Evaluation of *Alysicarpus* Germ Plasm for Resistance to Root-Knot Nematodes

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ABSTRACT

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Root-knot nematodes reproduce freely on alyceclover (*Alysicarpus vaginalis*) cv. Florida Common, limiting alyceclover establishment, forage production, and utility as a component of crop rotations. This study was conducted to identify alyceclover germ plasm with resistance to *Meloidogyne hapla* and *M. incognita.* Individual alyceclover seedlings planted in Cone-Tainers were infested with at least 2,300 eggs of *M. hapla* or *M. incognita* and grown for 77 days under glasshouse conditions at Overton, TX. Root systems were scored for galling on a 0 to 5 scale (0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls). Nematode egg production on selected alyceclover entries was evaluated and expressed as eggs per g of fresh root weight. Twenty-six alyceclover lines were evaluated in 1990, and five lines were reevaluated in 1991. Four alyceclover germ plasm groups were identified according to relative galling responses to *M. hapla* and *M. incognita.* Two groups were heavily galled by both nematodes, one group was not galled by *M. incognita* and was variable in response to *M. hapla,* and one group was galled by *M. incognita* and variable in galling response to *M. hapla.* Nematode egg production was often not related to gall response. *M. hapla* produced up to 62,000 eggs per g of fresh root weight on plants of breeding line FL-3 with no visible galls. Breeding lines of alyceclover were identified that contained individual plants resistant to either nematode species, but no single alyceclover line had resistance to both nematodes.

Additional keyword: screening

Alyceclover (*Alysicarpus* spp.) is a tropical legume with potential for increased use as a forage and pasture crop in the southern United States. This self-pollinated, annual legume produces high-quality forage and hay (1), is a valuable supplemental forage for wildlife (5), tolerates defoliation, and can be managed for reseeding.

Alyceclover cv. Florida Common (*Alysicarpus vaginalis* (L.) DC.) is currently the only alyceclover marketed in the United States and is highly susceptible to root-knot nematodes (*Meloidogyne* spp.), including the peanut root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood), the southern root-knot nematode (*M. incognita* (Kofoid & White) Chitwood), and the javanese root-knot nematode (*M. javanica* (Treub) Chitwood) (13,14). Variation in galling and egg mass production by *M. incognita, M. arenaria,* and *M. javanica* was reported for alyceclover germ plasm (14). No single source of resistance to these three *Meloidogyne* spp. has been identified in alyceclover germ plasm. The northern root-knot nematode (*M. hapla* Chitwood), also a serious pathogen of many forage legumes (7,8), often extends into cooler geographical regions than does *M. incognita* (11). Florida Common alyceclover is severely galled by *M. hapla* (G. R. Smith and J. L. Starr, *unpublished*).

Alyceclover germ plasm with multiple root-knot nematode resistance would be highly desirable for use as a reseeding summer forage crop or green manure crop in infested soils. The objectives of this research were to evaluate alyceclover plant introductions and breeding lines for galling response to infection by *M. hapla* and *M. incognita,* and to quantify reproduction of these nematodes on different alyceclover germ plasm sources.

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MATERIALS AND METHODS

Experiment I. Fifteen plant introductions (PI) and 10 breeding lines of *Alysicarpus* germ plasm were evaluated for resistance to *M. hapla* and *M. incognita* race 3. Florida Common alyceclover was included as a susceptible check (Table 1). Both nematode species were cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers), and nematode eggs were extracted by the NaOCl method (6). Nematode eggs were used for soil infestation within 2 h of extraction.

Seeds were placed on germination paper moistened with distilled water in petri dishes, and after radical emergence, each seedling was transplanted to a Super-cell Cone-Tainer (164 cm^3 volume; Stuewe and Sons, Inc., Corvallis, OR) in the greenhouse. Growth medium was a peat and sand mixture (2:1, vol/vol) amended according to soil test with 106 g of 0-0-49 (N-P-K), 208 g of 9-19.7-12.5 (N-P-K), and 1,767 g each of dolomitic lime and gypsum per cubic meter of media. Seedlings were transplanted on 14 and 15 June 1990 and inoculated with commercial cowpea bradyrhizobial inoculant on 18 June.

Factorial combinations of *Alysicarpus* lines and root-knot nematode species (26 × 2) were arranged in a completely random design with four to 28 replications. Forty of the 52 treatment combinations had at least five plants per replicate. Each individual Cone-Tainer was infested on 28 June 1990 with 2,300 eggs of either *M. hapla* or *M. incognita* suspended in 2 ml of water. Each Cone-Tainer was reinfested on 1 August 1990 with 1,800 *M. hapla* or 17,000 *M. incognita* eggs suspended in 2 ml of water. The Cone-Tainers were reinfested because we had no previous experience with this method of screening for nematode resistance and we wanted to ensure exposure of each seedling to the nematodes. The plants were fertilized at 2-week intervals with a 9-19-12 (N-P-K) nutrient solution and micronutrients (Peter's Soluble Trace Element Mix, W. R. Grace and Sons, Allentown, PA).

During 10 to 14 September 1990, all plants were removed from Cone-Tainers, and root systems were washed free of growth medium. Each root system was scored for nematode galls on a 0 to 5 scale, where 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls per root system (9). The NaOCl method (6) was used to extract nematode eggs from root systems of *Alysicarpus* lines with gall scores of 0 to 3, identified as potentially resistant, and from Florida Common alyceclover as the susceptible check. Gall score and egg production data were analyzed using analysis of variance, linear regression, and the Student-Newman-Keuls mean separation test (10).

Experiment II. Alyceclover breeding lines FL-3, FL-5, FL-7, and FL-9 were reevaluated for galling response and nematode egg production with *M. hapla* and *M. incognita.* Florida Common alyceclover was included as a susceptible check. Cultural conditions were as described for experiment I. Seedlings were transplanted and inoculated with commercial bradyrhizobial inoculant on 22 July 1991.

Factorial combinations of alyceclover lines and nematode species (5 × 2) were arranged in a completely randomized design with 12 to 48 replications. Each Cone-Tainer was infested with 3,000 eggs of *M. hapla* or *M. incognita* suspended in 2 ml of water on 24 July 1991. Both nematode species were cultured on Rutgers tomato, and nematode eggs were extracted for infestation by the NaOCl procedure (6).

During 8 to 11 October 1991, all plants were removed from Cone-Tainers and root systems were evaluated for galling and nematode egg production as described for experiment I. A 10 to 12 plant random subsample from each line–nematode treatment combination was evaluated for nematode egg production. Gall score and egg production data were analyzed using analysis of variance, linear regression, and the Student-Newman-Keuls mean separation test (10).

Table 1. Identification of *Alysicarpus* germ plasm by species, seed source, and origin

Entry	Germ plasm identification	Species	Seed source	Origin
1	PI 158805	*vaginalis*	S-9[a]	French Guiana
2	PI 189493	*vaginalis*	S-9	India
3	PI 217904	*vaginalis*	S-9	India
4	PI 219829	*vaginalis*	S-9	Sri Lanka
5	PI 257666	*rugosus*	S-9	Australia
6	PI 286530	*rugosus*	S-9	India
7	PI 257667	*vaginalis*	S-9	Australia
8	PI 316125	*vaginalis*	S-9	Australia
9	PI 322296	*vaginalis*	S-9	Brazil
10	PI 531076	*vaginalis*	S-9	Florida, USA[b]
11	PI 316124	*ovalifolius*	S-9	Australia
12	PI 200207	*rugosus*	S-9	Kenya
13	PI 213483	*rugosus*	S-9	India
14	PI 189492	*rugosus*	S-9	India
15	PI 283178	*rugosus*	S-9	India
C	FL Common	*vaginalis*	U. of Florida	
F1	FL-1	*vaginalis*	U. of Florida	
F2	FL-2	*vaginalis*	U. of Florida	
F3	FL-3[c]	*ovalifolius*	U. of Florida	
F4	FL-4	*vaginalis*	U. of Florida	
F5	FL-5[d]	*vaginalis*	U. of Florida	PI 217904
F6	FL-6	*vaginalis*	U. of Florida	
F7	FL-7	*vaginalis*	U. of Florida	
F9	FL-9	*vaginalis*	U. of Florida	
F10	FL-10	*vaginalis*	U. of Florida	
F11	FL-11	*rugosus*	U. of Florida	

[a] USDA-ARS Regional Plant Introduction Station, Experiment, GA.
[b] Released as FL-5, selection from PI 217904 (3).
[c] Released as FL-3 (2).
[d] Same as PI 531076.

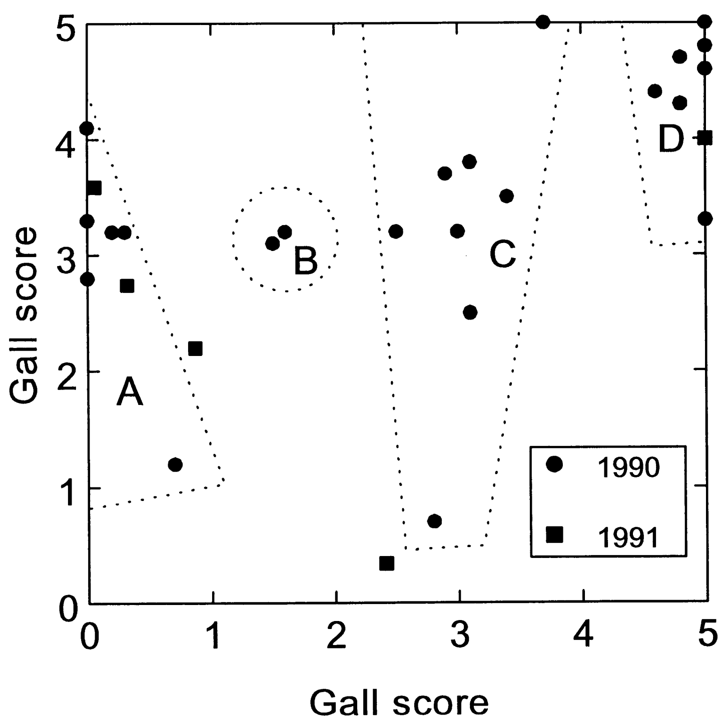

Fig. 1. Scatterplot of alyceclover germ plasm groups showing relative gall scores with both *Meloidogyne hapla* and *M. incognita.* The gall rating scale used was 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls per root system.

RESULTS AND DISCUSSION

Main effects and line-by-nematode species interaction were significant ($P < 0.001$) sources of variation for gall scores and egg production in both experiments. Data for each plant line were compared within nematode species.

Experiment I. Florida Common alyceclover and nine of the *Alysicarpus* lines evaluated in this experiment were highly susceptible to *M. incognita* (mean gall score >4.0; data not shown). These lines were heavily galled and had few secondary roots. Six lines had no or only a slight galling response to *M. incognita* (mean gall score <1.0), and three of these are related; PI 531076 is a selection from PI 217904 and is equivalent to FL-5 (3). No *M. incognita* galls were detected on the root systems of PI 217904 (n = 4), PI 316124 (n = 17), or FL-7 (n = 28). FL-3 and FL-5 responded to *M. incognita* as mixed or segregating lines. Fifty-two percent of the 27 individual FL-3 plants grown in media infested with *M. incognita* had no root

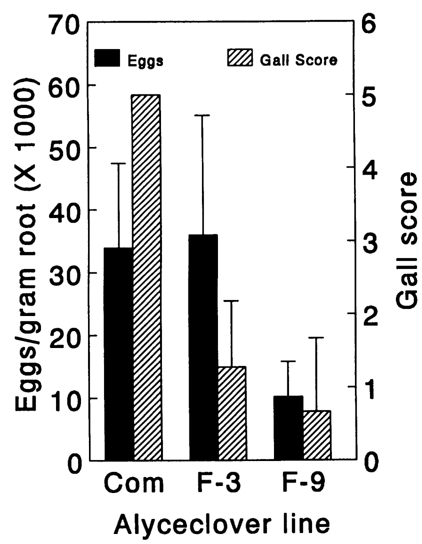

Fig. 2. *Meloidogyne hapla* egg production and gall scores of selected alyceclover entries in greenhouse tests conducted in 1990. The gall rating scale used was 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls per root system. Bars indicate +1 standard deviation.

galls, and the remaining plants had gall scores of 1 or 2. Among the 25 FL-5 plants evaluated for response to *M. incognita,* one plant had a gall score of 5, two plants had gall scores of 1, and 22 plants had no visible galls on the root systems.

Twelve *Alysicarpus* lines, including Florida Common, were highly susceptible to *M. hapla* (data not shown). FL-9 and FL-3 were the least galled by *M. hapla,* with mean gall scores of 0.7 and 1.2, respectively. Both FL-9 and FL-3 responded to *M. hapla* as mixed or segregating lines. Sixty-four percent of the 28 individual FL-9 plants inoculated with *M. hapla* had no galls on their root systems, and the remaining plants had gall scores of 1, 2, or 3. In contrast, 77% of the FL-3 plants inoculated with *M. hapla* had gall scores of 1 or 2, and 19% had no galls.

Separations of mean *M. incognita* gall scores based on the Student-Newman-Keuls test gave four distinct groups (P = 0.05; data not shown). These groups of *Alysicarpus* lines, visualized as a scatterplot (Fig. 1), were characterized as follows: group A produced few galls with *M. incognita* and was variable in response to *M. hapla*; group B was susceptible to both nematodes but was more sensitive to *M. hapla* than to *M. incognita*; group C was galled by *M. incognita* and variable in response to *M. hapla*; group D was galled with equal severity by both nematodes. Breeding lines FL-3, FL-5, and FL-7 were included in group A. Florida Common was in group D, and FL-9 was in group C.

Egg production by *M. hapla* was threefold higher on Florida Common and FL-3 alyceclover than on FL-9 (Fig. 2). Florida Common and FL-3 supported 34,000 and 36,000 *M. hapla* eggs per g of root fresh weight, respectively. This relatively high egg production on FL-3 was unexpected because gall scores were low on FL-3. *M. hapla* produced 10,160 eggs per g on FL-9, which generally distinguished FL-9 as more resistant to *M. hapla* than FL-3. The correlation between gall score and *M. hapla* egg production for FL-3 was 0.10 (P < 0.01) and for FL-9 was 0.55 (P > 0.05).

Mean egg productions by *M. incognita* on FL-3 and FL-7 were 18,160 and 540 eggs per g of root fresh weight, respectively (Fig. 3). The relatively high *M. incognita* egg production on FL-3 could not have been predicted based on mean gall scores. *M. incognita* produced between 9,000 and 28,000 eggs per g of root fresh weight on FL-3 plants with no galls. However, on FL-5 plants with no visible galls, *M. incognita* produced only 260 to 3,000 eggs per g. This nematode produced 33,000 eggs per g on the single FL-5 plant that had a gall score of 5. No FL-7 plants grown in media infested with *M. incognita* had visible root galls, and egg production by *M. incognita* on FL-7 ranged from 20 to 2,300 eggs per g of root fresh weight.

Experiment II. Florida Common alyceclover was badly damaged by *M. incognita.* At harvest, more than half of the Florida Common plants were dead, and the remaining plants had heavily galled, stunted root systems. The breeding lines FL-3, FL-5, and FL-7, which had slight galling responses to *M. incognita* in experiment I, exhibited similar reactions in

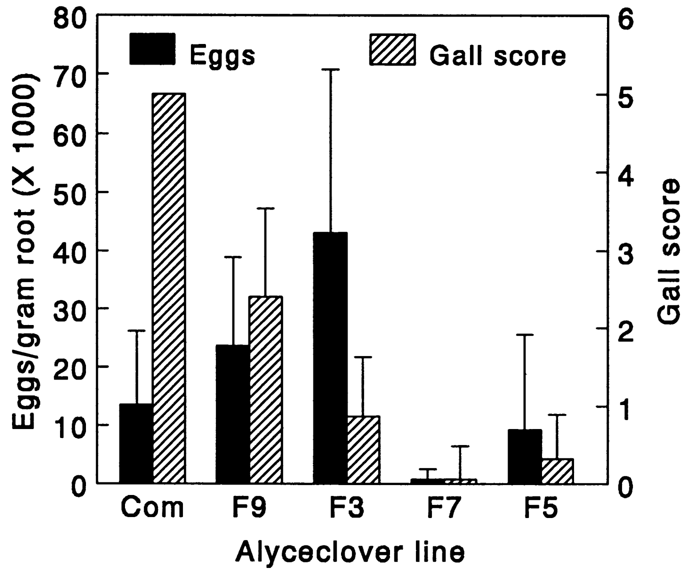

Fig. 4. *Meloidogyne incognita* egg production and gall scores of alyceclover entries from greenhouse tests conducted in 1991. The gall rating scale used was 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls per root system. Bars indicate +1 standard deviation.

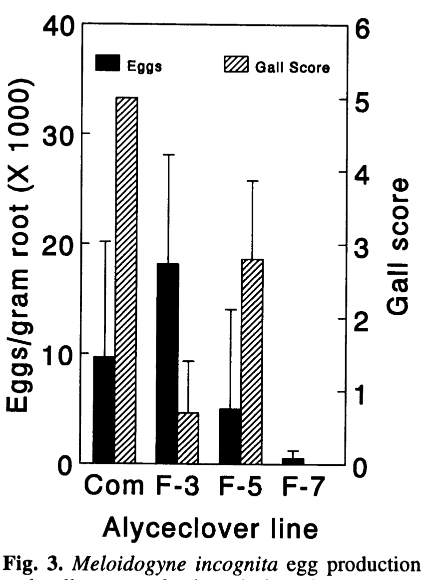

Fig. 3. *Meloidogyne incognita* egg production and gall scores of selected alyceclover entries in greenhouse tests conducted in 1990. The gall rating scale used was 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls per root system. Bars indicate +1 standard deviation.

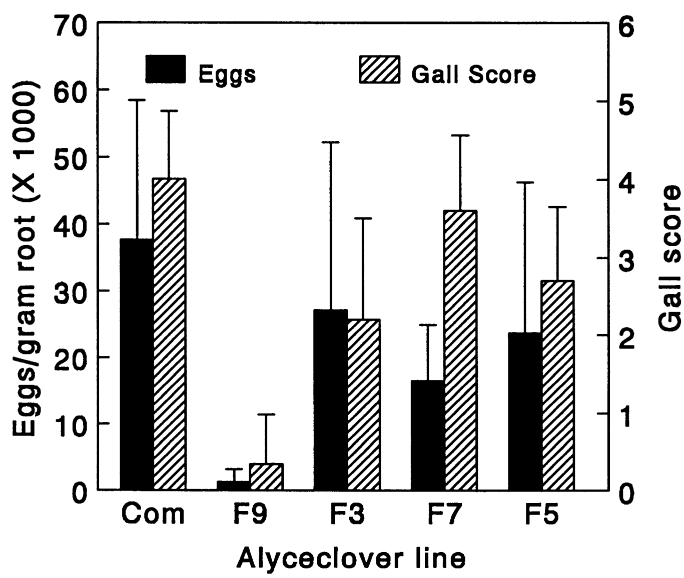

Fig. 5. *Meloidogyne hapla* egg production and gall scores of alyceclover entries from greenhouse tests conducted in 1991. The gall rating scale used was 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = more than 100 galls per root system. Bars indicate +1 standard deviation.

Table 2. Alyceclover response to *Meloidogyne incognita* and *M. hapla* in greenhouse experiments

Alyceclover line	*M. incognita*	*M. hapla*
Florida Common	Susceptible[a]	Susceptible
FL-3	Susceptible	Susceptible
FL-5	Partially resistant	Susceptible
FL-7	Resistant	Susceptible
FL-9	Susceptible	Partially resistant

[a] Plant response terms from Cook and Evans (4) are based on nematode reproduction, where Susceptible = high reproduction, Resistant = very low reproduction, and Partially resistant = intermediate level of reproduction.

experiment II (Fig. 4). Galls were noted on one of 49 FL-7 plants grown in media infested with *M. incognita.* Alyceclover lines FL-3 and FL-5 had gall scores similar to those noted in experiment I. FL-9 was galled by *M. incognita,* but plant-to-plant variation was high. Two FL-9 plants with no root galls were noted.

Florida Common and FL-7 were heavily galled and FL-5 was moderately galled by *M. hapla.* Although gall numbers were high, the root systems of these lines were similar to those of control plants in size and in number of secondary roots. *M. hapla* did not cause the severe root pruning and plant death associated with infection by *M. incognita.* As in experiment I, FL-3 and FL-9 were the least galled by *M. hapla,* with mean gall scores of 2.2 and 0.3, respectively (Fig. 5). No galls were detected on 72% of the FL-9 plants grown in media infested with *M. hapla.* In experiment II, the four breeding lines and Florida Common generally fit the same groupings used in experiment I to describe galling response to both *M. hapla* and *M. incognita* (Fig. 1).

Relatively low egg production by *M. hapla* on FL-9 was confirmed in experiment II (Fig. 5). Average productions were 1,384 and 37,760 eggs of *M. hapla* per g of root fresh weight for FL-9 and Florida Common, respectively. *M. hapla* egg production on individual plants of FL-9 ranged from 0 to 6,109 eggs per g of root fresh weight, with six of the 12 plants evaluated producing less than 20 eggs per g.

The root systems of Florida Common plants were so badly damaged by *M. incognita* that egg production was suppressed (Fig. 4). FL-9 and FL-3 allowed prolific reproduction of *M. incognita.* As noted for experiment I, FL-3 supported very high *M. incognita* egg production but did not show a strong galling response. Egg production by *M. incognita* was variable on FL-5. Individual plants of FL-5 supported from 0 to 45,333 eggs of *M. incognita,* with four of 12 plants producing less than 30 eggs per g of root fresh weight. FL-7 produced fewer *M. incognita* eggs per g of fresh root weight than any alyceclover line evaluated. No *M. incognita* eggs were detected on eight of 12 FL-7 plants evaluated.

The plant responses of FL-3, FL-5, FL-7, FL-9, and Florida Common to both nematodes are summarized in Table 2. Both FL-5 and FL-7 appear to contain individual plants with nearly complete resistance to *M. incognita,* characterized by lack of galling and low nematode reproduction. This confirms reports (14) of reduced galling and egg mass scores on FL-5 and FL-7 infected with *M. incognita* race 3 compared to Florida Common alyceclover. FL-5 and FL-7 were susceptible to *M. hapla.* In contrast, FL-3 had relatively low mean gall scores, but even individual plants without galls allowed high reproduction levels of both *M. incognita* and *M. hapla.* High nematode reproduction on alyceclover plants without galls indicates independence of nematode egg production and root galling. FL-3 could be defined as tolerant (4) to *M. incognita* based on its low galling response and low root damage. FL-3 was classified as susceptible to both nematodes based on nematode reproduction. FL-3 has been released by the Florida Agricultural Experiment Station as an alyceclover cultivar (2) resistant to *M. javanica* and *M. arenaria,* but susceptible to *M. incognita* race 3.

FL-9 was partially resistant to *M. hapla,* based on reduced reproduction of *M. hapla* compared with other *Alysicarpus* lines evaluated. Partial resistance is defined by Cook and Evans (4) as the support of intermediate levels of nematode reproduction. FL-9 was susceptible to *M. incognita,* as reported in earlier studies (14).

In a breeding program, partial resistance to *M. hapla* from FL-9 could be combined with *M. incognita* race 3 resistance from FL- 5 or FL-7. Both FL-9 and FL-7 have very poor seedling vigor (12), thus development of a nematode-resistant alyceclover cultivar using FL-7 or FL-9 as parents would require a concurrent effort to improve seedling vigor.

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