A number of seed harvesting and handling factors have been shown to influence seed quality significantly. Seed maturity at harvest is important to subsequent germination in many crops (5, 9, 11, 17, 24). Seed storage may be required to overcome dormancies (4, 20), but it has also been associated with membrane deterioration (12, 13), genetic deterioration (14, 19), and less-efficient physiological function (1, 2). Seed from fleshy fruit are often fermented to assist in their extraction from the surrounding pulp. This process has been shown to influence aspects of germination in strawberries (6) and tomatoes (22).

Freshly harvested cucumber seed generally exhibit satisfactory germination under optimum conditions. However, seed dormancies or after-ripening requirements have been reported for ‘Baroda’ and ‘Black Diamond’ (21, 25). Nienhuis and Lower (15) reported a dormancy of fresh seedlots that diminished with seed age when seeds from a heterogeneous cucumber population were germinated at 15°C. Such responses to seed aging were not observed at 25°C. They also reported that fermentation of seeds and fruit pulp for up to 4 days enhanced germination of seeds at 25°C, but further fermentation decreased the germination percentage.

The purpose of this investigation was to study the effects of seed maturity at harvest, fermentation duration, seed storage time, and germination temperature on germination of several cucumber populations. Of particular interest was the comparison between a population of compact plant types, cp/cp, and a similar population of vining plant types, Cp/- . Compact plant types produce small seeds that exhibit poorer seed germinability than those of conventional vining plant types (8).

Materials and Methods

Three genetically different cucumber populations were evaluated in this study: 1) a vining population of 25 individual gynoecious breeding lines and hybrids from the USDA cucumber breeding program (USDA Cp/-); 2) a heterogeneous population of 25 F2 families of vining genotypes from the Wisconsin Agricultural Experiment Station breeding program (WI Cp/-); 3) a heterogeneous population of 25 F2 families similar to the WI vining population in origin, but homozygous for the compact gene (WI cp/cp). Lines comprising the USDA Cp/- population had been developed by selecting for disease resistance in the greenhouse. In their development, fruit were harvested routinely 30 days after pollination and seed fermentation was seldom practiced. The WI populations were developed in the field; fruit were harvested at plant senescence, and fermentation durations of 2 to 4 days commonly were employed in the seed extraction procedure.

All seed evaluated in this investigation was harvested from plants grown in the field at the Hancock Experimental Farm, Hancock, Wisc., in Summer 1981. Each of the 3 populations was comprised of 25 plots. The USDA Cp/- population of breeding lines and hybrids was direct-seeded into the field on 27 May. Plots of the 2 WI populations were either cp/cp or Cp/- F2 segregants from each of 25 random F2 families seg-

regating for cp. These seeds were sown in the greenhouse, and plants were transplanted into the field on 22 June at the 3-leaf stage after classification for plant type. By 28 July, all plots were producing pistillate flowers, and developing fruit were removed. Fruit that subsequently developed were used in this investigation.

Fruits were sampled from each plot on 18 Aug., 25 Aug., 1 Sept., and 9 Sept. These dates were chosen to represent periods of about 3, 4, 5, and 6 weeks post-pollination. On each harvest date, 2 bulks of 25 well-developed fruit were uniformly sampled from the 25 plots constituting each of the 3 populations. Seed and pulp from each fruit bulk were mixed thoroughly in separate 10-liter plastic containers. About one-sixth of the slurry was sampled and immediately washed to separate developed seeds from undeveloped seed coats and pulp (3). The remainder of each bulk was placed in a 25°C incubation chamber and allowed to ferment. At 1, 2, 4, 8, and 12 days after fruit harvest the slurry was stirred and additional samples were collected and washed. All washed seed were spread on paper towels and air-dried at 22 ± 2°C, using a fan to insure rapid initial drying.

After one week of drying, three 50-seed samples were counted from each seedlot, weighed, and treated with Captan. Samples were placed on 2 layers of filter paper in 100-mm petri dishes and moistened with 4 ml of distilled water. The petri dishes then were covered, placed in large polyethylene bags to minimize evaporation, and incubated at 15°, 20°, or 25°C, respectively (± 2°C). Seed samples were monitored daily and numbers of germinated seed were recorded. Germination was defined as 5 mm of radicle protrusion. Data were recorded until germination had ceased for a 3-day period.

Remnant seed from each seedlot were stored in paper envelopes under laboratory conditions (22° ± 2°C) for about 6 months. At this time, six 25-seed samples from each seedlot were weighed, treated with Captan, and placed on filter paper in 60-mm petri
Fig. 2. Effects of fruit maturity at seed harvest, seed storage time, and fermentation time on germination percentage of seed from the WI Cpi — cucumber population at 15°, 20°, and 25°C.

Fig. 3. Effects of fruit maturity at seed harvest, seed storage time, and fermentation time on germination percentage of seed from the WI cp/cp cucumber population at 15°, 20°, and 25°C.

dishes. Two 25-seed samples of each treatment combination were randomized within blocks in 15°, 20°, or 25° growth chambers. Filter paper was moistened to capacity with distilled water, and petri dishes were covered with polyethylene to minimize evaporation. The slight difference in seed age (23–36 weeks) between seedlots was judged to be insignificant, based on results of a previous investigation (15). Growth chambers were maintained at 80% RH and were kept dark except for low-intensity light (about 140 μmol·s⁻¹·m⁻²) during the daily counting period. Germination data from the two 25-seed samples at each temperature were pooled to allow combined analysis of one-week-old and 6-month-old 50-seed samples.

Variables calculated for each 50-seed sample included seed weight, final percentage of germination, and mean number of days to germination (MDG). The MDG values were calculated using the following formula (16, 18):

\[
MDG = \frac{\sum_{i=1}^{n} (d_i - g_i)}{\sum_{i=1}^{n} g_i}
\]

where \(d_i\) = the number of days from sowing and \(g_i\) = the number of seeds germinating on that day. Seedlots failing to germinate were given a zero value for the germination percentage, and a missing value was assigned for MDG, since this parameter could not be evaluated. Analysis of variance (ANOVA) was conducted considering all factors except sampling terms as fixed effects.

Results

The main effects of populations, harvest dates, fermentation times, seed ages, and germination temperatures were all significant (\(\alpha = 0.01\)) sources of variability for germination percentage (Table 1). Furthermore, all 2-way, 3-way, and 4-way interactions between main factors were significant. Ninety-seven percent of the total observed variation was accounted for by this model. Main effects were responsible for 58% of the variation, and 2-way interactions accounted for an additional 27%. Percentages were transformed to angles, \(\arcsin(\sqrt{0.5\times \text{proportion}})\), to improve normality of the data (23). Analysis of transformed values did not produce results significantly different from the analysis in the original units.

Separate analyses within each population indicated that main effects of factors and their interactions were significant sources of variability for germination percentage. The single exception was the lack of effect of seed storage time in the cp/cp population. In this population, seed storage time also failed to interact with maturity at harvest and with the maturity × fermentation time interaction. An ANOVA was conducted excluding the first 2 fruit maturity samples to determine if differences in germination existed between the 5- and 6-week fruit maturities. These
maturities were not significantly different, and most interactions of other seed handling factors with fruit maturity also were nonsignificant in this subset of the data.

Because of complex interactions between handling factors, further interpretation of results was achieved by plotting mean values for each combination of seed handling procedures (Figs. 1–3).

Three-week fruit maturity. In the Cp/− populations (Figs. 1 and 2), little germination occurred for seed harvested from 3-week-old fruit. A response to fermentation was evident for these immature seedlots, since maximum germination percentages generally were observed for short fermentation times (1–4 days). Six months of storage significantly reduced germination of these immature seedlots. Compact (WI cp/cp) seedlots from 3-week-old fruit exhibited no germination under any conditions (Fig. 3).

Four-week fruit maturity. Seedlots harvested from 4-week-old fruit showed the most pronounced and complex responses to other handling factors. In the Cp/− populations, fresh seed (1-week storage) exhibited no germination at 15°C and only a low percentage at 20°C, but achieved up to 90% germination at 25°C. After six months of storage, Cp/− seedlots exhibited substantially improved germination at 15° and 20°C, but diminished germination at 25°C, particularly for those seedlots that had received little or no fermentation (0 and 1 day). Highly significant, positive responses to 2–4 days fermentation were observed with 6-month-old Cp/− seedlots. Compact seedlots from 4-week-old fruits (Fig. 3) exhibited low germination percentages and generally exhibited reduced germination with >2 days fermentation. Storage time decreased germination of cp/cp seedlots at all but the 15°C temperature regime.

Five- and 6-week fruit maturity. Since the patterns of germination response were generally similar at 5 and 6 weeks for each of the 3 populations, these maturities will be considered together. The germination percentage of Cp/− populations at 25°C was generally similar for fresh seed and stored seed. The 2 Cp/− populations, however, differed in their response to fermentation durations. Germination of the USDA Cp/− population was reduced dramatically by >8 days fermentation, a tendency not exhibited by the WI Cp/− population. At 20°C, both Cp/− populations exhibited significant increases in germination percentages for fresh seedlots with 8 or 12 days fermentation, but reduced or unchanged germination under the same conditions after 6 months of seed storage. No germination was obtained in Cp/− populations for fresh seedlots at 15°C, but seedlots exhibited >50% germination after 6 months of seed storage. Seedlots from 5- and 6-week-old cp/cp fruits consistently exhibited deleterious responses to long fermentation times, with as little as 4 days often being associated with significant reductions in germination percentage.

Although Cp/− populations exhibited >90% germination under some conditions when harvested at 4-, 5-, or 6-week postpollination, rates of germination varied significantly for different fruit maturities. Greater fruit maturities generally were associated with significantly more rapid rates of germination (fewer days to germination), as can be seen for WI Cp/− seedlots (Fig. 4). Fresh seed (1-week seed storage) exhibited very slow germination at 20°C, requiring an average of 18.5 days to germinate. After 6 months storage, germination rate at 20°C was accelerated from all fruit maturities. Seed from 4- and 6-week fruit maturities exhibited significantly more rapid rates of germination at 20°C with 2- and 4-day fermentation than if unfermented. This difference was true of both fresh seed and after 6 months of seed storage. However, the 5-week fruit maturity
exhibited opposite behavior for both seed ages, having significantly slower emergence at 20°C with 4 days fermentation than for 0 days fermentation.

Germination rates at 25°C were generally similar for both fresh seed and stored seed. Fermentation for short durations usually resulted in decreases in time to germinate, with increases in time to germinate often accompanying prolonged fermentation (8 or 12 days). The most rapid rates of germination were usually resulted in decreases in time to germinate, with increases in time to germinate often accompanying prolonged fermentation (8 or 12 days). The most rapid rates of germination were almost always obtained with one or 2 days fermentation. The pattern of response to fermentation of seeds from 5-week fruit maturity was similar to trends observed for 4- and 6-week maturity, unlike the situation observed at 20°C. The USDA Cp/ — population seedlots exhibited similar responses, except for a tendency for long fermentation durations to be associated with delayed germination (data not shown).

Seed weight increased significantly with each increase in fruit maturity for all 3 populations (Fig. 5). WI Cp/cp seedlots weighed about one-third as much as those of the genetically similar WI Cp/ — population. USDA Cp/ — seedlots were also heavier than WI Cp/ — seedlots at all fruit maturities. Seed weight was always greatest for unfermented seedlots, even when weight was recorded after 6 months of seed storage. Significant reductions in seed weight were observed in response to as little as one or 2 days fermentation for seeds from 3- and 4-week-old fruit of all 3 populations. The WI cp/cp population also exhibited significant reductions in seed weight with 12 days fermentation, even for seed from the advanced fruit maturities. Similar trends were evident for fresh seed weight (one-week storage) in all populations, but weights were consistently 3% to 6% greater for fresh seed than for seed stored 6 months in the laboratory (data not shown).

**Discussion**

Seed of the 2 Cp/ — populations might appear ‘‘mature’’ at 4, 5, or 6 weeks if this appraisal were based simply on a test of germination percentage under optimum conditions. However, differences between these maturities are evident under some circumstances with respect to seed weight, rate of germination, and tolerance to long fermentation durations. The rapid rates of germination observed with advanced maturities in this investigation may be of some importance in field stand establishment. Rapid germination may lead to reduced vulnerability to soil pathogens and improved uniformity of field stands and harvests (7, 10).

The 2 Cp/ — populations exhibited poorer germination percentages and slower germination rates at suboptimal temperatures (15°C or 20°C) when seedlots were fresh than when they had been stored for 6 months. This phenomenon has been reported previously for 15°C germination (15). Long fermentation times generally produced higher germination percentages of fresh seed at 20°C, but often decreased the germination of these same seedlots when evaluated 6 months later. Presumably, some factor involved in low-temperature dormancy of fresh seeds is diminished with long fermentation times, but fermentation also deleteriously influenced seed storage ability, germination rate, and germination percentage under some circumstances. Another anomalous fermentation response was observed for germination of Cp/ — seeds from the 4-week-old fruit. These rather immature seedlots germinated well at 25°C as unfermented fresh seed, but exhibited a marked reduction in germination under the same conditions after 6 months storage unless they had received one or 2 days of fermentation. These responses suggest the involvement of some form of dormancy. The rather general effect of fermentation in reducing seed weight was observed for fresh and 6-month-old seeds, suggesting it did not merely arise from an osmotic decrease in fresh seed moisture during fermentation. Instead, the embryo or fermenting microorganisms may be respiring appreciable amounts of some seed storage compounds during the fermentation process. The greatest seed weight reductions during fermentation were observed in the compact population and with less mature Cp/ — seedlots, situations in which seed coats appear to be poorly developed. For the mature, vining seedlots, fermentation durations of up to 4 days did not appear to be detrimental to seedlot quality at optimal germination temperatures. As little as 2 days fermentation may ease seed extraction markedly by degrading fruit pulp and improving the separation of seeds from pulp. Based on germination behavior alone, there is no evidence to suggest that this practice is harmful to seed quality. Further investigations into the effects of fermentation of cucumber seeds on emergence behavior and seed longevity would be helpful.

The observed differences between cucumber populations for responses to seed handling procedures may relate to methods employed in population development. The greater seed weight of the USDA Cp/ — population, relative to the WI Cp/ — population, may be due to inadvertent selection pressure for rapid seed development imposed by short maturation durations during...
population development. Likewise the intolerance of this population to long fermentation durations may relate to the fact that fermentation was seldom practiced in seed extraction during its development.

The dramatic differences between the WI cp/cp population and the genetically similar WI Cp/— population must be due to effects of the chromosome region including the compact locus. Greater fruit maturity was required to produce germinable seed in the compact population and >80% germination was not achieved under any conditions. Seed from cp/cp plants were also sensitive to fermentation, as indicated by consistent reductions in germination percentage and seed weight with >2 days fermentation. The maximum germination percentages for the compact population were obtained with unfermented fresh seed at 25°C.

Seed harvesting and handling parameters evaluated in this study exerted a profound influence on subsequent germination characteristics of seedlots. In developing a procedure to select for germination percentage or germination rate in any of the populations evaluated, seed harvesting and handling factors should be defined and controlled to maximize the ability to discriminate genetic differences. Failure to do so may result in the unnecessary confounding of a substantial degree of environmental variation with existing genetic variation.

Literature Cited