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Seasonal Changes in Starch Content in Trophopods of *Matteuccia struthiopteris*

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ABSTRACT.—Trophopods are modified stipe bases that function as starch-storage organs in a wide variety of mainly temperate ferns. Ever since they were first observed, the presence of trophopods has been explained by reference to seasonality and they have been assumed to provide nutrition for the rapidly expanding fern leaves in spring. We present the results of an analysis of the annual variation in starch content in *Matteuccia struthiopteris* (L.) Todaro, cultivated in the Leiden botanical garden. Our results show a distinct seasonal variation in starch content, suggesting that the presence of trophopods is indeed functionally linked to seasonality, but we do not find a decrease in starch content corresponding to the period of leaf expansion that would clearly link the depletion of stored starch to the expansion of new leaves.

KEY WORDS.—fern leaf expansion, overwintering buds, petiole, seasonality

The term "trophopod" was coined by Wagner and Johnson (1983), to denote "a petiole base that is specialized to accumulate food and to persist as a storage organ...". The first accurate description of this structure, however, is given by Eaton (1879) in his description of *Aspidium goldianum* Hooker. He describes the basic elements that characterize these structures: the thickness, the continuity with the rhizome, the persistence, and the starchy contents:

"The root-stock is creeping or ascending, several inches long, and nearly an inch thick. This thickness is made up, in considerable part, by the adherent bases of old stalks; the stalks being perfectly continuous with the root-stock, and so much crowded as to overlap each other. When fresh the root-stock is fleshy, and a longitudinal section of it shows that its substance passes so gradually into that of the stalk-bases, that no point of separation or distinction between the two can be selected. (...) The parenchymatous portion of the root-stock is loaded with starch in very minute grains, as may be easily proved by adding a drop of alcoholic solution of iodine to a thin slice of the root-stock

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placed under a microscope, when the grains will be presently seen to turn blue, the recognized sign of starch."

It is not surprising that he then correlated the presence of starch-containing structures with the annual growth of a new crown of leaves: "This abundance of nutritive material in the root-stock enables it to send up a fine circle of large leaves in the proper season of the year."

Eaton's observations have been confirmed in a number of studies, and over the course of the following century, they have been supplemented by observations of trophopods in a number of different species. Wagner and Johnson (1983) observed that trophopods are widespread in North American ferns, and that there is a correlation both with taxonomy and with ecology, as they are absent in several large taxonomic groups, as well as in obligatory rock-dwelling species. In passing, they also note that trophopods occur both in seasonal and in wintergreen taxa. In a more detailed study, Johnson (1986) noted that there may also be a correlation with climate, trophopods being absent in the predominantly tropical genus *Diplazium*, and present in related temperate groups. This conjecture is presented as fact by Lellinger (2002), who defined a trophopod as: "the enlarged, persistent, basal portion of a stipe that functions as storage organ, especially through the cold months, found in some ferns of temperate regions." Since then, however, anecdotal observations suggest that trophopods are also found in ferns from tropical climates. Vasco *et al*. (2013) report starch-filled trophopods in two tropical species of *Diplazium* in Central America. Hovenkamp (irregularly updated), in a study of the genera *Diplazium* and *Athyrium* on Mount Kinabalu (Malaysia, Sabah, Borneo) found that trophopods were regularly present in the *Athyrium* species growing on the temperate high slopes of the mountain, but also sporadically found in species from the closely related genus *Diplazium* growing on the warm-tropical lower slopes. Sundue and Rothfels (2014) state that trophopods are "perhaps best developed among predominantly temperate groups", but are "also present among sub-tropical and tropical species", and summarize that the relative prevalence of trophopods in tropical and temperate plants should be examined more thoroughly. An obvious functional interpretation for a higher prevalence of trophopods in temperate, seasonal, areas would be, as noted already by Eaton (1879), that they play a role in supporting the rapid expansion of new fern leaves in spring.

Here we attempt to confirm this functional explanation by examining the seasonal changes of the starch contents in *Matteuccia struthiopteris* (L.) Todaro (Fig. 1), which is native to the colder temperate regions of the Northern hemisphere (Odland *et al.*, 2004), where it may form large stands at least partly by vegetative reproduction. It is a deciduous plant, and expansion of the leaves in spring occurs in little more than four weeks (Von Aderkas and Green, 1986). In addition to leaves, in the course of the growing season, cataphylls, abortive leaves that consist of little more than the trophopods, develop as well as sporophylls, non-assimilating spore-producing leaves. All leaves have very distinct trophopods at the base of the stalk, which persist for an unknown number of years after the leaves are shed (Fig. 2).

FIG. 1. Seasonal changes in the aspect of the sampled *Matteuccia* patch in the Hortus botanicus Leiden in the sampled period. A – March 14, B – April 16, C – September 17, D – October 20.

As *M. struthiopteris* is a source for edible fiddleheads, which are commercially harvested from the wild (DeLong and Prange, 2008), some experiments have studied the effect of complete removal of croziers (fiddlehead harvest). Bergeron and Lapointe (2001) found that starch was the main form of carbohydrate reserve in trophopods when measured at the end of the growing season, and that complete defoliation early in the growing season could reduce this starch content by 88%.

MATERIALS AND METHODS

For a study site, we chose a patch in the Leiden botanical garden, where several hundreds of individual plants (crowns) occur on a surface of c. 25 m^2. As *Matteuccia* readily spreads by way of underground stolons, and the source of the original plant material could not be retrieved, the entire patch may well represent a single clone (Grzybowski and Kruk 2015). We sampled at roughly monthly intervals over the period February 2014 – February 2015, thus covering an entire growing season. Leaf expansion took place in late March and April; leaf dieback started at the end of September and was complete by the end of October (Fig. 1). From five separate crowns, we took samples of three or four trophopods on different levels of the crown, which we labeled L for trophopods from low down on the crown, M for those from middle level, H for the upper level and G for the uppermost, fresh, green ones. In total, 233

FIG. 2. Approximately medial longitudinal section through a crown of *Matteuccia struthiopteris*. R: rhizome, T: trophopod, scale bar 1 cm.

separate samples were analyzed because not all sampled crowns were robust enough to support the removal of four parts at different levels. Our intention was that by sampling in this way, the samples from the middle and upper level would correspond to the trophopods remaining from leaves that had abscised the previous year, lower-level trophopods to those from earlier years, and the uppermost ones to represent the metabolically most active part of the plant. In the months February to September the parts marked G corresponded to the dormant buds formed the previous year and the expanded leaves, in the months October to January these were the dormant buds formed during the sampling period. We did not distinguish between trophopods of foliage leaves and cataphylls, and whenever possible, we tried to avoid sampling from fertile leaves that are non-assimilating and therefore can be expected to have a different starch metabolism. The total number of well-developed crowns in the patch did not allow us to sample entire crowns destructively. Accordingly, some crowns may have been sampled repeatedly at different times, and the assignment of trophopods to categories L, M or H may have had some degree of arbitrariness.

For the analysis, the collected tissues were stored at –80°C until they were ground using a pestle and mortar under liquid nitrogen. The ground tissues were then transferred to 2 ml centrifuge tubes and placed in a freeze-dryer for 48 hours. For each sample, 30 mg dry tissue was accurately weighted and analyzed using the protocol of Smith and Zeeman (2006), using the Glucose (HK) Assay Kit obtained from Sigma-Aldrich. The data are available in the Dryad repository: doi:10.5061/dryad.3113c.

Results

Starch content of trophopods from all different levels of the rhizome increases roughly twofold over the summer period, when the leaves are metabolically active (Figs. 3, 4). There is a slight decrease between the values for March and April, but the major decrease occurs immediately after the maximum value has been reached in September and October. This is even more clearly visible in the green parts (Fig. 3). Here, the values for February to September refer to the overwintering buds and their expansion into new leaves, and the values for October to January show the changes occurring in the newly formed buds. The most dramatic decrease in starch content thus does not take place when the buds expand into new leaves (March–April), but occurs in the newly formed buds just after they have been formed on the plant.

Discussion

Variation in starch content on a single rhizome.—The starch content of trophopods at all four levels on the rhizome shows a clearly similar pattern of variation over the year (Fig. 3). Even taking into account that without destructive sampling of entire crowns, we could not confidently estimate the ages of all the sampled trophopods, and that the labeling of trophopods as from different levels may be partly erroneous, the general trend is clear. We expect therefore that this uncertainty does not affect our main results seriously. Our results then show that all trophopods, the older ones as well as the recently formed ones, are involved in the metabolism of the plant.

Seasonal variation.—Seasonal variation in starch storage shows a clear pattern (Figs. 3, 4). Over the growing season, there is an increase in starch content that amounts to a doubling or more, leading to starch contents at the end of the season that attain levels of around 40% of dry weight of the trophopod, even slightly higher for the newly formed dormant buds. These values are somewhat higher than the levels found at the end of the season in a more natural setting (Bergeron and Lapointe, 2001), which may be due either to differences in the analytical protocol, or to the better circumstances for the plants provided in our garden setting. It has been suggested that in wintergreen ferns, old leaves act as storage organs to support the emergence of new leaves (e.g., Landi *et al.*, 2014), and based on this, Eaton's (1879) expectation seems reasonable that in winter-dormant ferns new growth would be supported by the starch stored in trophopods. However, in our data leaf expansion is

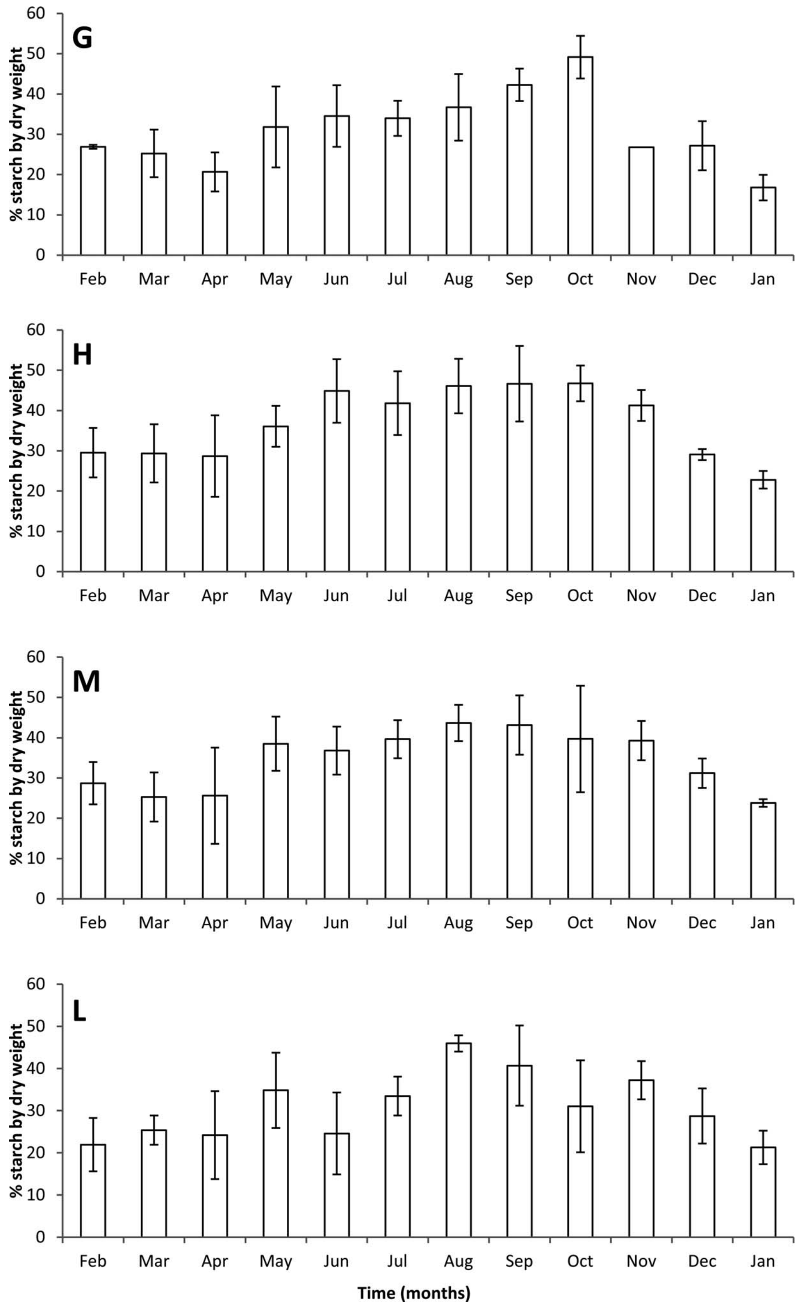

FIG 3. Seasonal changes at different levels (L=Low, M=Middle, H=High, G=Green) on the rhizome. Error bars correspond to one standard deviation in both directions.

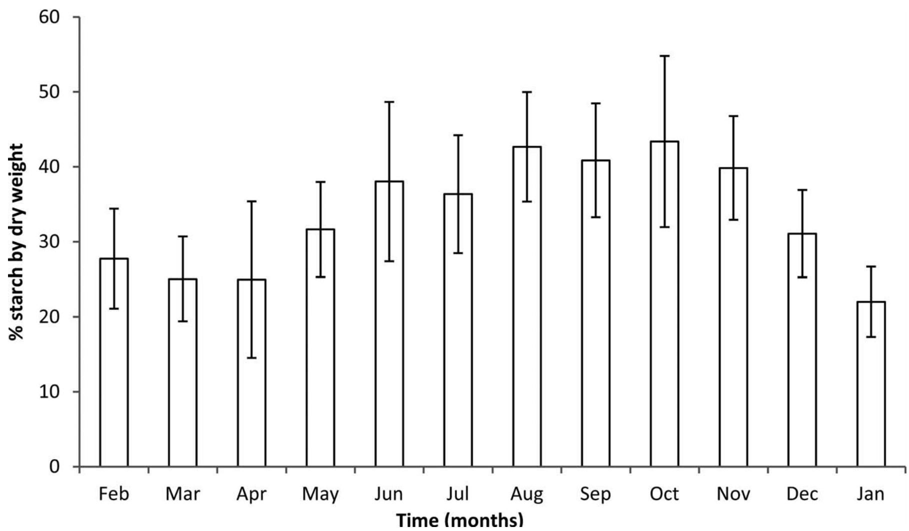

FIG 4. Seasonal changes in starch content pooled for all levels on the rhizome. Error bars correspond to one standard deviation in both directions.

unconnected to starch depletion. The main depletion of the starch reserves in trophopods of *Matteuccia* does not take place when the new leaves expand in spring, but immediately after the maximum level has been reached and the leaves have died back in autumn. At that time, there is no obvious destination for the carbohydrates that are thus released, which suggests that they are stored in a different location, or in a form that is not detected by Smith and Zeeman's (2006) protocol for starch analysis. In view of the very limited volume of the actual rhizome of a crown, compared to the aggregated volume of the trophopods, and the anatomical continuity between these organs, we think that storage of starch in the rhizome proper is not a likely explanation of the observed depletion of the trophopods. Thus, while distinct seasonal variation in starch contents suggests a functional link between the metabolism of the trophopod and the seasonality of the plant, the exact nature of this link requires further investigation into alternative modes of storage for carbohydrates through the dormant season.

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