GERMINATION ECOLOGY OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ, EUPHORBIACEAE) IN TRADITIONAL AGROECOSYSTEMS: SEED AND SEEDLING BIOLOGY OF A VEGETATIVELY PROPAGATED DOMESTICATED PLANT[1]

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Benoît Pujol, Guillaume Gigot, Gérard Laurent, Marina Pinheiro-Kluppel, Marianne Elias, Martine Hossaert-McKey, *and* **Doyle McKey** *(Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, 1919 route de Mende, 34293 Montpellier Cedex 05, France).* GERMINATION ECOLOGY OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ, EUPHORBIACEAE) IN TRADITIONAL AGROECOSYSTEMS: SEED AND SEEDLING BIOLOGY OF A VEGETATIVELY PROPAGATED DOMESTICATED PLANT. *Economic Botany 56(4):366–379, 2002. Cassava is clonally propagated, but Amerindian farmers also use plants from volunteer seedlings to prepare stem cuttings. Although sexual reproduction plays a role in cassava's evolution it is poorly studied. We examined one aspect of cassava reproductive ecology, seed dormancy and germination. Volunteer seedlings emerge from a soil bank of seeds produced during the previous cycle of cultivation that remain ungerminated through the fallow period, then germinate synchronously after vegetation is slashed and burned. Laboratory experiments showed that germination can be enhanced by mechanical scarification and also by dry heat treatment, suggesting that burning after field clearing could help break dormancy. Germination was also stimulated by high temperatures (35°C) that in nature indicate bare soils, and inhibited by temperatures (25°C) close to those in soil shaded by vegetation and by light. Seeds of both wild and domesticated cassava exhibit physiological dormancy, an adaptation for germination in periodically disturbed habitats. In addition to these preadaptations, preliminary results also suggest specific adaptations of domesticated cassava to the distinctive disturbance regimes of swidden agriculture.*

Key Words: *Manihot esculenta* Crantz; Euphorbiaceae; soil seed bank; seed dormancy; seed germination; shifting cultivation; fire ecology; agroecology.

Cassava, the most important of tropical root and tuber crops, is propagated strictly vegetatively by means of stem cuttings. However, in traditional agroecosystems based on swidden cultivation in Amazonia (Boster 1984; Elias, Panaud, and Robert 2000; Elias, Rival, and McKey 2000; Elias et al. 2001a,b; Salick, Cellinese, and Knapp 1997; Sambatti, Martins, and Ando 2001) and elsewhere (McKey et al. 2001), farmers allow volunteer seedlings that appear in fields to grow. These seedlings are the result of a series of processes that escape human control. This begins with the open pollination of flowers and extends to the dispersal, dormancy, and germination of seeds. Along with plants grown from cuttings, some plants that originated as seedlings are used by farmers to prepare stem cuttings for propagation of the next generation. These products of sexual reproduction are important in the evolutionary dynamics of this clonally propagated crop (Cury 1993; McKey and Beckerman 1993; Elias 2000; Elias, Panaud, and Robert 2000; Elias, Rival, and McKey 2000; Elias et al. 2001a; Sambatti, Martins, and Ando 2001; McKey et al. 2001). Patterns of genetic diversity suggest that incorporation of volunteer seedlings is frequent and leads to an increase in intravarietal genetic diversity (Elias 2000; Elias, Panaud, and Robert 2000; Elias, Rival, and McKey 2000; Elias et al. 2001a,b; Sambatti, Martins, and Ando 2001). Aside from the genetic consequences, use of plants originating from seedlings may confer an important agronomic advantage. Because systemic pathogens

are usually not transmitted by seed, seedlings are often relatively free of viral and other pathogens that tend to accumulate in vegetatively propagated plants (Lozano and Nolt 1989). Plants originating as seedlings may thus supply healthier cuttings (McKey et al. 2001).

In the farms of Makushi Amerindians in Guyana, volunteer seedlings of cassava emerge from a bank of dormant seeds in the soil established during the previous cycle(s) of swidden cultivation 3–30 (average about 10) years ago (Elias and McKey 2000). Ants may be important in the constitution of the seed bank, burying seeds and thus protecting them from surface-foraging seed predators (Elias and McKey 2000). As with many other Euphorbiaceae (e.g., Bülow-Olsen 1984; Lisci and Pacini 1997; Passos and Ferreira 1996), cassava seeds possess a seed appendage termed the caruncle that functions as an ant-attractant elaiosome (oil body). Burial of seeds and formation of a persistent seed bank are important features in the biology of many ant-dispersed plants, enabling seeds to escape predators during prolonged dormancy and to survive fires by escaping lethal temperatures at the soil surface. Buried seeds of many such plants are able to perceive environmental variation and respond by timing germination to coincide with the favorable conditions that follow fires (Baskin and Baskin 1998).

When the field is cleared and burned, cassava seeds that have persisted during the fallow period since the previous crop germinate in large numbers about two weeks after burning. The regeneration niche of volunteer seedlings is restricted to newly burned fields; any rare seedlings appearing later in cultivated fields appear not to survive. Any that do survive in these less favorable conditions are highly unlikely to be selected for cuttings, which are always chosen from large, healthy, vigorous plants (Elias, Rival, and McKey 2000). Incorporation of volunteer seedlings by farmers into germplasm for stem cuttings means that natural selection can act to maintain traits of seeds that ensure their germination at times favorable for seedling survival. What is known of the ecology of *Manihot esculenta* subsp. *flabellifolia,* considered by many to be the wild ancestor of cassava (Allem 1999; Olsen and Schaal 2001), suggests a regeneration niche adapted to periodic disturbances. This and related *Manihot* spp. are light-demanding plants of forest gaps or of savanna or savanna-forest ecotone (Allem 1994). These considerations lead to the hypothesis that domesticated cassava could have inherited a germination biology that was ''pre-adapted'' to Amazonian shifting agriculture.

Has natural selection in agricultural ecosystems led not only to evolutionary maintenance of adaptive traits of seeds, but also to evolutionary modification of the germination biology of domesticated cassava? In natural ecosystems that shaped the evolution of wild cassava, environments are characterized by a continuum in the frequency and intensity of disturbance, ranging from small forest gaps, sometimes with partial vegetation cover, to large clearings created by disturbances such as fire. In contrast, in agroecosystems based on swidden cultivation, disturbance regimes (e.g., size, intensity, and frequency of disturbances) are simpler and form greater environmental contrasts. The critical distinction between environments where cassava seedlings can survive and those where they cannot is simple: field (even more restrictively, a newly burnt field) or fallow. Seeds of domesticated cassava may thus have been selected to germinate only under the narrow range of environmental conditions associated with newly cleared fields, and to avoid germination in old fields and fallows, which present a relatively broad range of environments as a function of the age and development of vegetational cover.

Existing information on domesticated cassava and its wild relatives is consistent with an adaptive interpretation of their germination ecology. First, in domesticated cassava, high temperatures stimulate germination, via two different effects. (1) Dry heat treatments of 60°C or more, often continued over a period of days or weeks, enhance the proportion of seeds that germinate (M. A. Guzman, CIAT, Cali, Colombia, pers. comm.). (2) Under moisture conditions favorable for germination, maximum germination of cassava seeds occurred only when temperature for part of the day exceeded 36°C and the mean temperature was at least 33°C (Ellis, Hong, and Roberts 1982, 1985). For wild *Manihot* spp., an alternating temperature regime of 26/38°C (16 h/8 h) is claimed to promote germination (Nassar and Teixeira 1983; for a review see Ellis, Hong, and Roberts 1985). As in many other studies, it is unclear whether germination of *Manihot* seeds is triggered by fluctuation between high and low temperatures, or simply by the high tempera-

tures reached during at least part of the 24-hour cycle (Baskin and Baskin 1998; Ellis, Hong, and Roberts 1982, 1985).

The studies conducted so far on cassava germination have been motivated more by the purely technical question of how to increase germination rates (and thereby obtain more seedlings) in breeding programs, than by the goal of understanding germination ecology in field populations. Consequently, effects of various factors have often been tested on an ad hoc basis, with little reference to conditions experienced in the field. Such reference is necessary for ecologically meaningful germination experiments (Baskin and Baskin 1998). Secondly, experiments have often been conducted using seeds of improved commercial varieties of cassava. Because sexual reproduction via incorporation of volunteer seedlings appears to play a negligible role in populations of these varieties, selection maintaining seed and seedling traits may have been relaxed. The seed and seedling biology of ''modern'' varieties may thus not be representative of the germination ecology of rustic landraces in swidden agriculture practiced by Amazonian Amerindians, the ecological setting in which volunteer seedlings are important and which has shaped much of the evolution of this crop.

In this study, we investigate germination ecology of cassava seeds produced by populations of local varieties grown in shifting agriculture by Makushi Amerindians in Guyana. Volunteer seedlings are known to be important in evolutionary dynamics of these populations (Elias, Panaud, and Robert 2000; Elias, Rival, and McKey 2000; Elias et al. 2001a,b; McKey et al. 2001). We pose the following questions:

(1) Could fire, used to clear fields, facilitate germination of seeds in the soil seed bank? We could not test this question directly. Instead, we tested the effect on germination of a prolonged dry heat treatment. If such treatment does enhance germination, the mechanisms responsible depend on the type of dormancy. To test whether physical dormancy is involved, we examined whether the same effect obtained by prolonged dry heat could be obtained by mechanically scarifying seeds, without the heat treatment. We also compared water uptake of cassava seeds in which the seed coat was scarified or not.

(2) What is the effect of light on germination of cassava seeds? Because light penetrates only a few mm into soil (Baskin and Baskin 1998), most seeds buried by ants probably germinate under conditions of complete darkness. We reasoned that light should not be required for germination of cassava seeds. In many light-demanding pioneer species, light quality affects germination, which is inhibited by far red light typical of that filtered by vegetation cover and stimulated under red light typical of gaps inside the forest (Vazquez-Yanes 1980). Two previous studies claimed that exposure to red light (600–700 nm) before being sown in darkness (Nartey 1978) or sown under incandescent light (Mendes 1981) improved germination of cassava seeds in comparison to exposure to incandescent light. We question the ecological relevance of this finding for cassava, because these studies, like all other studies conducted on cassava so far, did not compare germination in darkness with other treatments (in some studies [e.g., Mendes 1981; Ellis, Hong and Roberts 1982] light conditions used in the experiments are not specified). The positive effect of red light could have been due to the lower quantity of radiation received by seeds exposed to this light compared to normal light, rather than to any difference in light quality.

(3) What is the effect of the caruncle on seed germination? Few studies have examined the effect of presence or absence of the elaiosome on germination of ant-dispersed seeds (Baskin and Baskin 1998). In *Ricinus communis* L. (Euphorbiaceae), the caruncle plays a role in water exchange, channeling water from the developing seed to reduce its water content and channeling water from humid soils into the imbibing seed, enhancing germination (Bianchini and Pacini 1996). In some ant-dispersed Euphorbiaceae, such as *Mercurialis annua* L. (Pacini 1990) and *R. communis* (Lagôa and Pereira 1987), the caruncle impedes germination by physical and/or chemical mechanisms, and germination occurs rapidly only if the caruncle is removed. In cassava, we considered it unlikely that the caruncle would have any positive effect, because in natural conditions it is physically removed by ants, or decomposed during the seed's tenure in the soil seed bank, long before the seed germinates.

(4) Could relatively high soil temperatures be used by cassava seeds as a cue to trigger their germination in newly cleared and burnt fields, whereas lower temperatures, close to those of shaded soil surfaces in the lowland tropics,

maintain seed dormancy? Using a combination of pre-treatments and treatments found to produce high germination rates in the first experiment (mechanical scarification, darkness, removal of the caruncle), we varied only the temperature at which seeds were placed to imbibe (25°C, representative of temperatures in shaded soils, and 35°C, representative of soil with little or no vegetational cover).

(5) Is the response of seeds of wild cassava to potential germination cues similar to that of seeds of domesticated cassava? Because few seeds of wild cassava were available, only their response to one factor could be tested. The response tested was not that to dry heat pre-treatment, of which an important effect could be thermal scarification of the seed coat, but rather the response of already scarified seeds to incubation at temperatures that in nature would be experienced in soils with (25°C) or without (35°C) vegetational cover. We examined whether scarified seeds of domesticated cassava, whose regeneration niche is restricted to newly burnt fields, have a more stringent requirement for high temperature to trigger germination than do those of wild cassava. Because seeds of wild and domesticated cassava could only be obtained from one or two localities for each taxon, this experiment must be regarded as preliminary.

MATERIALS AND METHODS

MATERIALS

Seeds of domesticated cassava used in the experiments were collected from fields of Makushi Amerindian farmers of bitter cassava, in the village of Rewa (4°02′ N, 58°35′ W), North Rupununi district, Guyana. The study site, agricultural practices, and varietal composition of cassava grown by Rewa villagers are described by Elias and McKey (2000) and Elias, Rival and McKey (2000). Seeds of wild cassava were collected from two populations of *M. esculenta* ssp. *flabellifolia,* the taxon indicated by recent studies to be the ancestor of domesticated cassava (Olsen and Schaal 1999, 2001). The first population was located near the village of Toka, the other near the village of Annai (for locations see Elias and McKey 2000). Mature capsules, dry and ready to dehisce, were collected from plants haphazardly over a period of about two weeks (for domesticated cassava) and on two separate one-day visits to the wild populations. Capsules

were placed in paper bags in the sun and allowed to explode. Seeds were separated from the capsule fragments and stored dry at room temperature in plastic bags. All seeds were collected in November 1998. Seeds were kept at room temperature under dark conditions until January 1999. At this time, they were moved into a cold room at 5°C, where they were stored in darkness until being used in the experiments. Germination experiments were conducted in July 1999 (experiment 1), August 1999 (experiment 2) and February–March 2000 (experiment 3).

METHODS

To test whether the stored seeds were viable, we first conducted (July 1999) 2,3,5-triphenyl tetrazolium chloride viability tests on a haphazardly selected subsample of 20 seeds following the protocol of Kearns and Inouye (1993). After 3 h seeds were scored for staining of the embryo. Of the 20 seeds tested, one was dead; its seed coat was normal but empty. The remaining 19 seeds were viable, as indicated by the bright pink staining of the cotyledons and the rest of the embryo.

To determine whether dry heat treatment enhances germination of domesticated cassava, and whether mechanical scarification of the seed coat has the same effect, we compared germination of seeds subjected to each of these treatments with that of a control group. Before the experiment, seeds were cleaned in a weak solution of bleach (5ml.l^{-1}) containing a few drops of liquid soap, then washed two times with deionized water and dried at room temperature. Three lots of 200 seeds each were prepared. The first lot was subjected to dry heat treatment. Following the procedure used to prepare seeds of domesticated cassava for germination at the CIAT (International Centre for Tropical Agriculture, Cali, Colombia), seeds were kept in an oven at 65°C during 14 days. In the second lot, each seed was mechanically scarified. Using a hand-held precision electric drill fitted with a small bit, a small area (<1 mm^2) of seed coat was removed at the end of the seed opposite the attachment of the caruncle. Scarification at this location enabled us to avoid damaging the embryo. The third lot constituted a control with no pre-treatment.

Each of the three lots was divided into four sub-lots of 50 seeds, each subjected to a different combination of two treatments: light (12 h

light and 12 h darkness, or permanent darkness) and caruncle (intact or removed). Each sub-lot was divided into five replicate groups of 10 seeds each. Seeds were placed by group on a sheet of moist filter paper enclosed in a booklet of two corrugated plastic cards held together by elastic bands. Booklets were placed vertically (with the end of the seed bearing the caruncle at the bottom) in a plastic container with sufficient de-ionized water to keep the filter paper saturated with moisture. Containers were placed in an incubation chamber maintained at 35℃ by a thermostat; a thermometer placed in the chamber allowed periodic checking of temperature. The artificial daylight was given by a small fluorescent warm white tube (8W). Seeds were checked for germination once daily. These checks were conducted under normal room lighting (common white fluorescent tubes). For the ''darkness'' treatment, green safe light (Baskin and Baskin 1998) was not available. Thus seeds in the darkness treatment received artificial light for about 30 minutes during each daily check.

A seed was scored as having germinated when the radicle was seen protruding through the split testa. The experiment ran for 21 d. Seeds that had not germinated at the end of the experiment were tested for viability using tetrazolium.

Probability of germination of seeds subjected to different combinations of treatments was analyzed using the GENMOD procedure of SAS v8 (SAS 1996), for binomial distribution and Type III analysis. Pre-treatment (mechanical scarification, dry heat, control), light (darkness, light/dark cycle) and caruncle (present, removed) were used as explanatory variables. We also tested for a ''booklet'' effect. We used backwards selection to identify the factors with the most significant effects in the full model. Data on time to germination (considering only seeds that did germinate) was analyzed using a type III generalized linear model (GENMOD procedure of SAS v8 [SAS 1996]) for Poisson distribution (log linear model) in a backwards selection model using the same explanatory variables.

The second experiment was conducted to examine the effect of temperature during imbibition on germination of seeds of domesticated cassava. In this experiment, all seeds used were subjected to a combination of treatments shown in the first experiment to lead to high rates of germination: mechanical scarification of the seed coat, removal of the caruncle, and darkness. Although leaving the caruncle intact led to somewhat higher germination in the first experiment (an effect seen mostly in the presence of light), removal of the caruncle reduced fungal contamination of germinating seedlings. Only the temperature during imbibition was manipulated. Five lots of seeds (23, 20, 20, 20, and 18 seeds) totaling 101 seeds were set to germinate at 35℃, as in the first experiment, and five other lots (four lots of 20 seeds and one with 18 seeds) totaling 98 seeds were set to germinate at 25℃. Each lot was placed in a separate booklet, as in the first experiment. Probability of germination at the two temperatures and time to germination were compared as in the first experiment.

The third experiment compared the effect of temperature during imbibition on germination of seeds of domesticated (210 seeds) and wild (70 seeds) cassava. As in the second experiment, all seeds were mechanically scarified, the caruncle was removed, and seeds were set to germinate in darkness. For each taxon, half the seeds were kept at 35℃ and half at 25℃. The small number of seeds of wild cassava available for the experiment did not allow replication of different booklets for each taxon. Seeds of domesticated and wild cassava were thus mixed on the same booklet, and the position of each seed was randomized. Probability of germination was analyzed as in the first experiment, using taxon and temperature as explanatory variables.

Mean time to germination was compared among the three experiments using ANOVA, taking into consideration only those seeds of each experiment that were subjected to the same combination of treatments (mechanical scarification, caruncle removed, darkness, 35℃).

Two complementary experiments were also conducted. First, to test the effect of our storage conditions on seed viability, in June 2001 we conducted tetrazolium tests on two lots of 20 seeds. One lot was from the stock of seeds that was used for all experiments. These seeds had been stored in darkness at 5℃ since January 1999, and had not been used in any of these experiments. The other lot was taken from seeds that had been used in experiment 1 and had not germinated. These seeds had been in darkness at 5℃ since July 1999. Second, in an attempt to determine whether seed coats of cassava are impermeable to water, resulting in physical dor-

TABLE 1. PROPORTION OF SEEDS OF DOMESTICATED CASSAVA THAT GERMINATED FOLLOWING DIFFER-ENT COMBINATIONS OF PRE-TREATMENTS AND TREATMENTS (EXPERIMENT 1)

Pre-treatment	Light	Caruncle present	Caruncle removed
Mechanical scarification: 148/200 (74%)	12:12 light:dark: 67/100 (67%)	40/50 (80%)	27/50 (54%)
	24 h dark: 81/100 (81%)	44/50 (88%)	37/50 (74%)
Dry heat (65°C dry heat for 14 days): 110/200 (55%)	12:12 light:dark: 43/100 (43%)	28/50 (56%)	15/50 (30%)
	24 h dark: 67/100 (67%)	34/50 (68%)	33/50 (66%)
Control (no pre-treatment): 45/200 (22.5%)	12:12 light:dark: 21/100 (21%)	15/50 (30%)	6/50 (12%)
	24 h dark: 24/100 (24%)	12/50 (24%)	12/50 (24%)
TOTAL: 303/600 (50.5%)	**Total** 12:12 light:dark: **131/300** (43.7%) **Total** 24 h dark: **172/300** (57.3%)	**Total: 173/300** (57.7%)	**Total: 130/300** (43.3%)

mancy, we compared water uptake by seeds that had been mechanically scarified and seeds that had been left intact. This test was conducted in May 2002 using seeds of domesticated cassava from the same stock used in the other experiments. Four lots of 20 seeds each were tested, one for each combination of two variables, temperature (25°C or 35°C) and seed coat treatment (scarified or intact). Seeds were weighed, placed in booklets under saturated humidity conditions, and then re-weighed after 24 h and 92 h of imbibition. A seed was scored as being imbibed if it gained more than 0.01 g of mass.

RESULTS

Table 1 presents the results for proportion of seeds germinated under the 12 different combinations of pre-treatments and treatments in Experiment 1. Table 2 presents the results of the

TABLE 2. RESULTS OF THE GENERALIZED LINEAR MODEL (PROC GENMOD OF SAS v8 [SAS 1996], USING BINOMIAL DISTRIBUTION) PERFORMED ON PROBABILITY OF GERMINATION IN EXPERIMENT 1. TYPE III ANALYSIS ON THE MODEL THAT WAS RETAINED AFTER BACKWARDS SELECTION PERFORMED ON THE COMPLETE MODEL.

| Source	df	χ^2	P
Pre-treatment	2	120.43	0.0001
Caruncle	1	15.47	0.0001
Light	1	14.06	0.0002
Caruncle $\times$ light	1	6.02	0.0141

generalized linear model performed on these results. There was no booklet effect. All other factors tested contributed significantly to explaining the variance. There was also a significant interaction between light and caruncle treatments. Among the different combinations of treatments, probability of germination was highest (88%) for seeds scarified mechanically, with the caruncle intact, and kept in darkness, and lowest (12%) for seeds subjected to no pre-treatment, with the caruncle removed, and kept in a 12 h: 12 h light:dark regime.

EFFECT OF PRE-TREATMENTS

Type of pre-treatment had the greatest effect on probability of germination (Table 2). Both dry heat and mechanical scarification of the seed coat greatly increased the probability of germination compared to controls (no pre-treatment; see Table 1). Probability of germination was higher with mechanical scarification than with dry heat (PROC GENMOD, SAS [SAS 1996]; least squares means comparison, $P < 0.0001$), and higher with dry heat than with no pre-treatment ($P < 0.0001$). Seeds subjected to each of these pre-treatments responded similarly to the light treatment and to removal of the caruncle.

EFFECT OF LIGHT

Probability of germination was significantly higher in the 'darkness' treatment than under a regime of 12 h light and 12 h dark (Table 2). Although the interaction between light and pre-treatment was not significant, the depressing ef-
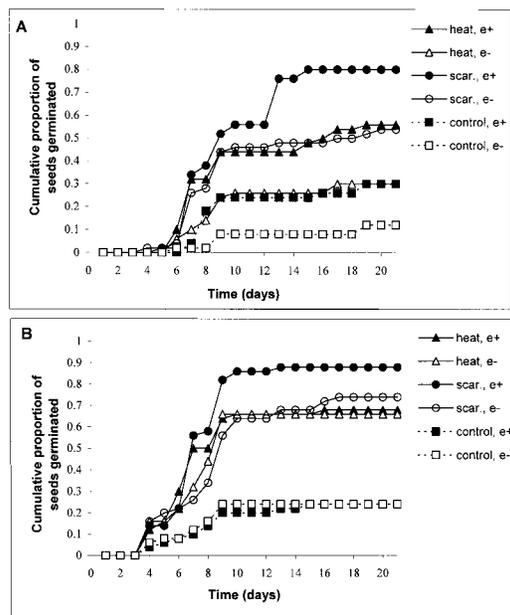
Fig. 1. Time course of germination of seeds of domesticated cassava for each of the 12 combinations of treatments, experiment 1. A: with 12 h light and 12 h darkness; B: darkness. N = 50 seeds tested in each of the 12 combinations of treatments.

EFFECT OF THE CARUNCLE

Probability of germination was significantly higher when the caruncle was intact than when it had been mechanically removed. This effect did not vary among pre-treatments. However, there was a significant interaction between this treatment and light (Table 2). Removal of the caruncle had little negative effect in darkness, but substantially reduced the proportion of seeds that germinated in light (Table 1).

SPEED OF GERMINATION

Figure 1 shows the time course of germination in Experiment 1. Germination began four days after the start of the experiment. Peak germination was between 5–10 days for all treatments. Germination in all combinations of treatments had ceased by 20 days. The experiment was terminated after 21 days. Considering only seeds that did germinate, there was relatively little variation in speed of germination among pre-treatments and treatments (Table 3; Fig. 1). Of the factors tested, only light significantly affected speed of germination, with seeds left in darkness germinating more rapidly (mean $\pm$ s.d.: 7.4 $\pm$ 2.6 d) than those maintained in a 12 h light: 12 h dark cycle (mean $\pm$ s.d.: 9.5 $\pm$ 3.7 d). This difference was significant ($\chi^2_1 = 37.7$, $P < 0.0001$). The effect of light on speed of germination was similar for all three pre-treatments, and did not depend on presence or absence of the caruncle (Table 3).

fect of light on probability of germination appeared to be less pronounced in the control (no pre-treatment) than in the two pre-treatment groups (Table 1).

TABLE 3. TIME TO GERMINATION (IN DAYS; CALCULATED ONLY FOR SEEDS THAT GERMINATED) OF SEEDS OF DOMESTICATED CASSAVA SUBJECTED TO DIFFERENT COMBINATIONS OF PRE-TREATMENTS AND TREATMENTS, EXPERIMENT 1. NUMBERS OF SEEDS GERMINATED FOR EACH COMBINATION ARE PRESENTED IN TABLE 1.

Pre-treatment	Light	Caruncle present	Caruncle removed
Mechanical scarification: 8.5 $\pm$ 3.0	12:12 light:dark: 9.2 $\pm$ 3.7	9.4 $\pm$ 2.8	9.1 $\pm$ 3.8
	24 h dark: 7.7 $\pm$ 2.7	7.3 $\pm$ 2.0	8.3 $\pm$ 3.5
Dry heat (65°C dry heat for 14 days): 7.9 $\pm$ 3.1	12:12 light:dark: 9.2 $\pm$ 3.7	9.3 $\pm$ 4.0	9.1 $\pm$ 3.4
	24 h dark: 6.9 $\pm$ 2.1	6.9 $\pm$ 2.4	7.0 $\pm$ 2.0
Control (no pre-treatment): 8.9 $\pm$ 4.0	12:12 light:dark: 10.5 $\pm$ 4.5	10.1 $\pm$ 4.2	11.8 $\pm$ 5.7
	24 h dark: 7.5 $\pm$ 2.7	8.1 $\pm$ 3.3	6.9 $\pm$ 2.1
TOTAL: 8.3 $\pm$ 3.2	**Total** 12:12 light:dark: 9.5 $\pm$ 3.7 **Total** 24 h dark: 7.4 $\pm$ 2.6	**Total**: 8.3 $\pm$ 3.1	**Total**: 8.2 $\pm$ 3.4
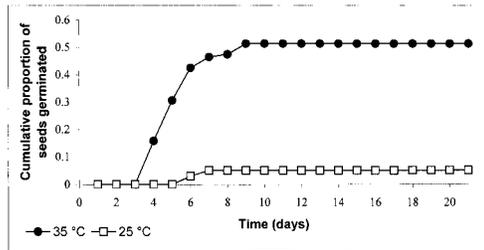
Fig. 2. Time course of germination of seeds of domesticated cassava in experiment 2. Squares: 25°C (N = 98 seeds tested); circles: 35°C (N = 101 seeds tested).

TABLE 4. RESULTS OF THE GENERALIZED LINEAR MODEL (PROC GENMOD OF SAS v8 [SAS 1996], USING BINOMIAL ERROR AND TYPE III ANALYSIS) PERFORMED ON PROBABILITY OF GERMINATION IN EXPERIMENT 3, USING AS EXPLANATORY VARIABLES TEMPERATURE (25°C OR 35°C) AND TAXON (WILD OR DOMESTICATED CASSAVA).

Source	df	χ^2	P
Temperature	1	45.25	0.0001
Taxon	1	15.53	0.0001
Temperature × taxon	1	8.68	0.0032

VIABILITY OF SEEDS THAT DID NOT GERMINATE DURING THE EXPERIMENT

Following experiment 1, tetrazolium tests were conducted on 60 seeds that did not germinate during the experiment (5 seeds were taken from each of the 12 combinations) and on 10 seeds taken from the original stock and not subjected to the experiment. These tests showed that 56 of the ungerminated seeds (93%) and nine of the control seeds (90%) were still viable, suggesting that none of the treatments used in the experiment killed any significant number of seeds.

EFFECT OF TEMPERATURE ON GERMINATION OF SEEDS OF DOMESTICATED CASSAVA (EXPERIMENT 2)

A much larger proportion of seeds (mechanically scarified, caruncle removed, left in darkness) germinated at 35°C (52 of 102 seeds, 51.5%) than at 25°C (5 of 98 seeds, 5.1%). This difference was significant (χ^2_1 = 17.2, P < 0.0001). Within each temperature, there was no booklet effect. For those seeds that did germinate, germination was somewhat more rapid at 35°C (mean ± s.d.: 5.4 ± 1.4 d) than at 25°C (mean ± s.d.: 6.4 ± 0.5 d) (Fig. 2). This difference was significant (PROC GENMOD, SAS [SAS 1996]; type III analysis, for Poisson distribution: χ^2_1 = 17.17, P < 0.0001).

COMPARISON OF THE EFFECT OF TEMPERATURE ON GERMINATION OF SEEDS OF DOMESTICATED AND WILD CASSAVA (EXPERIMENT 3)

As in the second experiment, a much larger proportion of seeds of domesticated cassava germinated at 35°C (40 of 105 seeds, 38.1%) than at 25°C (0 of 105 seeds). The same was true for

seeds of wild cassava, with 17 of 35 seeds (48.5%) germinating at 35°C and 7 of 35 seeds (20%) germinating at 25°C. Results of the generalized linear model performed on these data are presented in Table 4. There was no booklet effect. Both temperature and taxon (wild or domesticated) significantly affected probability of germination, and the interaction between the two was also significant. A smaller proportion of seeds of domesticated cassava germinated overall, and the effect of temperature on probability of germination was more marked for domesticated than for wild cassava. Least squares means comparisons (PROC GENMOD, SAS [SAS 1996]) showed that probability of germination was lower at 25°C than at 35°C for both wild (P = 0.014) and domesticated cassava (P < 0.0001). Probability of germination of wild and domesticated cassava was not different at 35°C (χ^2_1 = 1.2, P = 0.28), but was higher for wild cassava than for domesticated cassava at 25°C (χ^2_1 = 8.9, P = 0.0028). Figure 3 shows the time
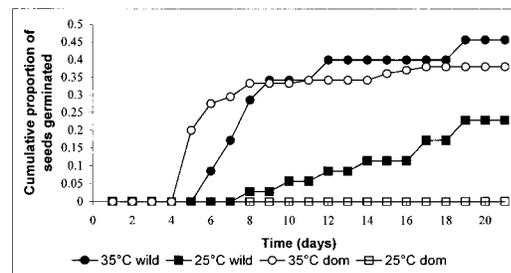
Fig. 3. Time course of germination in experiment 3. Open symbols: Domesticated cassava; closed symbols: wild cassava. Squares: 25°C; circles: 35°C. For domesticated cassava, N = 105 seeds tested for each temperature treatment; for wild cassava, N = 35 seeds tested for each temperature treatment.

TABLE 5. RESULTS OF THE GENERALIZED LINEAR MODEL (PROC GENMOD OF SAS V8 [SAS 1996], USING A LOGIT TRANSFORMATION FOR ANALYZING BINOMIAL DISTRIBUTIONS) PERFORMED ON THE PROPORTION OF SEEDS THAT TOOK UP WATER AFTER 24 AND 92 H, USING AS EXPLANATORY VARIABLES TEMPERATURE (25°C OR 35°C) AND PRE-TREATMENT (SCARIFIED OR UNSCARIFIED).

Imbibition period	After 24 hours			After 92 hours		
Source	df	χ^2	P	df	χ^2	P
Temperature	1	0.90	0.3418	1	0	1
Pre-treatment	1	6.76	0.0093	1	5.76	0.0164
Temperature $\times$ pre-treatment	1	0.02	0.8761	1	0	1

course of germination in this experiment. At 35°C, seeds of domesticated cassava germinated more rapidly (mean ± s.d. = 6.8 ± 3.3 d) than did those of wild cassava (mean ± s.d. = 9.4 ± 4.0 d). Seeds of wild cassava germinated more slowly at 25°C (mean ± s.d. = 14.5 ± 4.2 d) than at 35°C. Differences were significant (least squares means comparisons) for all three possible pairwise comparisons (wild vs. domesticated, at 35°C: $P = 0.0458$; wild at 25°C vs. wild at 35°C: $P = 0.0056$; domesticated at 35°C vs. wild at 25°C: $P < 0.0001$).

PROPORTION OF SEEDS GERMINATED AND TIME TO GERMINATION AS A FUNCTION OF TIME SINCE COLLECTION

Comparing all three experiments, and taking into account only seeds of domesticated cassava subjected to the same combination of conditions (seeds mechanically scarified, caruncle removed, seeds left to imbibe in darkness at 35°C), the proportion of seeds germinated decreased significantly over time, from 74.0% in experiment 1 (conducted 8 mo after seeds were collected) to 51.5% in experiment 2 (9 mo after collection), to 38.1% in experiment 3 (15 mo after collection). Under the conditions of storage, there thus seemed to be a decline in the ability to germinate of cassava seeds over time. The cause of this decreased ability to germinate was not seed death. Of 20 seeds from the base stock, stored since January 1999, 18 were viable in June 2001. All of the 20 ungerminated seeds from experiment 1 that were tested for viability were still alive in June 2001. These results demonstrate that decreased ability to germinate over time was due to reinforced dormancy of seeds under the conditions of storage.

Again comparing only seeds subjected to the same combination of treatments (mechanical scarification, caruncle removed, darkness, 35°C),

mean time to germination was significantly different among experiments (ANOVA, $F_{2, 128} = 11.84$, $P < 0.0001$), being highest in the first experiment, lowest in the second experiment, and intermediate in the third experiment. Least squares means comparisons showed that mean time to germination was significantly different between each pair of experiments (experiments 1 and 2: $P < 0.0001$; experiments 1 and 3, and experiments 2 and 3: $P = 0.0489$). Inspection of the cumulative number of seeds germinated over time (Figures 1–3) shows that germination began at about the same time in all experiments (day 4 in the first two experiments, day 5 in the third), but continued over periods very different among experiments. In experiment 1, in which the highest proportion of seeds germinated (74%), germination continued until day 17. In experiment 2, in which only 51.5% of seeds germinated, the last germination observed occurred on day 9. However, the pattern suggested by the comparison of experiments 1 and 2 did not continue over time. In experiment 3 (38.1% germination), germination continued until day 17, as in experiment 1.

IMBIBITION OF CASSAVA SEEDS

Mechanical scarification had a significant effect on the probability of water uptake by seeds. Temperature did not influence whether seeds imbibed or not, and there was no significant interaction between temperature and scarification (Table 5). After 24 h, 87.5% of scarified seeds had imbibed water, whereas 62.5% of unscarified seeds had imbibed. After 92 h, all of the scarified seeds and 90% of the unscarified seeds had imbibed.

Considering only seeds that did imbibe, the amount of water absorbed after 24 h was not affected by any of the variables studied. Despite a tendency ($\chi^2_1 = 3.52$, $P = 0.0606$), water up-

take did not significantly differ between scarified and unscarified seeds after 24 h (means 15% and 11%, respectively, of initial weight). However, after 92 h, there was a significant effect of scarification on amount of water absorbed (means 23% and 17% of initial weight, for scarified and unscarified seeds, respectively). Water uptake by imbibed seeds after 92 h was significantly increased by mechanical scarification ($\chi^2_1 = 7.85$, $P = 0.0051$). Imbibition was thus enhanced by scarification but did not require it.

DISCUSSION

MANIHOT ESCULENTA AS A DISTURBANCE-ADAPTED PLANT

The germination ecology of domesticated cassava exhibits several traits characteristic of plants adapted to disturbed habitats. Seeds remain viable and dormant for long periods if conditions are unfavorable for germination and seedling growth. Dormancy is largely physiological in nature (as in many Euphorbiaceae: Baskin and Baskin 1998), being responsive to temperature and to light conditions. Germination only occurs when temperatures are relatively high, which in nature would occur only in soil lacking a vegetation cover, signaling conditions likely to be favorable for germination. This is similar to light-demanding species that use high soil temperature, or high and variable soil temperature, as a cue indicating exposed sites lacking vegetational cover (Baskin and Baskin 1998; Egley 1995; Fenner 1995; Keeley 1991; Thompson and Grime 1983). Germination is also enhanced by darkness. A further indication of physiological dormancy is the increasingly deep dormancy observed with progressively long periods of seed storage at low temperatures.

Germination ecology of cassava appears to be particularly well suited to habitats in which fire is a frequent type of disturbance. Having remained dormant for years in fallow vegetation, large numbers of cassava seeds rapidly germinate in newly burned fields. Several traits of germination ecology appear to constitute a functionally linked set of adaptations to fire.

First, dry heat stimulates germination of cassava seeds. Although physiological mechanisms may play a role in this response, part of the effect may be due to enhanced passage of water into the seed (reviewed in Baskin and Baskin [1998]), because mechanical scarification replaced the effect of dry heat treatment. Our imbibition experiment showed that whereas imbibition was enhanced by mechanical scarification, most unscarified seeds eventually imbibed some water. We cannot rule out the possibility that seed coats of seeds used in the experiment had become permeable as a result of our storage conditions (cf. Baskin and Baskin 1998). Although physical dormancy is so far unknown in seeds of euphorbiaceous species, it should be tested for in further studies of cassava using more natural conditions of seed storage. Seeds of some plants are known to combine physical and physiological dormancy (Baskin and Baskin 1998).

Second, cassava seeds possess adaptations for ant dispersal. Burial of cassava seeds by ants (Elias and McKey 2000), as typically occurs in many fire-adapted plants (Baskin and Baskin 1998), may protect seeds not only from surface-foraging predators, but also from lethal temperatures that may occur on the soil surface during fire (Espalader and Gomez 1996; Bülow-Olsen 1984; Passos and Ferreira 1996; Baskin and Baskin 1998). The negative response of cassava seeds to light is related to the importance of seed burial in its germination ecology. Seeds of *Ricinus communis,* another myrmecochorous Euphorbiaceae, are also negatively photoblastic (Lagôa and Pereira 1987). Also, like most other seeds that germinate more readily in darkness, cassava seeds are relatively large compared to those of other disturbance-adapted plants, reflecting the requirement for larger amounts of reserves when seeds germinate deep in the soil, than when they require light and germinate only near the soil surface (Baskin and Baskin 1998).

RESPONSE TO LIGHT AND TO REMOVAL OF THE CARUNCLE

Presence of light decreased the probability of germination, and slowed the speed of germination when it did occur. At least under the conditions in our experiment, light thus appeared to have a negative effect, exerted by unknown physiological mechanisms, on cassava seeds. Interestingly, light had little effect on probability of germination of seeds in which the caruncle was left intact, and of seeds that were not subjected to any pre-treatment (Table 1). In negatively photoblastic seeds that have been studied, the embryo and/or endosperm are the light-sensitive organs (Baskin and Baskin 1998). In our experiment, mechanical scarification would cer-

tainly have increased the amount of light transmitted to these organs. Fissuring of seed coats by dry heat, and removal of the caruncle, could have had the same effect. Thus the interactions of these treatments with light may all be due to a single common mechanism. In nature, seeds in the soil quickly lose the caruncle. Because under natural conditions seeds are buried in soil and thus normally germinate in darkness, absence of the caruncle is unlikely to have a negative effect on germination.

Once dormancy was broken, light also had a negative effect on the speed of germination. In contrast to the results for proportion of seeds germinated, the effect of light on speed of germination was not modified by any of the other factors tested. We suggest the following explanation for this result. Germination involves splitting of the seed coat. Light, in combination with other treatments, affects the probability that this will happen, but once germination did begin, the seed coat split and embryos and/or endosperm of all seeds in the light treatment were exposed to light, regardless of what other treatments are applied. Thus seeds that germinated in light all did so at the same speed.

A shortcoming of our experiment was that pre-treatments and preparation of the experiment were conducted in normal light. Light perceived during this phase could thus have reduced the difference between the two light treatments. However, the effect of light appeared to depend on treatments that allowed transmission of light across the seed coat, suggesting that light received by seeds before treatments to the seed coat may have had little effect. Another shortcoming of our experimental design is that periodic examination of seeds in the darkness treatment was conducted under normal light, not green light, to which plants are insensitive. Seeds were thus exposed to a few minutes of light every day when checked for germination. Some seeds are extremely light-sensitive, a brief pulse of light sufficing to trigger germination, presumably because it signals proximity to the surface (Baskin and Baskin 1998).

Although such possibilities cannot be excluded for cassava seeds, it is clear that their response to light is overwhelmingly negative. Because these shortcomings noted in our experimental design all tend to reduce the contrast between the light and darkness treatments, we suggest that their most likely effect is to lead to an underestimation of the negative impact of light. Our results would thus likely be strengthened, rather than weakened, by an improved experimental design.

COMPARISON OF GERMINATION IN THE THREE EXPERIMENTS

Our results showed a decrease in the probability of germination over time, although tetrazolium tests revealed that stored seeds were still alive in June 2001. We suspect that this decrease is due to the unnatural conditions of storage (at 5°C beginning January 1999). In nature, large numbers of cassava seeds stored in soil seed banks remain capable of germination after fallow periods ranging from 3 to 30 y, and anecdotal evidence suggests that seeds may remain viable for at least 45 y (Elias, Rival, and McKey 2000). Under our conditions of storage, seeds appear to have developed a deeper dormancy less easily overcome by our treatments. For seeds of many species, requirements for germination in the case of physiological dormancy are known to be affected by storage conditions (Baskin and Baskin 1998). Unnatural conditions of storage, or uncontrolled differences between experiments that may have escaped our notice (for example, the amount of time seeds were exposed to light during daily checks), may also be responsible for the significant differences among experiments in mean time to germination, differences for which we have no other explanation. In future experiments, seeds should be stored under conditions closer to those experienced by dormant cassava seeds in nature.

COMPARISON WITH WILD CASSAVA: IMPLICATIONS FOR THE EVOLUTION OF DOMESTICATION

Our results suggest that seeds of domesticated cassava may have a more stringent temperature requirement for germination, once they have been scarified, than those of wild cassava. This could be evidence of an adaptive response to disturbance regimes in slash-and-burn agriculture, in which only a narrow subset of environmental conditions is suitable for germination. Our results also suggest that under these favorable conditions seeds of domesticated cassava germinate more rapidly than those of wild cassava. This could represent an adaptation to the field environment, characterized by relatively rich resources but also intense competition.

These results require confirmation by experiments including replicates of several populations of each taxon. If confirmed, they imply that traits relating to seed dormancy and germination are part of the domestication syndrome of this vegetatively propagated crop. Whether the germination ecology of domesticated cassava includes specific adaptations to agriculture or only pre-adaptation, it is clear that selection on seed dormancy and dispersal during domestication acted on cassava in a way very different from its action on these traits in seed-propagated, annual crops, in which mechanisms for dormancy and dispersal were lost (Baskin and Baskin 1998).

The germination ecology of cassava may yield insights on the process of domestication. Our results are consistent with the notion that domesticated cassava evolved from a fire-adapted, fire-following ancestor (Tardy 1998), probably initially restricted to the more seasonal habitats in which lightning-caused fires are frequent (Pyne 1998). Favored by anthropogenic fires, the wild ancestor of cassava could have been one of the resources that responded strongly to use of fire in habitat management by pre-agricultural peoples. Cultivation of cassava by stem cuttings may have originated via attempts by foraging peoples to supplement the density in newly burned areas of spontaneous seedlings and regrowth of top-killed plants. Such supplementation may have been most crucial, and most feasible, in wetter habitats where virtually all fires were anthropogenic (Pyne 1998). In any case, cassava was eminently pre-adapted to slash-and-burn agriculture, which enabled spread of this plant into habitats much wetter than those occupied by its wild ancestors. The spread of this major crop plant may thus reflect the working of the same human actions postulated to have dramatically shifted the distribution of many wild and semidomesticated plant species in Amazonia (Balée 1993).

APPLICATIONS OF SEXUAL REPRODUCTIVE ECOLOGY IN MANAGING VEGETATIVELY PROPAGATED CROPS

Unmanaged sexual reproduction is a key process in the demographic and genetic dynamics of populations of several vegetatively propagated crop plants grown in traditional agroecosystems. Incorporation of volunteer seedlings into germplasm for vegetative propagation is impor-

tant in crops such as sweet potato (Yen 1974), potato (Johns and Keen 1986), and ensete (Shigeta 1996), as well as in cassava. Germination and establishment of volunteer seedlings that escape human control, and their incorporation into propagation material, have been little studied. Information on these phenomena is crucial to understanding evolution in populations and for designing on-farm strategies of conservation of crop plant genetic diversity in traditional agroecosystems (Brush 1995; McKey et al. 2001).

This information could also be useful in scientific breeding programs. Local varieties of crop plants constitute genetic resources that are often poorly represented in ex situ collections (Elias, Panaud, and Robert 2000; Roa et al. 1997; Sambatti, Martins, and Ando 2001; Second et al. 1997; Soleri and Smith 1995). Knowing how to manipulate these resources is a prerequisite for their efficient use. Seed biology, in particular factors controlling germination, is a relatively poorly understood area in cassava breeding (Biggs, Smith, and Scott 1986; Fregene, Ospina, and Roca 1999). Finally, understanding this aspect of cassava reproductive ecology is important to estimate the potential for spread of transgenes in crop populations following introduction of genetically modified varieties (Ellstrand, Prentice, and Hancock 1999; Wilson 1990).

LITERATURE CITED

Allem, A. C. 1994. The origin of *Manihot esculenta* Crantz (Euphorbiaceae). Genetic Resources and Crop Evolution 41:133–150.

———. 1999. The closest wild relatives of cassava (*Manihot esculenta* Crantz). Euphytica 107:123–133.

Balée, W. 1993. Indigenous transformation of Amazonian forests. L'Homme 33:231–254.

Baskin, C. C., and J. M. Baskin. 1998. Seeds. Ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego.

Bianchini, M., and E. Pacini. 1996. The caruncle of *Ricinus communis* L. (castor bean): its development and role in seed dehydration, rehydration, and germination. International Journal of Plant Sciences 157:40–48.

Biggs, B. J., M. K. Smith, and K. J. Scott. 1986. The use of embryo culture for the recovery of plants from cassava (*Manihot esculenta* Crantz) seeds. Plant Cell, Tissue and Organ Culture 6:229–232.

Boster, J. S. 1984. Classification, cultivation, and selection of Aguaruna cultivars of *Manihot esculenta* (Euphorbiaceae). Advances in Economic Botany 1: 34–47.

Brush, S. B. 1995. In situ conservation of landraces in centers of crop diversity. Crop Science 35:346–354.

Bülow-Olsen, A. 1984. Diplochory in *Viola*: a possible relation between seed dispersal and soil seed bank. American Midland Naturalist 112:251–260.

Cury, R. 1993. Dinâmica evolutiva e caracterização de germoplasma de mandioca (*Manihot esculenta* Crantz), na agricultura autóctone do Sul do Estado de São Paulo. Master's thesis, Univ. São Paulo Esc. Super. Agric.–Luiz de Queiroz, Piracicaba, Brazil.

Egley, G. H. 1995. Seed germination in soil: dormancy cycles. Pages 529–544 *in* J. Kigel and G. Galili, eds., Seed development and germination. Marcel Dekker, New York, NY.

Elias, M. 2000. Sélection naturelle, sélection humaine, et diversité chez une plante domestique propagée de façon végétative: le cas de la culture du manioc par les Indiens Makushi du Guyana. Thèse, Université Montpellier II, Montpellier, France.

———, **D. McKey, O. Panaud, M. C. Anstett, and T. Robert.** 2001a. Traditional management of cassava morphological and genetic diversity by the Makushi Amerindians (Guyana, South America): perspectives for on-farm conservation of crop genetic resources. Euphytica 20:143–157.

———, **and** ———. 2000. The unmanaged reproductive ecology of domesticated plants in traditional agroecosystems: an example involving cassava and a call for data. Acta Oecologica 21:223–230.

———, **O. Panaud, and T. Robert.** 2000. Assessment of genetic variability in a traditional cassava (*Manihot esculenta* Crantz) farming system, using AFLP markers. Heredity 85:219–230.

———, **L. Penet, P. Bertolino, P. Vindry, D. McKey, O. Panaud, and T. Robert.** 2001b. Unmanaged sexual reproduction in a vegetatively propagated crop, cassava (*Manihot esculenta* Crantz) and dynamics of genetic diversity assessed with microsatellite markers. Molecular Ecology 10: 1895–1907.

———, **L. Rival, and D. McKey.** 2000. Perception and management of cassava (Manihot esculenta Crantz) diversity among Makushi Amerindians of Guyana (South America). Journal of Ethnobiology 20:239–265.

Ellis, R. H., T. D. Hong, and E. H. Roberts. 1982. An investigation of the influence of constant and alternating temperature on the germination of cassava seed using a two-dimensional temperature gradient plate. Annals of Botany 49:241–246.

———, ———, **and** ———. 1985. Handbook of seed technology for gene banks. 2 vols. International Board for Plant Genetic Resources, Rome.

Ellstrand, N., H. C. Prentice, and J. F. Hancock. 1999. Gene flow and introgression from domesticated plants into their wild relatives. Annual Review of Ecology and Systematics 30:539–563.

Espalader, X., and C. Gomez. 1996. Seed production, predation and dispersal in the Mediterranean myrmecochore *Euphorbia characias* (Euphorbiaceae). Ecography 19:7–15.

Fenner, M. 1995. Ecology of seed banks. Pages 507–528 *in* J. Kigel and G. Galili, eds., Seed development and germination. Marcel Dekker, New York, NY.

Fregene, M., J. A. Ospina, and W. Roca. 1999. Recovery of cassava (*Manihot esculenta* Crantz) plants from culture of immature zygotic embryos. Plant Cell, Tissue and Organ Culture 55:39–43.

Johns, T., and S. L. Keen. 1986. Ongoing evolution of the potato on the altiplano of western Bolivia. Economic Botany 40:409–424.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.

Keeley, J. E. 1991. Seed germination and life history syndromes in the California chaparral. Botanical Reviews 57:81–116.

Lagôa, A. M. M. A., and M. F. A. Pereira. 1987. The role of the caruncle in the germination of seeds of *Ricinus communis.* Plant Physiology and Biochemistry 25:125–128.

Lozano, J. C., and B. L. Nolt. 1989. Pests and pathogens of cassava. Pages 169–182 *in* R. P. Kahn, ed., Plant protection and quarantine, Vol. II. Selected pests and pathogens of quarantine significance. CRC Press, Boca Raton, Florida, USA.

McKey, D., and S. Beckerman. 1993. Chemical ecology, plant evolution and traditional manioc cultivation systems. Pages 83–112 *in* C. M. Hladik, A. Hladik, H. Pagezy, O. F. Linares, G. J. A. Koppert and A. Froment, eds., Tropical forests, people and food, UNESCO, Paris.

———, **L. Emperaire, M. Elias, F. Pinton, T. Robert, S. Desmoulière, and L. Rival.** 2001. Gestions locales et dynamiques régionales de la diversité variétale du manioc en Amazonie. Génétique, Sélection et Evolution 33(Suppl. 1):S465–S490.

Mendes, R. A. 1981. Melhoramento na germinação de semente botânica de mandioca (*Manihot esculenta* Crantz). Pages 523–539 *in* Anais i Congresso Bras-

ileira de Mandioca, vol. 1. Pesquisas agronômicas. Embrapa-Did, Salvador, SBM, Brazil.

Nartey, F. 1978. *Manihot esculenta* (cassava, tapioca, manioc, mandioca, yuca): cyanogenesis, ultrastructure and seed germination. Munksgaard, Copenhagen.

Nassar, N. M. A., and R. P. Teixeira. 1983. A quebra da dormência da semente das espécies selvagens da mandioca. *Manihot* spp. Ciência e Cultura 35:630–632.

Olsen, K. M., and B. A. Schaal. 1999. Evidence on the origin of cassava: phylogeography of *Manihot esculenta.* Proceedings of the National Academy of Sciences, USA 96:5586–5591.

———, and ———. 2001. Microsatellite variation in cassava (*Manihot esculenta,* Euphorbiaceae) and its wild relatives: further evidence for a southern Amazonian origin of domestication. American Journal of Botany 88:131–142.

Pacini, E. 1990. *Mercurialis annua* L. (Euphorbiaceae) seed interactions with the ant *Messor structor* (Latr.), Hymenoptera: Formicidae. Acta Botanica Neerlandica 39:253–262.

Passos, L., and S. O. Ferreira. 1996. Ant dispersal of *Croton priscus* (Euphorbiaceae) seeds in a tropical semideciduous forest in southern Brazil. Biotropica 28:697–700.

Pyne, S. J. 1998. Forged in fire: history, land, and anthropogenic fire. Pages 64–103 *in* W. Balée, ed., Advances in Historical Ecology. Columbia University Press, New York.

Roa, A. C., M. M. Maya, M. C. Duque, J. Tohme, A. C. Allem, and M. W. Bonierbale. 1997. AFLP analysis of relationships among cassava and other *Manihot* species. Theoretical and Applied Genetics 95:741–750.

Salick, J., N. Cellinese, and S. Knapp. 1997. Indigenous diversity of cassava: generation, maintenance, use and loss among the Amuesha, Peruvian upper Amazon. Economic Botany 51:6–19.

Sambatti, J. B. M., P. S. Martins, and A. Ando. 2001. Folk taxonomy and evolutionary dynamics of cassava: a case study in Ubatuba, Brazil. Economic Botany 55:93–105.

SAS. 1996. SAS/STAT User's guide, Release 6.12. Cary, N.C.: SAS Institute.

Second, G., A. C. Allem, R. A. Mendes, L. J. C. B. Carvalho, L. Emperaire, C. Ingram and C. Colombo. 1997. Molecular marker (AFLP)-based *Manihot* and cassava numerical taxonomy and genetic structure analysis in progress: implications for their dynamic conservation and genetic mapping. African Journal of Root and Tuber Crops 2:140–147.

Shigeta, M. 1996. Creating landrace diversity: the case of the Ari people and ensete (*Ensete ventricosum*) in Ethiopia. Pages 233–268 *in* R. Ellen and K. Fukui, eds., Redefining nature: ecology, culture and domestication. Oxford University Press, Oxford.

Soleri, D., and S. E. Smith. 1995. Morphological and phenological comparisons of two Hopi maize varieties conserved in situ and ex situ. Economic Botany 49:56–77.

Tardy, C. 1998. Paleoincendies naturels, feux anthropiques et environnements forestiers de Guyane française du tardiglaciaire à l'Holocène récent. Thèse, Université Montpellier II, Montpellier, France.

Thompson, K., and J. P. Grime. 1983. A comparative study of germination responses to diurnally-fluctuating temperatures. Journal of Applied Ecology 20:141–156.

Vazquez-Yanes, C. 1980. Light quality and seed germination in *Cecropia obtusifolia* and *Piper auritum* from a tropical rain forest in Mexico. Phyton 38:33–35.

Wilson, H. D. 1990. Gene flow in squash species. BioScience 40:449–455.

Yen, D. E. 1974. The sweet potato and Oceania: an essay in ethnobotany. Bernice P. Bishop Museum Bulletin 236, Bishop Museum Press, Honolulu.