programs to produce high quality seed and to introduce genes conferring resistance to common soybean diseases would be beneficial. In the meantime, under the conditions of this work, seed treatment with fludioxonil + mefenoxam offered an immediate approach to improve seedling establishment. In addition, the emergence rate was improved the most by the seed treatment for several cultivars that were generally slow to emerge. The extent to which such improvements in crop emergence influence crop yield merits study. Nonetheless, crop establishment is essential for the development of viable edamame production in the U.S. Seed treatment with fludioxonil + mefenoxam, at the rate currently registered for use on grain-type soybean, offers one approach to help vegetable growers improve crop emergence.

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