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The Effect of Temperature on Seed Quality and Quantity in Crosses between European (*Populus tremula*) and Hybrid Aspens (*P. tremula* × *P. tremuloides*)

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Hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) plantations are expanding in Fennoscandia and the Baltic countries; however, the possible effects of plantations on the native European aspen (*P. tremula*) and the level of gene flow between European and hybrid aspen have not been investigated. We studied seed quantity and quality in intraspecific and interspecific crosses of the European and hybrid aspens over a two year period. In order to study whether elevated temperatures due to climate change would benefit the species differently, we performed the crosses in different temperatures. In both years, interspecific crosses produced more seeds with higher quality than intraspecific crosses. This result was most distinct in crosses between female hybrid aspen and male European aspen. In higher temperatures, relative germination difference between hybrid aspen seeds and seeds from *P. tremula* × *P. tremula* crosses seems to increase. These results suggest that hybrid aspen may have a significant genetic impact on the European aspen, and this effect may be strengthened by climate warming.

Keywords climate change, gene flow, germination, hybrid vigour
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1 Introduction

The European aspen (*Populus tremula* L.) is the only native *Populus* species in northern Europe. It is an important species for boreal forest biodiversity, for example, in Finland more than 200 other species use dead or living aspen trees as a source of nutrition or as a habitat (Siitonen 1999, Kouki et al. 2004). Aspens are dioecious, anemophilous and anemochore trees, which are able to reproduce both sexually and asexually through root suckers. Asexual reproduction is generally more important due to low seedling survival (Latva-Karjanmaa et al. 2003), but sexual reproduction may not be as uncommon as has previously been thought (Suvanto and Latva-Karjanmaa 2005).

Hybrid aspen (*P. tremula* × *P. tremuloides* Michx.) is a man-made hybrid between the European aspen and the North American trembling aspen. Hybrid aspen does not occur in the wild, because the two parent species are naturally geographically isolated from one another (i.e. live on different continents). The hybrid aspen has been planted in Fennoscandia since the 1940s, first to serve as a raw material for the match industry and later for the paper industry (Rytter and Stener 2005). Aspens, and especially hybrid aspen, represent excellent material for high quality paper mainly because of their fibre qualities (Ranua 2002) and fast growth rate (Yu and Pulkkinen 2003). Currently, plantations are established using only hybrid aspen, with about 1500 ha being planted in Finland and Estonia during the period 1997–2003 (Holm 2004). The hybrid aspen grows fast (Hynynen et al. 2002), and it may be a better competitor for space than the European aspen. Additionally, the flowering of European and hybrid aspen occurs simultaneously in Finland (R. Jaatinen pers. com. 2010) and hybrid aspen can cross with European aspen. This indicates that *P. tremuloides* genes can introgress wild populations of *P.tremula*, but the level of this gene flow has not been studied in Fennoscandia or Baltic countries. Introgression of foreign genes has, however, been reported in other *Populus* species (Vanden Broeck et al. 2004), and gene flow in poplars may be quite effective (Imbert and Lefèvre 2003). Furthermore, the number of hybrid aspen clones cultivated in the Nordic countries is rather small, and the clones are often closely related due to few siring *P. tremuloides* individuals. The low genetic diversity of hybrid aspens may narrow the gene pool of European aspen when gene flow between European and hybrid aspen occurs.

There are differences in the level of hybridisation between *Populus* species. In general, artificial crosses between different sections in the genus *Populus* are more difficult than crosses within sections (Villar et al. 1987). In addition, the backcrossing rate may also vary. In a hybrid zone involving *P. tremula* and *Populus alba* L. in the Danube valley near Vienna, Austria, crosses occur mainly between *P. tremula* males and *P. alba* females, and almost all hybrids are backcrosses towards *P. alba* (Lexer et al. 2005). Studies on North American *Populus* hybrid zones have found no cytonuclear incompatibilities between the species studied irrespective of the direction of the cross (Paige et al. 1991). Studies on linkage mapping suggest that barriers to hybridisation between species are due to differences in particular genes, not to the incompatibility of chromosomes (Cervera et al. 2001).

Interspecific hybrids are generally considered to be less fit than their parents (reviewed by Burke and Arnold 2001), but there is in fact much variation depending on the species and the environment (Arnold et al. 2001, Burke and Arnold 2001, Johnston et al. 2001). Recombinant phenotypes can even outperform their parents, at least in some habitats (Vila and D'Antonio 1998, Burke and Arnold 2001). The mechanisms leading to increased fitness may be segregation of additive genetic factors and to a lesser extent epistasis (Burke and Arnold 2001). The fitness of the hybrids is also dependent on the level of hybridisation. Kirk et al. (2005) have found that early hybrid generations do better than natural hybrids which have experienced intercrossing and backcrossing through several generations.

One factor that may influence the relative performance of hybrids is temperature, and hence the ongoing climate warming may have consequences for the interaction between the European aspen and the hybrid aspen. The prolonged growing season, relatively warm winters and increased precipitation together with high frequencies of more extreme weather conditions (Jylhä et al. 2009) can affect the plants' frost hardening, dehardening and especially flowering. Especially in the northern hemisphere, the climate is expected to change rapidly. For instance, in Finland the average temperature is predicted to rise by 2 to 6°C by the year 2100. Additionally, changes in precipitation are forecasted (Jylhä et al. 2009). Environmental conditions are also known to affect reproduction, for instance, in *Betula pendula* Roth temperature affects pollen-tube growth rate (Pasonen et al. 2000). Warming climate may promote interspe-

Table 1. Locations of female and male aspens used in the crossings and the location of each tree. All sites are in southern Finland.

Sex	Species	Locality	Latitude	Longitude	Year
F	*P. tremula*	Riihimäki	60°40’27”	24°38’09”	2003
F	*P. tremula*	Nurmijärvi	60°36’39”	24°28’38”	2003, 2004
F	*P. tremula*	Riihimäki	60°40’27”	24°38’09”	2003, 2004
F	*P. tremula*	Nurmijärvi	60°18’11”	24°42’53”	2004
F	Hybrid	Karkkila	60°30’55”	24°20’58”	2003
F	Hybrid	Karkkila	60°30’55”	24°20’58”	2003
F	Hybrid	Loppi	60°36’59”	24°25’55”	2003, 2004
F	Hybrid	Vihti	60°33’09”	24°31’18”	2004
F	Hybrid	Loppi	60°37’33”	24°24’24”	2004
F	Hybrid	Loppi	60°37’15”	24°24’01”	2004
M	*P. tremula*	Riihimäki	60°40’27”	24°38’09”	2003
M	*P. tremula*	Loppi	60°36’38”	24°31’09”	2003, 2004
M	*P. tremula*	Riihimäki	60°40’27”	24°38’09”	2003, 2004
M	*P. tremula*	Loppi	60°37’19”	24°27’14”	2004
M	Hybrid	Karkkila	60°30’55”	24°20’58”	2003
M	Hybrid	Karkkila	60°30’55”	24°20’58”	2003
M	Hybrid	Loppi	60°36’39”	24°28’38”	2003
M	Hybrid	Karjalohja	60°15’22”	23°44’04”	2004
M	Hybrid	Janakkala	60°53’12”	24°35’33”	2004
M	Hybrid	Loppi	60°37’33”	24°24’24”	2004

cific crossings and/or increase survival of hybrids by changing competition interactions between the species. The growth of the hybrid aspen commences earlier in the spring and continues later in the autumn than the growth of the European aspen (Yu et al. 2001), which has partly restricted the planting of hybrid aspen to the southern parts of Fennoscandia. If the growing season becomes longer and winters become warmer, the cultivation of the hybrid aspen may become possible further north. All these factors may strengthen the impact of the hybrid aspen on the European aspen and the entire forest ecosystem.

The purpose of this study was to measure the quantity and quality of seeds produced in the crosses between the European and hybrid aspens (F_2 and backcrosses) in different temperature treatments to assess the likely consequences of gene flow between European and hybrid aspen. Experiments were conducted in different temperatures to investigate whether the temperature dependence of seed germination is different in the European and hybrid aspens.

2 Materials and Methods

2.1 Experimental Design

Crosses between the European and hybrid aspens were produced in the springs of 2003 and 2004. All hybrid aspens originated from crosses between *P. tremula* females and *P. tremuloides* male. We used four European aspen females and four males and six hybrid aspen females and six male clones from different locations in southern Finland (Table 1). In late March we collected about ten 2 to 2.5 m long branches with many flower buds from the upper third of each parent tree.

First, the branches of the test trees were set aside for a preparatory phase of 2 days. This phase was necessary in order to force the branches to flower. We used temperatures which have been found to give the best results in our previous flowering experiments (starting from 2002, R. Jaatinen pers. com.). The branches were placed in water-filled buckets in a greenhouse, where the day temperature was 10°C and the night temperature was 8°C. The branches and floor of the greenhouse were watered 2–3 times a day to prevent the branches from withering. After two days, the temperature in the greenhouse was changed. In 2003, the section housing the male branches was set at 10°C for the night and 15°C for the day, whereas for the female flowers the night temperature was set at 12°C and the day temperature at 16°C. In 2004, the day temperatures were set as described in Table 3 and the night temperatures were 4–5°C lower than the respective day temperatures. At this point the actual experiment started.

The basic procedure of the crossings was as follows (see Table 2). In 2003, we used six female clones and six branches of each clone. The number of male clones was also six. Each female branch was used in two crosses with two different male clones, and each male clone was crossed with two female branches of the same female clone. Thus there were 12 crosses (bags) within one female clone. Male clones were crossed randomly with one female clone, i.e. the males and females were not selected based on any genetic or phenotypic characters. The produced seeds were collected and combined from two branches / female clone (see Table 2).

In 2004, we had approximately 50 female branches, which were divided, on average, into eight branches per clone. We had seven female clones, which were crossed with six male clones using all possible combinations. Each temperature condition had, on average, two female branches per female clone, and all males were crossed with both of these two branches; thus the number of crosses per male clone per condition and female clone was two. The seed material from these two bags was combined as in 2003.

We had to use partly different parents in the second year, because not all trees flowered in both years. All males and females represented different clones. The individuals used for the crosses were not selected because of any clonal properties; we simply used the flowering individuals that were available.

In 2003, all crosses were performed in the same temperature to study the numbers and viability of the seeds produced. In 2004, the crosses were done under different temperatures (Table 3). All test temperatures are within the normal temperature variation predominant in nature at the time of aspen flowering and seed development.

The male branches were covered with large paper bags 4–5 days after they had been moved to the greenhouse. The male flowers matured in 3–4 days, after which the mature pollen was extracted, using a vacuum, through a hole made in the bag, dried and bottled. The female branches were bagged one week after the transfer to the greenhouse and pollination was carried out 2–3 days after bagging. The maturity of the female flowers was checked on the basis of the status of the pistils. Pollination was done by injecting the pollen inside the bags when the pistils were clearly visible. We used 12 cm^3 of pollen for each bag. Pollination was repeated after two days. Pollen used in all the crosses was similar in age, and stored after its collection for a maximum of a three days. The pollination bags were removed 4–5 days after the second pollination and branches were treated with pesticides (malathion and pyrethrin). The number of flowers was counted. Catkins were gathered ca. 20 days after pollination, when the seed hair was visible and the seeds started to shed, which showed that the seeds were mature. The seed material was cleaned as described in Latva-Karjanmaa et al. (2003).

Table 2. Crossing design (one female clone as an example).

Female clone	Branch	Male clone					
x	1	a					d
x	2	a	b				
x	3		b	c			
x	4			c	d		
x	5				d	e	
x	6					e	d

Table 3. Temperature during the crossing experiments.

Year and treatment no.	Mean temperature °C At flowering (T_F)	At seed development (T_D)	Average
2003	13.1	18.6	15.8
2004/1	11.9	13.1	12.5
2004/2	11.9	17.1	14.5
2004/3	13.7	15.7	14.7
2004/4	13.7	17.4	15.5

2.2 Measured Variables and Statistical Analysis

We measured several genotype*genotype level seed variables; germination percentage (GER%), number of seeds per flower (SEED/FL), number of germinated seeds per flower (GSEED/FL), weight of the seeds in grams per branch (WEIGHT) and the number of seeds per branch (SEEDNO). To measure the germination percentage we sampled 20 mg of seed material from each seed lot. The seeds were germinated on a Plantek-25 base. We counted the number of germinated seeds after six days.

To meet the assumption of normality, we used square-root transformation for SEEDNO and logarithmic transformation for WEIGHT, SEED/FL and GSEED/FL. We used general linear models (univariate analysis of variance; SPSS for Windows 12.0.1.) to explain variation in GER%, SEED/FL, GSEED/FL, WEIGHT and SEEDNO by the species and gender and their interaction (data for 2003). The results for 2004 were analysed with nested ANOVA (Systat version 9), using both genders, flowering and development (nested within flowering temperature) temperatures as well as their interactions as explanatory variables. In both models the explanatory variables were fixed factors.

3 Results

European aspen females produced significantly ($p<0.05$) more seeds (WEIGHT, SEEDNO, SEED/FL) and also more viable seeds (GSEED/FL, p=NS), when crossed with hybrid aspen males and this was the same when hybrid aspen females was crossed with European aspen males (Fig. 1, Tables 4 and 5). The type of males did not have a significant effect on the independent variables in either of the two years, but male × female interaction was significant for WEIGHT and SEEDNO in the first year and for SEEDNO, SEED/FL and GSEED/FL in the second year (Table 5).

The results regarding seed quality and quantity were different for the two years. In 2003, when all seeds developed in the same temperature, hybrid aspen females produced more seeds and the seeds were also more viable than the seeds of the European aspen (Table 4). The most seeds were produced in the crosses between hybrid aspen females and European aspen males (Table 4, Fig. 1A). However, in 2004, European aspen females produced more seeds than hybrid aspen females (Fig. 1B).

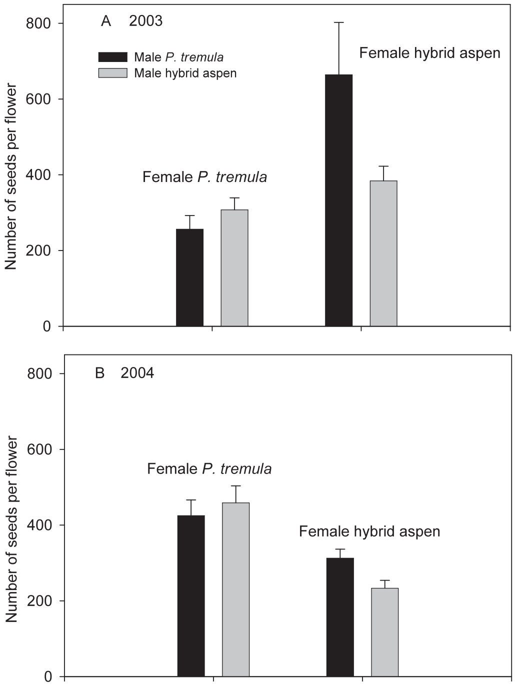

Fig. 1. Mean number of seeds per flower (with SE) in 2003 (A) and 2004 (B).

Temperature during seed development affected GER%, SEED/FL and GSEED/FL (Table 5). SEED/FL was highest at development temperatures of 15.7 to 17.1°C (Fig. 2). Interaction (Female × T_F) (T_F = temperature at flowering) explained some of the variation in SEEDNO and interaction (Female × $T_D(T_F)$) (T_D = temperature at seed development) in GER% (Table 5). Temperature during flowering had a significant effect only on SEEDNO, thus more seeds at higher temperatures (Table 5).

The highest seed viability was achieved in temperature treatments 2 ($T_D = 11.9°C$, $T_F = 17.1°C$) and 3 ($T_D = 13.7°C$, $T_F = 15.7°C$). The number of seeds that germinated increased with elevated seed development temperature (Fig. 2), but the

Table 4. Means (0), standard errors (SE) and numbers (n) for different variables in the different types of crosses (female is presented first) in 2003. The variables are: GER% = germination percentage, SEED/FL = number of seeds / flower, GSEED/FL = number of germinated seeds / flower, WEIGHT = weight of seeds (g) / branch, and SEEDNO = number of seeds / branch. Tre = *P. tremula*, hyb = hybrid aspen.

Variable		tre × tre	tre × hyb	hyb × tre	hyb × hyb
GER%	0 ± SE	39.9 ± 5.90	48.7 ± 6.79	63.0 ± 3.70	57.6 ± 6.75
	n	17	16	17	18
SEED/FL	0 ± SE	256 ± 35.9	273 ± 36.6	664 ± 138	384 ± 38.7
	n	17	18	17	18
GSEED/FL	0 ± SE	106 ± 22.0	164 ± 30.1	399 ± 89.5	240 ± 33.3
	n	17	16	17	18
WEIGHT	0 ± SE	1.08 ± 0.079	1.56 ± 0.313	2.83 ± 0.448	1.75 ± 0.256
	n	18	18	17	18
SEEDNO	0 ± SE	5 790 ± 1140	9 030 ± 1670	16 600 ± 2870	12 200 ± 2110
	n	18	18	17	18

Table 5. Analysis of variance (2003) and nested ANOVA (2004) for the effects of female (F) and male (M) “species”, temperature during flowering (T_F) and during development (T_D) and those interactions that were statistically significant for at least one of the variables. Significant P values are shown in bold. The variables are as in Table 3.

Source	GER%			SEED/FL			GSEED/FL			WEIGHT			SEEDNO		
	df	MS	P	df	MS	P	df	MS	P	df	MS	P	df	MS	P
2003															
F	1	4332	**.009**	1	653	**.000**	1	3.02	**.002**	1	.485	**.013**	1	1.013	**.005**
M	1	50	.773	1	110	.132	1	.053	.675	1	.011	.704	1	.021	.680
F×M	1	851	.237	1	96	.159	1	.937	.082	1	.513	**.011**	1	.670	**.021**
2004															
F	1	605	.315	1	1032	**.000**	1	2.767	**.003**	1	.879	**.005**	1	6121	**.016**
M	1	1491	.116	1	14	.544	1	.059	.655	1	.204	.169	1	3363	.074
T_F	1	73	.727	1	55	.231	1	.270	.341	1	.402	.054	1	5089	**.028**
$T_D(T_F)$	2	25435	**.000**	2	243	**.002**	2	8.445	**.000**	2	.205	.150	2	2474	.096
M×F	1	718	.274	1	263	**.010**	1	2.225	**.007**	1	.099	.337	1	5312	**.025**
F×T_F	1	1164	.164	1	130	.067	1	0.094	.574	1	.406	.053	1	5258	**.026**
F×$T_D(T_F)$	2	2292	**.023**	2	57	.227	2	.726	.089	2	.018	.848	2	17	.983

responses of the crosses to elevated temperature were different. When the temperature was elevated by 3–4°C, crosses with hybrid aspen had a higher increase in germination than crosses within the European aspen.

4 Discussion

Both European and hybrid aspens produced more seeds and the seeds were more viable when crossed with the other species (see Fig. 1, Table 4). Temperature affected the quantity and quality of aspen seeds, but particular conditions had differing effects on the seed yield in different crosses (see Table 5). In higher temperatures, the crosses with hybrid aspen produced seeds with the highest viability (see Fig. 2).

Temperature during flowering and seed development affects the number of seeds and influences seed quality (see Table 5). In general, higher temperatures lead to higher germinability (Gutterman 1992). However, the literature about temperature effects on germination in broadleaved trees is scarce. In herbaceous plants, the development temperature resulting in the highest germination has been shown to depend on the germination

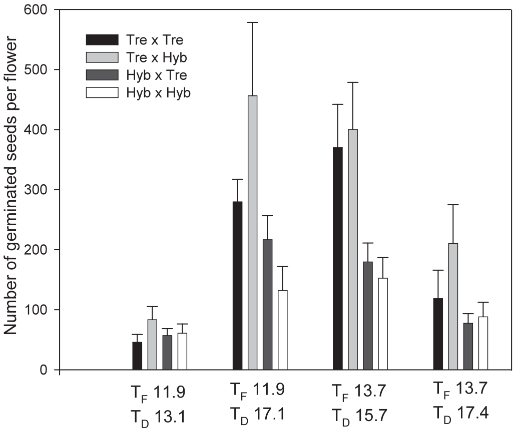

Fig. 2. Mean number of germinated seeds per flower (with SE) obtained from different cross types (female is presented first) in different temperatures. Tre = *P. tremula*, hyb = hybrid aspen, T_F = flowering temperature (°C), T_D = seed development temperature (°C).

temperature at which the seeds were tested (Gutterman 1992). Aspen seeds have a wide tolerance of temperatures during germination. In *P. tremuloides*, depending on the study, a range in temperatures from 0°C to 39°C has resulted in high germination (reviewed by McDonough 1985), with the optimum temperature being between 15°C and 25°C (McDonough 1979). Although we seem to have found the optimum development temperature range for high seed viability in aspen (see Fig. 2), we cannot entirely exclude the possibility that the differences in seed crop were due to genetic differences of the parents.

The results would, of course, have been clearer if the experiment had been done using several replications and exactly the same individuals in both years. Unfortunately, we did not have enough regulated greenhouse space to carry out the experiment using more replications. Since we used individual trees growing in nature, we could not remove too many branches from the tree without damaging its crown and future growth. Furthermore, since not all the individuals flower every year, we were not able to use a more sophisticated experimental design, such as complete balanced factorial design. Another difficulty with this kind of study is that no reliable method to measure the germination ability of aspen pollen exists; thus the pollen viability has to be determined indirectly based on the viability of seeds.

Masting (synchronised reproduction of individuals, Houle 1999, Shibata et al. 2002) leads to large year-to-year variation in seed production in aspen (El-Ghazaly et al. 1993, Barnes et al. 1998). Masting and variable weather conditions in different years could partly explain the differences in seed yield between the years in our experiment. Although the flowering of European and hybrid aspen in Finland is synchronous, in second level hybrids (e.g. F_2 or backcrosses) the temporal pattern of reproduction may be more complex.

In general, maternal effects control much of the variation in seed production. For instance, seed germination is almost entirely determined by maternal effects in many cultivated plants (Roach and Wulff 1987). Burgess and Husband (2004) found, in their studies on mulberries, that maternal effects determined seed set and germination, while the male, and the interaction between male and female, had no effect on either of these characteristics. The mechanisms of maternal effects may include maternal nuclear contributions to the endosperm, non-genetic influence of the maternal environment on offspring or maternally inherited cytoplasm and cytonuclear interactions (Burgess and Husband 2004). The endosperm of aspen seeds is very small and thus it is unlikely that it would have much effect on seed quantity and quality. The effect of maternally inherited cytoplasm seems also improbable, because the hybrid aspen has derived its cytoplasm and organelles from the European aspen. Thus the explanation of maternal environment as the main cause for better seed production seems most likely. Hauser et al. (1998) suggest that maternal influence of F_1 plants would be more beneficial to the offspring than that of the pure mother species (in their study *Brassica rapa* L.) due to the more vigorous vegetative growth in hybrids compared to mother species. Growth and competition experiments with one- and two-year old European aspens and F_2-aspen hybrids has shown that especially the F_2-hybrid, where European aspen is the mother and hybrid aspen is the father, has a better height growth and competition ability than European aspen, particularly when the temperature during growing season is increased (P. Pulkkinen, pers.

com. 2010).

At higher temperatures, the crosses involving the hybrid aspen produced seeds with higher germinability than the European aspen. This has important implications for gene flow between the European and hybrid aspens. Because pollen travels further than seeds, hybrid aspen pollen flow has more importance than its seed flow for the invasion by the hybrid aspen. E.g. Burczyk et al. (2004) have recorded abundant pollen mediated gene flow between poplar populations. Invasiveness can evolve, and hybridisation between species can induce further evolution (Ellstrand and Schierenbeck 2000). Genes of the hybrid aspen could be advantageous for adaptations to changing climate and thus favour hybridisation between the European and hybrid aspens. However, even though the hybridization may be favoured by future climate conditions (see Jylhä et al. 2009), the key point for the real ecological impact is the survival and development of seed in nature. In the present conditions, the sexual reproduction of European aspen is not very common compared to vegetative reproduction (Latva-Karjanmaa et al. 2003).

In conclusion, simultaneous flowering of European and hybrid aspen as well as improved germinability of seeds produced or sired by hybrid aspen in high temperatures can promote gene flow between European and hybrid aspen. The increasing planted area of hybrid aspen in Fennoscandia and especially in the Baltic countries together with climate change, which enables the cultivation of hybrid aspen in more northern areas, realizes the threat of foreign gene flow from hybrid to European aspen. However, the real importance of hybridization should be verified by comparing the effect of different environmental conditions (e.g. temperature, moisture, CO_2) to the relationship between generative and vegetative reproduction.

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