Developmental fate of the cyanogenic glucoside linamarin in Costa Rican wild lima bean seeds

WHEN a seed germinates, we expect the defensive secondary compounds it contains to be transferred intact to the growing seedling, or variously decomposed to produce resources for the growing seedling. The seeds of wild indigenous Costa Rican lima beans (*Phaseolus lunatus* L.) contain about 3.45% fresh weight of linamarin[1], a cyanogenic glucoside that can enzymatically decompose to produce 0.37% fresh weight hydrocyanic acid (HCN). We report here that, after germination, the amount of linamarin in the total seedling (roots, cotyledons, and shoots) remains essentially constant during a 26-day experimental period and moreover corresponds to the amount of linamarin present in the seed before germination (Fig. 1).

To determine the fate of this defensive secondary compound in young seedlings, we germinated seeds and monitored the linamarin content of the seedlings for 26 days. While the amount of linamarin per seedling remained constant, the distribution of the glucoside present initially only in the seed changed dramatically with time. For example, after 6 days 42% of the total linamarin in the seedling was contained in the cotyledons, 27% was in the new leaves and shoot tip, 20% was in the stem and 11% was in the roots. After 14 days, 69% of the linamarin was in the leaves, and after 16 days the cotyledons were devoid of linamarin and dropped off. The change in fresh weight of the different plant parts, expressed as per cent of the plant's total weight, paralleled the change in linamarin for each different part. That is, the cotyledons decreased in weight each day by an amount corresponding to 5% of the total weight of the seedling and lost 4.4% of the total linamarin each day. Roots gained 2% in weight and 0.5% in linamarin each day while stems lost 0.9% in weight and 0.4% in linamarin each day.

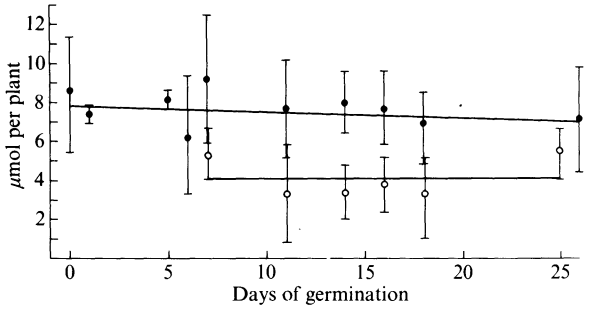

Fig. 1 The linamarin content of individual seeds or seedlings of wild lima beans (*Phaseolus lunatus*). Seeds were germinated by pricking the thin seed coat with a pin and soaking overnight in distilled water. Seedlings were grown in vermiculite at 24 °C with a 14-h photoperiod. On the 7th day of growth the cotyledons were carefully removed from 15 seedlings (○) to permit comparison with untreated seedlings (●) whose cotyledons remained on the plant for ~2 weeks. Linamarin content was determined by grinding weighed fresh seeds or seedlings in liquid nitrogen with a mortar and pestle. The ground sample was immediately transferred to 2 ml of 0.1 M phosphate buffer (*p*H 6.8) containing the enzymes emulsin and linamarase in the outside compartment of a centre well flask; the centre well contained 1 ml of 1 M NaOH. After 24 h in a shaking water bath at 37 °C, the contents of the centre well were assayed for HCN, a product of the enzymatic decomposition of linamarin. The method used[6] detects HCN in the range 0.01–0.15 μmol. Qualitative examination of 80% (v/v) ethanolic extracts by gas liquid chromatography[7] established that linamarin was the only cyanogenic glycoside present either in seed or seedlings. Seeds were hand-shelled from mature dry pods collected from a large wild plant in March, 1976 at Finca La Pacifica, near Cañas, Guanacaste Province, Costa Rica. An average seed weighted 62 mg (s.d. 13 mg, $n = 25$). Seeds from this plant were polymorphic; 60% were black and 30% brown. The black seeds tended to be slightly heavier, to have a higher instantaneous germination rate, and to possess a thicker seed coat than the brown seeds. Only black seeds were used in the study reported here.

The data displayed in Fig. 1 suggest that the plant either transfers its linamarin intact from the seed (cotyledons) or breaks it down and resynthesises it in equal amounts during new growth of the seedling. To determine what happens, the cotyledons were removed from 15 plants after 7 days of growth. The excised cotyledons contained approximately 4.5 μmol linamarin, and the glucoside content of the seedlings dropped to about 4.0 μmol per plant. Although the treated seedlings continued to grow and develop, their linamarin content remained constant throughout the experiment. This could be explained by assuming that the plant uses only the breakdown products of the linamarin for resynthesis in the growing seedling. However, the breakdown products of the aglycone of linamarin are acetone and HCN, compounds which are not readily converted to valine, the demonstrated primary precursor of linamarin in other linamarin-containing species[1]. We therefore prefer the more parsimonious explanation that the linamarin is transferred intact from the cotyledons to the growing seedling.

The degree to which plants synthesise secondary compounds as they change and develop, as opposed to shifting the location of compounds already synthesised, should be an important consideration in the pattern and budget of chemical defenses against herbivores. In *Heteromeles arbutifolia*, a chaparral evergreen shrub in California, the cyanogenic glucoside in the immature fruit is transferred to the ripening seed, and thus the same chemicals probably serve as a defence in consecutive developmental stages[2]. On the other hand, seeds of *Sorghum vulgare* do not contain a cyanogenic glucoside but the seedlings synthesise large amounts of it (dhurrin) in the first 5 days of growth[3]. Some kinds of secondary compounds are probably removed from deciduous tree foliage before the leaves are shed; presumably they can be used in other parts of the plant or in leaf production in later seasons.

Seeds contain a large variety of secondary compounds which are undoubtedly defensive for the seed[4], amongst other possible functions. While it is easy to postulate that such secondary compounds are stored as resources for developing seedlings, this has never been demonstrated. In the present study we have demonstrated that they may also be defense resources for the developing seedling. In short, if the seed is a container whose volume or weight is to be minimised (for dispersal purposes), the parent plant produces a higher quality package for the seedling if it puts the intact, defensive molecule into the seed than if it puts in the raw materials for synthesis of that molecule.

With this view in mind, there are at least three selective forces acting on the quantity of a given kind of secondary compound to be found in a seed. First, there are the levels that the parent plant can donate considering the total number of seeds it is producing and its own needs. Second, there are the levels necessary to deter the relevant herbivores from eating the seeds. Third, there are the levels needed to give enough defensive compounds to the seedling to meet its herbivore challenges. The first selective force will act to minimise the compound level and the second two to maximise it; however, the second two need not necessarily have the same optimal level. For example, co-evolutionary selection by a bruchid beetle known to feed on lima bean seeds (D.H.J., unpublished) may drive the concentration of linamarin in the seed up until it is higher than that which is needed by the seedling. At this point, the levels of linamarin in the seedling may have increased to a point well over that which is needed to deter an insect that eats seedling leaves. Conversely, strong selection on the seedling for increased linamarin content by a defoliator may drive the linamarin content of the seed to a high enough level that the bruchid larvae can no longer survive in the seed. This would be a real-life example of the theoretical manner in which herbivores competitively interact through the

medium of the resource budget of the plant in evolutionary time[5].

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