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This is the Accepted version of the following publication

Uddin, Md Nazim, Robinson, Randall, Rashid, MH and Asaeda, T (2017)
Seasonal variations of carbohydrates in Pueraria lobata related to growth and phenology. *Weed Biology and Management*, 17 (2). 103 - 111. ISSN 1445-6664

The publisher's official version can be found at
<http://onlinelibrary.wiley.com/doi/10.1111/wbm.12124/abstract>
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RESEARCH PAPER

Seasonal variations of carbohydrates in *Pueraria lobata* related to growth and phenology

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The seasonal changes of non-structural carbohydrates (NSCs), water-soluble carbohydrates (WSCs) and starch concentrations were examined in *Pueraria lobata* throughout its life cycle in order to elucidate the potential points in the seasonal growth and phenological cycles for its improved control. The study was carried out at two different habitat zones with distinct soil conditions in the riparian zone of the Tama River, Tokyo, Japan. One zone, with sandy soil, had lower levels of available nitrogen and phosphorous, while the second zone contained high levels of organic matter and nutrients. The highest and lowest concentrations of the NSC pools in the roots of *P. lobata* were observed in December and August, respectively, at both study sites. The lowest NSC reserves were attained at the end of the vegetative stage, with the highest NSC reserves found at the end of the reproductive stage. The WSCs gradually decreased from April to August in the roots and then increased until February. The starch concentrations of all the plant organs followed the same pattern as for the WSCs. The stems and leaves did not show the same pattern as did the roots. The total carbohydrate concentrations and pools did not show significant variation between the two sites, suggesting that the soil conditions had very little effect on carbohydrate production in the different organs of the plant. The study suggests that to control *P. lobata* effectively, the above-ground biomass should be removed at any date between the end of August and early September.

Keywords: non-structural carbohydrate, riparian zone, soil characteristics, starch, water-soluble carbohydrate.

The forms of carbohydrate that are stored vary among plant species, type of storage tissue and time of year (Adams *et al.* 2013; Rosas *et al.* 2013). Starch is one of the most common storage carbohydrates and is converted readily to soluble sugars when needed for growth (Loescher *et al.* 1990). Two organs in plants account for the majority of stored carbohydrates – the stems and coarse roots (Wardlaw & Willenbrink 1994) – but reserves also can be present in the fine roots, foliage, fruits and buds (Chapin *et al.* 1990). In general, reserve

carbohydrates are stored as starch in the roots and as sucrose in the stems (Weyand & Schultz 2006). One of the main uses of these carbohydrate reserves is to support growth and respiration prior to growth initiation after dormancy (overwintering), as well as for recovery after disturbance, such as herbivory or fire (Kozłowski 1992; Sakai *et al.* 1997). Depending on the species, one-half to two-thirds of the stored reserves can be used to support growth (Kozłowski 1992). Reserves are usually markedly depleted during bud-break and the subsequent growth in spring, are replenished during the growing season and then gradually are used in respiration during the overwintering season (Loescher *et al.* 1990). The carbohydrate reserves are mobilized and shifted from the roots to the shoots in anticipation of the breaking of dormancy and leaf-out in spring.

Pueraria lobata (Willd.) Ohwi (commonly called “kudzu”), is a climbing, invasive legume that can grow

Communicated by N. Yoshino

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Received 30 May 2016; accepted 17 April 2017

doi:10.1111/wbm.12124

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to 10 m high and has become a great economic and environmental concern in several parts of the world (Sun *et al.* 2006; Rashid *et al.* 2010b; Asaeda *et al.* 2011). The species is a rapid-growing perennial and characteristically possesses large stolons and tuberous roots that allow it to reproduce vegetatively. *Pueraria lobata* has the ability to grow in nutrient-poor soil due to its association with *Rhizobium* bacteria (Asaeda *et al.* 2015). It is deciduous and overwinters in the form of underground rhizomes during winter, regenerating from the stolons and tuberous roots in spring. *Pueraria lobata* shows a high adaptability to different habitat conditions and exhibits rapid growth in a range of climatic conditions, from tropical to boreal. Rashid *et al.* (2010a) found that it produces monospecific stands by using allelopathic mechanisms. Once established in an area, *P. lobata* begins to reshape the entire landscape, enshrouding and slowly killing the surrounding fields and forests and destroying habitat for the associated wildlife (Forseth & Innis 2004) and thereby it has a great impact on native plant diversity (Frye *et al.* 2012). By the late 1970s, *P. lobata* had become recognized as a common weed in most parts of the world (Everest *et al.* 1999; Rashid *et al.* 2010b). The United States Department of Agriculture added *P. lobata* to its list of noxious weeds by 1998 (USDA 2014).

The capability of a perennial species to store carbohydrates plays an essential role in its ability to support canopy growth in spring until a sufficient leaf area is attained in order to meet the carbon demands from photosynthesis (Smith *et al.* 2003; Smith & Zeeman 2006). *Pueraria lobata*, being perennial, is reliant on non-structural carbohydrate (NSC) reserves to ensure its ability to regenerate rapidly in spring. Starch makes the reserve for plants and its reserve has been associated with the vigor and regrowth of perennial plants (Pellegrino *et al.* 2014; Vriet *et al.* 2014; Martínez-Vilalta *et al.* 2016) and the patterns of starch dynamics enable plants to adapt to long/short day conditions (Scialdone & Howard 2015). Starch storage is also viewed as more important and sensitive than the concentrations of other non-structural carbohydrates, in terms of the sprouting ability of woody plants (Von Fircks & Sennerby-Forsse 1998). The concentration of water-soluble carbohydrates (WSCs) determines the immediate functions (e.g. osmotic) and its level should be above a critical threshold during the growth period of a plant (Dietze *et al.* 2014).

It is postulated that the rapid growth of *P. lobata* in spring might exhaust carbohydrate storage and therefore make the plant, at least temporarily, weakened. By understanding the phenology of the target plant and the fate of carbohydrates, there is the possibility to

identify any potential “weak points” in the life cycle. Previous research on other plant species has demonstrated successfully that timing the control technique to the target plant’s low point in carbohydrate storage can increase the effectiveness of management (Richburg 2005; Pennington & Sytsma 2009).

A better understanding of the seasonal cycles of plants might produce insight into the timing of biological, herbicidal and mechanical control techniques in order to maximize their effectiveness. Specially, management techniques that are timed to coincide with reductions in stored carbohydrates could reduce the ability of the target plant to regrow after treatment or to survive an overwintering period. The period of storage, amount and location of carbohydrate deposits for use in the current and subsequent seasons have been identified as a priority research area for timed intervention and control (Steen & Larsson 1986). Asaeda *et al.* (2008) indicated that quantitative studies that determine material budgets and resource allocation patterns across the growing season are of paramount importance for understanding plant biology and management techniques. The natural changes in carbohydrate reserves have been studied in many plant species (Cook 1966), but the seasonal variation in carbohydrate reserves is not the same for all species. Therefore, it is necessary to establish the carbohydrate cycle for each study species, as a plant’s ability to sprout after cutting could depend on its reserve carbohydrate levels. To date, little is known about the specific details of carbohydrate allocation in *P. lobata* plants.

The objective of this study was to investigate the seasonal dynamics of carbohydrates in the plant organs (leaf, stem and root) of *P. lobata* in two contrasting soils over the season. Based on this information, potential physiological weaknesses in the growth cycle of this plant might be suggested.

MATERIALS AND METHODS

Location, soil characteristics and vegetation history of the study sites

The study was part of research that was carried out from 2008 to 2009 at Tama River and published by Rashid *et al.* (2013). The observations were conducted at two locations in Tokyo, Japan: (i) near Fuchu Yotsuya Bridge (35°39’46”N, 139°26’15”E), denoted by Fuchu; and (ii) on the flood plain downstream of the Ohgurigawa River junction (35°38’59”N, 139°28’32”E), denoted by Ohguri (Fig. 1). These sites are situated at 34.6 and 33.6 km up from the Tama River mouth, respectively. Both locations were

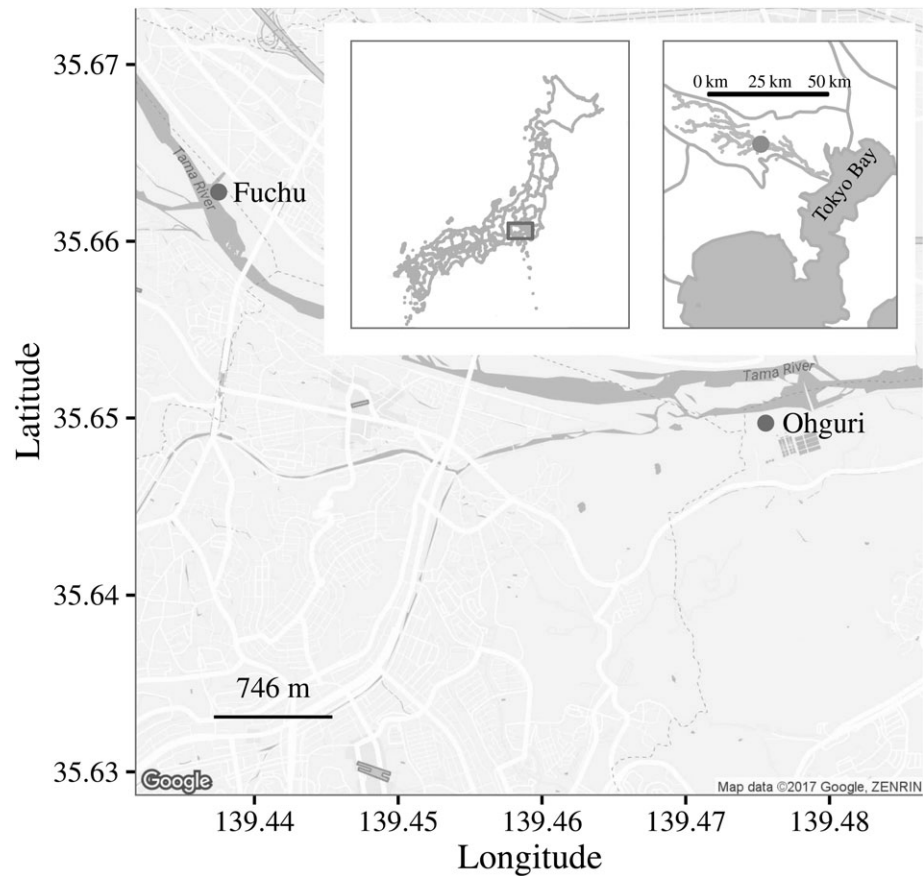


Fig. 1. Location of the study areas (Fuchu and Ohguri, Japan). The inset maps show the positions of the study locations in Japan (left) and along the Tama River (right).

relatively flat, although slightly inclined from the bank to the channel. However, these locations are located at slightly different elevations: 0.3–0.9 m in Fuchu and 3.0–3.5 m in Ohguri from the normal water level. The inundation frequency was more frequent at the Fuchu study site, while Ohguri was rarely inundated, even when the river was under high flood. The largest flood that was experienced in the last 20 years occurred in September 2007. During this flood, both study sites suffered with flood scour and large colonies of vines, mostly *P. lobata*, disappeared. The Tama River typically transports large amounts of sand that accumulates on the flood plain during floods. Thus, the soil properties differ between locations, depending on the frequency of the inundation and deposition.

The soil of the Fuchu site was mostly sandy and gravelly and supported a relatively low plant biomass. The upper part of the soil column of Fuchu was gravelly with overlying thin sand layers. The lower parts of the soil column were composed of accumulated sand layers, which were finer the deeper they occurred in the layer. Ohguri was slightly more elevated than Fuchu and relatively homogeneous, with a vegetative cover of dense

herbs, with the substrate composed mostly of silt. Ohguri soil, in contrast, had not accumulated flood deposition but had accumulated silt through sheet erosion from adjacent areas. As this site did not experience frequent flooding events, the soil was more or less homogenous in physical and chemical nature. The organic matter content in Ohguri was higher than in Fuchu and had higher nitrogen and phosphorous concentrations. Rashid *et al.* (2013) described the soil characteristics in detail. Though *Miscanthus sacchariflorus* and other pioneering species were primarily inhabitant, *P. lobata* and *Sicyos angulatus* have been reported to have invaded these sites in recent years (Asaeda *et al.* 2011; Uchida *et al.* 2012; Rashid *et al.* 2013). There was a large flood in 2007 in Tama River that washed away all the colonies of *P. lobata* from Fuchu and Ohguri. Therefore, all the studied colonies of *P. lobata* were young and not older than 2 years.

Sampling and chemical analyses

The study was carried out from April, 2008 to June, 2009, with sampling at monthly intervals. Three plants or ramets (Fig. 2) were randomly selected in apparently

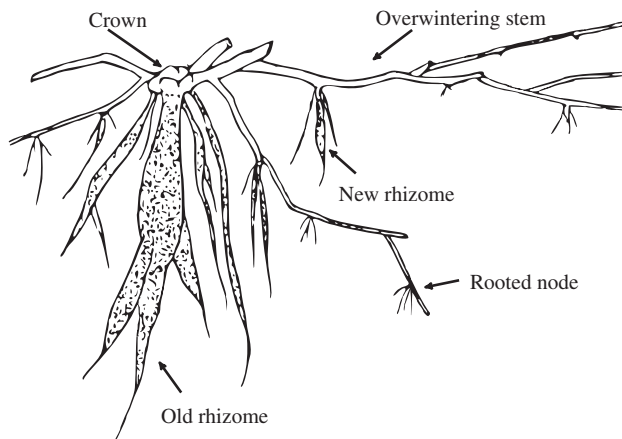


Fig. 2. Stem and below-ground organs of a typical ramet of *Pueraria lobata* (modified from Lewontin [1969]).

homogenous area at each site. At each observation, the shoots, below-ground organs and surrounding sediments of the selected ramets were carefully dug out to a depth of at least 1 m to obtain all of the below-ground tissues. The depth of the *P. lobata* roots was not significantly different within each of the two sites. The materials at the bottom of the hole were sieved carefully to ensure that there was no remaining plant material. At the same time, soil samples were collected and tightly sealed in plastic vials. All of these samples were put into a plastic bag for transportation to the laboratory.

In the laboratory, all the plant samples were rinsed with pressurized water. Then, they were sorted into leaves and stems for the above-ground tissues and roots for the below-ground tissues. The plant and soil samples were dried at 80°C in the oven for >3 days until the weight was constant. The dry weight was measured separately for each organ. The oven-dried samples were ground to a 2 mm mesh size by using a Wiley mill and

were stored in sealed plastic vials until the chemical analyses were conducted.

The moisture content of the soils was determined by the gravimetric method (Reynolds 1970). For this, a specific portion of all the soil samples was used before drying. All the soil samples then were air-dried and the particle sizes were determined by using sieves according to the protocol of ASTM International (2002). The pH of the soil was measured in a 1:5 (soil : water) paste by using a digital pH meter (HM-25R; DKK-TOA Corporation, Tokyo, Japan). The soil organic matter was estimated by the loss-on-ignition method (Heiri *et al.* 2001). Potassium, calcium, magnesium, sodium, copper and zinc were extracted by Mehlich-3 extractant (Mehlich 1984) from the oven-dried soil samples by using the methods that were stipulated by Ziadi and Tran (2007). The samples subsequently were measured by using an atomic absorption spectrophotometer (AA-6300; Shimadzu, Tokyo, Japan) at the respective wave lengths that were specified for each metal. The total carbon and total nitrogen were determined by using a CHN (carbon, hydrogen and nitrogen) analyzer (CHN Corder MT-5; Yanaco, Kyoto, Japan). The total phosphorus was determined by the molybdenum blue colorimetric method (Murphy & Riley 1962) after digestion with $\text{H}_2\text{SO}_4\text{-HClO}_4$ (APHA 1998).

The amounts of NSCs, WSCs and starch (Fig. 3) were measured by using the phenol-sulphuric acid method (Kabeya & Sakai 2005). An acid extraction was made with a solution of 0.4 N H_2SO_4 for the NSCs. Each sample (~6 mg) was placed in a 100 mL round-bottomed flask with 50 mL of acid and refluxed for 1 h in a boiling water bath (Yamoto Scientific Company, Ltd., Kyoto, Japan). In the case of the WSCs, only 50 mL of distilled water was used. The hot solution was filtered through filter paper (No. 42; Whatman International, Ltd., Maidstone, UK). The filtrate was cooled and diluted to volume with distilled water. The carbohydrate content of the filtrate was determined spectrophotometrically (HACH-4800; Hach Company, Loveland, CO, USA) at 485 nm by using the phenol-sulphuric acid method. A glucose solution was used as the calibration standard. A correction factor of 0.9 was used to convert the glucose equivalents to starch (Latt *et al.* 2001). The results were expressed as a percentage on a dry weight basis. The NSC pools (i.e. the total amount of NSCs) were calculated by multiplying the concentration of NSCs by the dry biomass of each organ (Busso *et al.* 1990).

Statistical analyses

The data analyses were carried out by using R (R Development Core Team 2010). The raw data of all the variables were checked for normal distribution

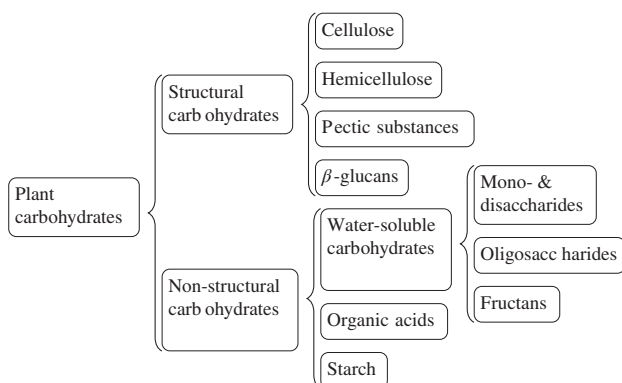


Fig. 3. Major carbohydrate fractions in plants (Whittaker 1953; Gregory *et al.* 1991).

with the one-sample Kolmogorov–Smirnov test, as well as for homogeneity of the variances with Levene’s test, and when necessary, arcsin transformation was performed. The results are presented as the mean \pm standard deviation ($n = 3$). The data were subjected to a one-way ANOVA, followed by the Duncan’s multiple range test in order to evaluate the mean difference at $P \leq 0.05$. A map of the observation sites was prepared by using the “ggmap” package of R (Kahle & Wickham 2013).

RESULTS AND DISCUSSION

Non-structural carbohydrate concentrations

The fractions of NSCs that were included in this study were the WSCs and starch. The allocation of plant carbohydrates among different organs and their storage have been associated with critical functions, such as photosynthesis, growth and maintenance (Percy *et al.* 1996). Chapin *et al.* (1990) reported that plants tend to store carbohydrates under a nutrient-deficit condition at the cost of growth. Thus, carbohydrate metabolism is always connected to the habitat conditions and gives an understanding of the plant’s growth.

A very clear seasonal trend was found of increments or decrements of the NSCs in the roots. From the beginning of bud breaking (April–May), the concentration of NSCs in the roots decreased until the onset of the reproductive stage (August–September) (Fig. 4). From August to September, the NSC concentration in the roots increased until December (i.e. during fall). With the onset of senescence, the NSC concentration in the roots showed a decreasing trend until bud-breaking next April. However, no consistent seasonal dynamic in the NSC concentration in the leaves and stems was found (Fig. 4). During the early stage of growth, a low NSC concentration was found in the current stem and it increased a little, with a fluctuation trend throughout the growing period. In contrast, the NSC concentration in the roots declined in the growing period, then followed a gradual increment through the remainder of time. Between the two sites, the maximum level of NSCs in *P. lobata* was observed in the roots (48%), followed by the stems (24%) and leaves (22%) in Ohguri. The minimum levels of NSCs also were recorded in Ohguri, with values of 13, 12 and 9% in the roots, stems and leaves, respectively. In Fuchu, the maximum NSC concentrations in the roots, stems and leaves were 47, 25 and 20%, respectively, whereas the minimum values that were found in these organs were 17, 14 and 12%, respectively.

The WSCs in the roots showed a decrease from May to August, reaching a minimum level of 5%, after which the WSCs started to increase until March, with fluctuations in Fuchu (Fig. 4). The stems and leaves did not show the same pattern as that of the NSC concentration. The maximum concentration of WSCs was found in the roots (21%), followed by the stems and leaves. The leaves had a very low concentration of WSCs (7%). These results are compatible with other studies, where all species accumulated high root carbohydrate concentrations in fall (Chinnasamy & Bal 2003; Asaeda *et al.* 2006; Asaeda *et al.* 2008). The seasonal pattern of WSCs in Ohguri followed the same pattern as in Fuchu (Fig. 4). The roots had a higher value (24%), followed by the stems (16%) and leaves (11%) in Ohguri.

The lowest starch concentration in the *P. lobata* roots occurred in July in Ohguri and in October in Fuchu. The seasonal concentration of starch in plant organs in Ohguri exhibited a similar pattern to that of the plants in Fuchu. The root starch concentration at both sites attained their highest level in February after following fluctuating trends until December (Fig. 4). The roots had a higher accumulation of starch (24% in Fuchu, 23% in Ohguri), followed by the stems (19% in Fuchu, 14% in Ohguri) and leaves (14% in Fuchu, 13% in Ohguri). The carbohydrate reserve trends of the roots also were reported by McLaughlin *et al.* (1980) and Latt *et al.* (2001) for starch in five multipurpose tree species. These authors found that the carbohydrate concentrations decreased as active growth resumed during the wet season. Generally, the roots showed the highest accumulation, followed by the stems and leaves. In the observations of this study, the NSC, WSC and starch concentrations showed no significant difference between the two sites ($P < 0.05$). Similar findings have been found by at least one other study (Schaberg *et al.* 2000).

Non-structural carbohydrate pool sizes

The root reserve does not contribute extensively to the seasonal regrowth of vines; the seasonal reserve is stored in the stem tissue and is used for this purpose (van der Maesen 2002). Therefore, the concentrations of soluble sugars (WSCs) contribute to the seasonal growth dynamic of vine plants. In contrast, the carbohydrate pools of the vine depend more on the biomass than the carbohydrate concentrations. As the dry mass of the leaves, stems, roots and rhizomes changes seasonally, the pool size of different carbohydrate fractions is also dynamic. Thus, studying the carbohydrate pool

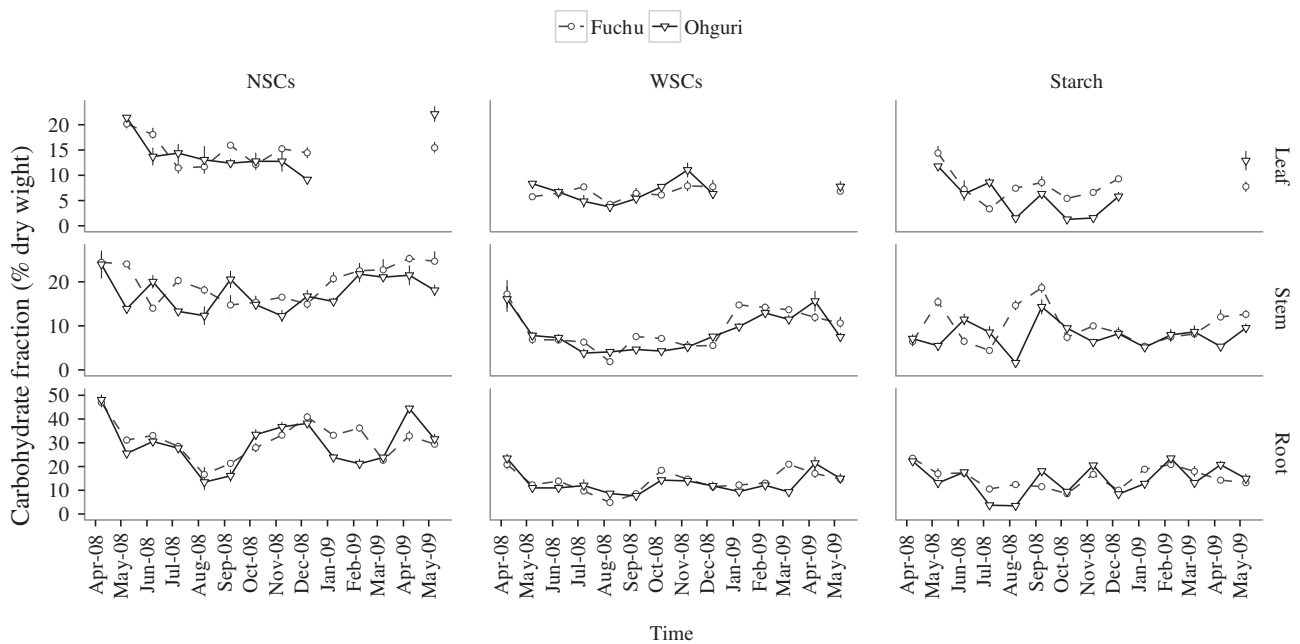


Fig. 4. Seasonal changes in the non-structural carbohydrate (NSC) fractions in the plant organs of *Pueraria lobata* in Fuchu and Ohguri, Japan. The vertical bar that is associated with each data point is the standard deviation of the mean ($n = 3$). WSCs, water-soluble carbohydrates.

dynamics gives a clear picture of the source–sink relationship of the storage among different organs.

The NSC pool size in the leaves and the size increased gradually at both sites from the early vegetative period (April) to the end of the vegetative period (September) (Fig. 5). The NSC pools increased more in the leaves (237%), followed by the stems (95%) in Fuchu through the vegetative period (Fig. 5). However, this trend was the opposite at the Ohguri site, where the NSC pools' increment in the leaves was 66% and that of the stems was 297% at the end of the vegetative period. The gradual increment of the leaf and stem pool size at both sites was related mainly to an increase in the leaf and stem biomass (Rashid *et al.* 2013) during the vegetative period, rather than as a result of a commitment of resources to the roots.

The dynamics of the root NSC pools of the Fuchu and Ohguri populations followed a similar fluctuating pattern during the vegetative period (Fig. 5), except that the root biomass was lower in Fuchu than in Ohguri, possibly related to the fact that the soil in Fuchu is less fertile than in Ohguri. During the reproductive period (September–December), the NSC pools declined drastically in the leaves (92% in Fuchu, 86% in Ohguri), reflecting a loss of carbohydrates from the leaves and an accumulation in the roots via the stems. The NSC pools in the stems, though, had a fluctuating trend in both sites,

decreasing slightly at the later stage of the overwintering period, indicating the storage of carbohydrates in the roots in preparation for spring growth. These findings are in accord with other researchers (Cook 1966; Humphreys & Robinson 1966; Chapin *et al.* 1986), who found that the carbohydrate pools in the leaves and stems increased gradually through the growing season.

During the vegetative period, the WSC pools increased in the leaves and stems but their concentration remained almost unchanged at both sites. The WSCs were measured at the highest levels in the stems, followed by the roots and leaves during this period (Fig. 5). There was no significant difference between the Fuchu and Ohguri sites with regard to the WSC pools during the vegetative period ($P = 0.341$). The WSC pools decreased during the reproductive stages and were most pronounced in the leaves, followed by the stems. In the roots, the WSC concentration decreased only at the later stage of the reproductive period at both sites. A variation in the WSCs with the season is related in part to the environmental effects on plant processes and partly to the plant development stages (Fulkerson & Donaghy 2001). The WSCs accumulated in the roots and stubble of perennial ryegrass plants during stem elongation, for instance, and decreased during seed-head development due to the conversion of the WSCs to starch

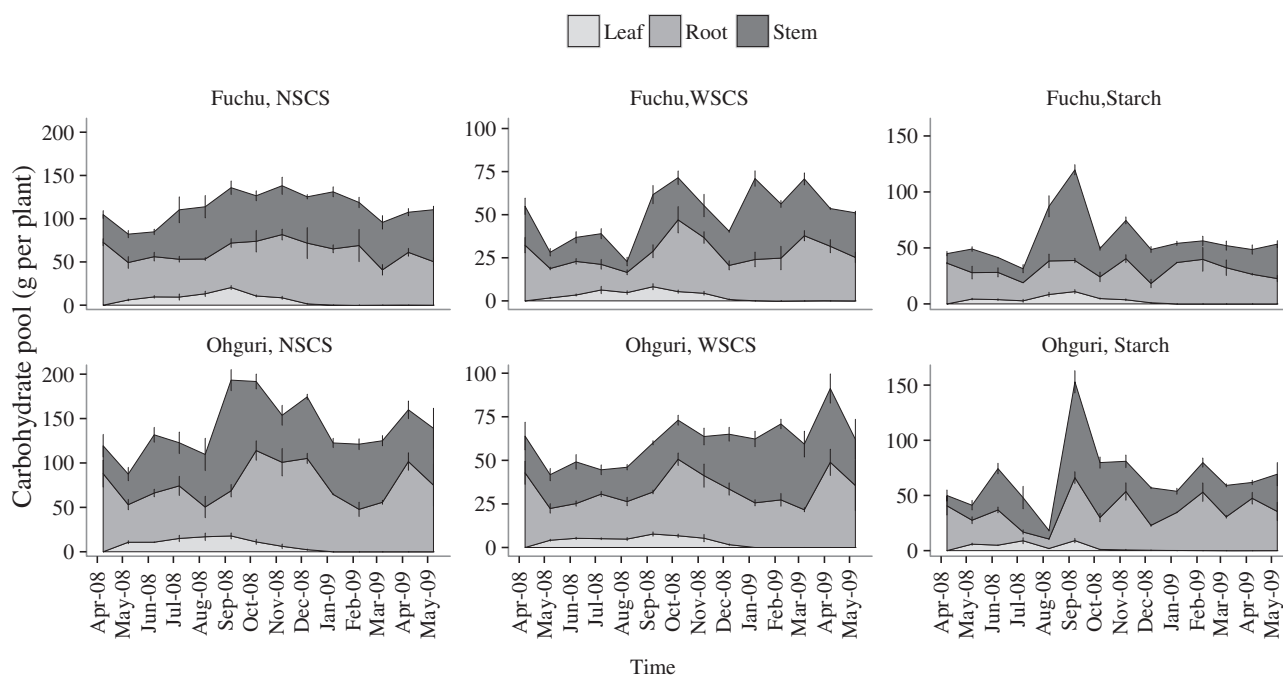


Fig. 5. Seasonal changes in the pools of non-structural carbohydrate (NSC) fractions in the plant organs of *Pueraria lobata* in Fuchu and Ohguri, Japan. The vertical bar that is associated with each data point is the standard deviation of the mean ($n = 3$). WSCs, water-soluble carbohydrates.

and its storage in the seeds (Waite & Boyd 1953). The results of this study are supported by the findings of Sullivan and Sprague (1943), who observed that during defoliation, the underground organs of perennial ryegrass received carbohydrates as storage. There is a marked variation in the WSC accumulation during regrowth with regard to season. The similarity of carbohydrate concentrations in the plants at the two study sites indicates that the populations did not differ substantially in their carbohydrate concentrations. The clear-cut seasonal course of the concentrations of both the WSC reserves and the NSC reserves corresponds to patterns that have been reported previously (Granéli *et al.* 1992; Guthruf *et al.* 1993).

Starch represented the greatest part of the NSC pools. The starch pools in the roots decreased during the early vegetative stage of the plants at both sites and reached the minimum level in July (16.19 g per plant in Fuchu, 7.93 g per plant in Ohguri) (Fig. 5). Conversely, the amount of starch in the stems and leaves fluctuated, increasing at the end of the vegetative period. For example, the starch pools increased more in the stems (857% in Fuchu, 835% in Ohguri), followed by the leaves (154% in Fuchu, 54% in Ohguri) (Fig. 5), in the vegetative stage. For the period covering the reproductive stages, starch decreased more in the leaves

(90% in Fuchu, 107% in Ohguri), followed by the stems and roots. From the beginning to the end of the overwintering period (December–April), the starch pools in the roots increased by ~27 and 110% in Fuchu and Ohguri, respectively, whereas it decreased by ~65 and 57% in the stems in Fuchu and Ohguri, respectively. There was a decrease of starch in the roots and stems at the end of the vegetative growth in the Ohguri site but it increased again at a later period in the reproductive stages that coincided with a decrease in the starch levels of the leaves. At the later stage of the dormant (overwintering) period, the NSC pools in the stems and roots decreased slightly (Fig. 5) at both sites. This loss can be associated with the respiration of the overwintering organs (rhizome and stem) (Ino *et al.* 2003).

The most striking differences among the plant organs were observed in the pattern of the carbohydrate (NSCs, WSCs and starch) storage for *P. lobata*. It stored the NSCs in the stems and roots. A declining trend of the NSCs was found in the root tissues during the growing season until August, while it increased during fall at both sites. There was no significant variation in the carbohydrate concentrations between the two populations, even though they grew in vastly different soil conditions.

Management implications

The NSCs are important in initiating regrowth when photosynthetic tissues are non-existent or are inadequate to supply both respiration and growth demands. Carbohydrate storage is a characteristic feature of most plants, particularly perennials. It has been hypothesized that invasive plant control might be improved if management efforts are applied when carbohydrate reserves are low in plant organs, especially the roots. *Pueraria lobata* is a climbing perennial legume that has become invasive and naturalized in several parts of the world. The invasive success of *P. lobata* is related, in part, to its production of large underground storage roots that are composed largely of starch and other carbohydrates. Vigorous sprouting and biomass production of the plant depend on the carbohydrate reserve.

For kudzu and other invasive vines, it is common practice to cut or mow the colony during the early vegetative stage (June–July) of the plant when it is manageable. Terrill *et al.* (2003) reported that the removal of the above-ground vegetation of *P. lobata* during the vegetative growth stage does not reduce herbage production. In fact, the plant might grow more biomass. The reason for this response of *P. lobata* to mowing can be explained in view of the findings that cutting the above-ground biomass during this period does not affect the storage carbohydrate pool (starch) of the underground rhizome. Therefore, the starch storage pool becomes larger than the previous year. The current study found that the NSCs in *P. lobata* changed remarkably in its phenological cycle and they were highest and lowest in the roots during its overwintering and reproductive period, respectively. The NSCs translocate to the rhizome at the end of September. Therefore, the end of August to early September would be the best time for cutting the biomass and a successful eradication program should include repeated herbage removal for consecutive years in order to deplete the carbohydrate storage of a *P. lobata* ramet.

ACKNOWLEDGMENTS

The first author was supported in part by a Grant-in-Aid for Japanese Society for the Promotion of Science (JSPS) Fellows (10F00378) from the JSPS, Tokyo, Japan. The research also was supported by a grant from the Joint Japan World Bank Graduate Scholarship Program, Washington, DC, USA, as a postgraduate scholarship for the second author.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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