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Seasonal Fluctuations of Lectins in Barks of Elderberry (*Sambucus nigra*) and Black Locust (*Robinia pseudoacacia*)[1]

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MAKUTA NSIMBA-LUBAKI AND WILLY J. PEUMANS*
Laboratorium voor Plantenbiochemie, Kuleuven, Kardinaal Mercierlaan 92, 3030 Leuven, Belgium

ABSTRACT

Elderberry (*Sambucus nigra*) and black locust (*Robinia pseudoacacia*) agglutinins, which are abundantly present in the bark of both species, display seasonal fluctuations with regard to their content in this tissue. These seasonal changes result apparently from a circa-annual rhythm of lectin accumulation and depletion during autumn and spring, respectively. Because the bark of trees can be considered as a type of vegetative storage tissue, the results suggest that bark lectins behave as typical storage proteins.

Plant lectins represent an extended class of proteins or glycoproteins classified in a single (artificial) group on the basis of their unique ability to recognize and bind specific sugars (for reviews, see refs. 4 and 11). During the past decades numerous phytohemagglutinins have been purified and characterized with respect to their biochemical, physicochemical and biological properties. Although in the past most of the work has been devoted to seed lectins, evidence has accumulated that phytohemagglutinins occur also in such vegetative organs as tubers (2), root stocks (15), phloem exudates (1), roots (18), rhizomes (14, 16) and leaves (10). Interestingly, living bark of at least two tree species, namely black locust (*Robinia pseudoacacia*) (6) and elderberry (*Sambucus nigra*) (3) were found to contain high concentrations of lectin. In both species, indeed, the bark lectin represents up to 5% of the total protein content of the tissue (3, 6). Bark tissue of deciduous woody perennials is a major site of nitrogen accumulation at the end of the growing season and represents the major source of nitrogenous compounds at the beginning of growth during the next spring (13, 17). Most of the stored nitrogen is incorporated in proteins (7, 8, 12, 17), which accumulate during autumn when the amino acids derived from leaf protein breakdown are translocated to the bark and made available for protein synthesis at this particular storage site. When the plants resume growth after overwintering, the bark storage proteins are degraded into amino acids, which then are translocated to the growing tissues to satisfy their high nitrogen demand. Since both elderberry and black locust lectins represent a considerable portion of the total bark protein, the question arises whether these particular proteins might also be involved in the process of nitrogen storage in this tissue.

In this paper, we report the distribution of elderberry and black locust lectins in different tissues and seasonal fluctuations of the lectin content of the bark. Evidence is presented that both agglutinins are predominantly located in the bast, where their concentration is subject to a circa-annual rhythm of accumulation and depletion.

MATERIALS AND METHODS

Material. Bark and other tissues were obtained from locally growing elderberry (*Sambucus nigra*) and black locust (*Robinia pseudoacacia*) trees. Small pieces of bark were stripped with a knife from stems, branches, or roots at different times of the year (as indicated in the tables and figures). The age of stems and branches was deduced from the branching pattern or determined by counting the annual rings. Other tissues (*e.g.* flowers, fruits) were collected at the appropriate times as indicated.

Extraction. Small pieces (about 200 mg) were cut from bark samples stripped of the suberized (dead) outer layers and extracted in a mortar with 10 volumes (v/w) of phosphate-buffered saline (PBS: 1.5 mM KH_2PO_4, 10 mM Na_2HPO_4, 3 mM KCl, 140 mM NaCl, pH 7.4). Homogenates were centrifuged in an Eppendorf microcentrifuge (4 min, 10,000*g*) and the resulting supernatants used as such for determination of the agglutination titer. Extracts from other tissues were prepared similarly.

Agglutination Assays. Agglutination assays were performed as previously described (16). Titers are expressed as the highest dilution at which the extracts still agglutinated a 1% suspension of the trypsin-treated group A red blood cells.

Purification of Elderberry and Black Locust Bark Lectins. *S. nigra* agglutinin and *R. pseudoacacia* agglutinin were purified by affinity-chromatography on fetuin-agarose essentially as previously described (3).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Crude extracts and purified lectins were analyzed by SDS-PAGE using a discontinuous system as described by Laemmli (9) on a 12.5 to 25% acrylamide gradient gel.

RESULTS

Distribution of Elderberry and Black Locust Lectins over Different Tissues. Both elderberry and black locust bark are rich sources of lectin. According to earlier reports, SNA[2] and RPA represent up to 5 and 10% of the total protein content of bark extracts from elder and black locust, respectively (3, 6). To find out whether SNA and RPA are bark-specific proteins, their presence and relative concentrations were determined in other parts of the trees. As shown in Tables I and II, SNA and RPA do not occur exclusively in the bark but are found in all vegetative tissues examined. Moreover, the same tables show that during late spring (when the trees are flowering), the lectin content of the bark (of at least new shoots) is not higher than that of other vegetative tissues. However, during winter, the lectin content of bark is many times higher than that of any other tissue, which

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[2] Abbreviations: SNA = *Sambucus nigra* agglutinin; RPA = *Robinia pseudoacacia* agglutinin.

Table I. *Distribution of Lectin in Different Tissues and Organs of Elderberry during Different Seasons*

Tissue	Titer of Extracts[a]		
	First week of June 1983	First week of September 1983	Last week of November 1983
Leaves			
Young blades	320		
Expanded blades	80	80	
Petioles	20	20	
Flowers			
Peduncles	80		
Petals	320		
Sepals	80		
Styles	240		
Stamens	240		
Pollen grains	0		
Fruits, Juice		160	
Seeds		80	
Stems			
Pith	5	10	10
Wood	10	20	160
Bark	160	1,280	12,800
Roots			
Wood	40	80	80
Bark	320	2,000	7,500
Root tips	1	1	1

[a] All extracts were prepared by homogenizing the tissues in 10 volumes of PBS.

Table II. *Distribution of Lectin in Different Tissues and Organs of Black Locust during Different Seasons*

Tissue	Titer of Extracts[a]		
	First week of June 1983	First week of September 1983	Last week of November 1983
Leaves			
Young blades	240		
Expanded blades	120	80	
Petioles	40	40	
Flowers			
Peduncles	60		
Petals	80		
Sepals	80		
Styles	80		
Stamens	60		
Pollen grains	0		
Fruits, pods		80	2.5
Seeds		1,280	1,280
Stems			
Wood	80	80	160
Bark	320	2,000	20,000
Roots			
Wood	120	120	120
Bark	240	3,000	15,000
Nodules	20	20	40

[a] All extracts were prepared by homogenizing the tissues in 10 volumes of PBS.

indicates that there must be dramatic seasonal changes of the lectin content of the bark. To check whether the agglutination activity of the extracts from different tissues was really due to SNA and RPA, extracts from all tissues were assayed for agglutination activity in the presence of 5 mM lactose and 50 mM *N*-acetylgalactosamine, respectively (under which conditions SNA and RPA, respectively, are completely inhibited). In no case was any agglutination activity left in either elderberry or in black locust extracts, which indicates that the lectin activity we observed had to be ascribed to SNA or RPA. Moreover, upon immunodiffusion against SNA-antiserum, extracts from different elderberry tissues yielded precipitin lines which completely fused with that of purified SNA (results not shown). It is noteworthy that seeds of both elderberry and black locust also contain lectins. However, these seed lectins are definitely different than those found in vegetative tissues (M Nsimba-Lubaki, unpublished data).

Seasonal Variation of Lectin Content of Elderberry and Black Locust Barks. Seasonal variations of the lectin content of bark tissue were followed by determining the agglutination activity of bark extracts prepared from tissue samples taken at regular intervals throughout the year. As shown in Figure 1, the lectin content of new shoots (*i.e.* those sprouting during the spring) remains low during summer but increases dramatically during the autumn. The lectin content then remains at the same (high) level during the winter until it starts to decrease during late spring, whereafter it rapidly falls back to very low levels during the summer. In the second growing season the lectin content likewise remains low during the summer months but very strongly increases during the autumn. It can be concluded, therefore, that both in elderberry and black locust the lectin content is subject to remarkable seasonal variations. To find out whether the lectin content of bark of older stems also changes seasonally, bark samples were taken from stems aged up to 20 years and lectin contents determined. As shown in Figure 2, a to c, the same seasonal changes occur in bark of stems of all ages. However, it appears that the disappearance of the lectin during the summer decreases as a function of age. The data obtained with elderberry bark of different ages (Fig. 2, a and b) are compiled in Figure 3 showing seasonal variations of the lectin content over a period of several years. Although this figure is somewhat artificial, it clearly demonstrates a circa-annual rhythm of lectin accumulation and disappearance during au-

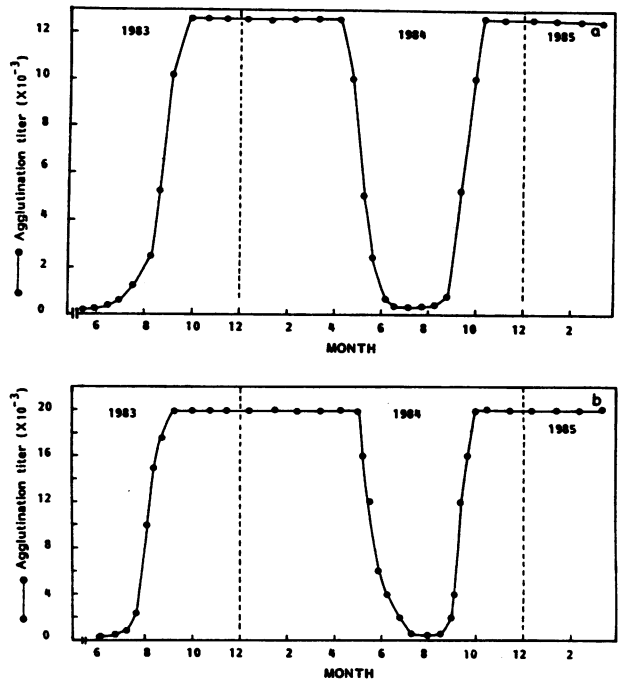

FIG. 1. Seasonal variation of the lectin content of elderberry (a) and black locust (b) barks. Bark samples were taken at regular intervals from shoots sprouted in the spring of 1983 from June 1983 until April 1985. The lectin content of the bark was estimated by determining the agglutination titer of the extracts.

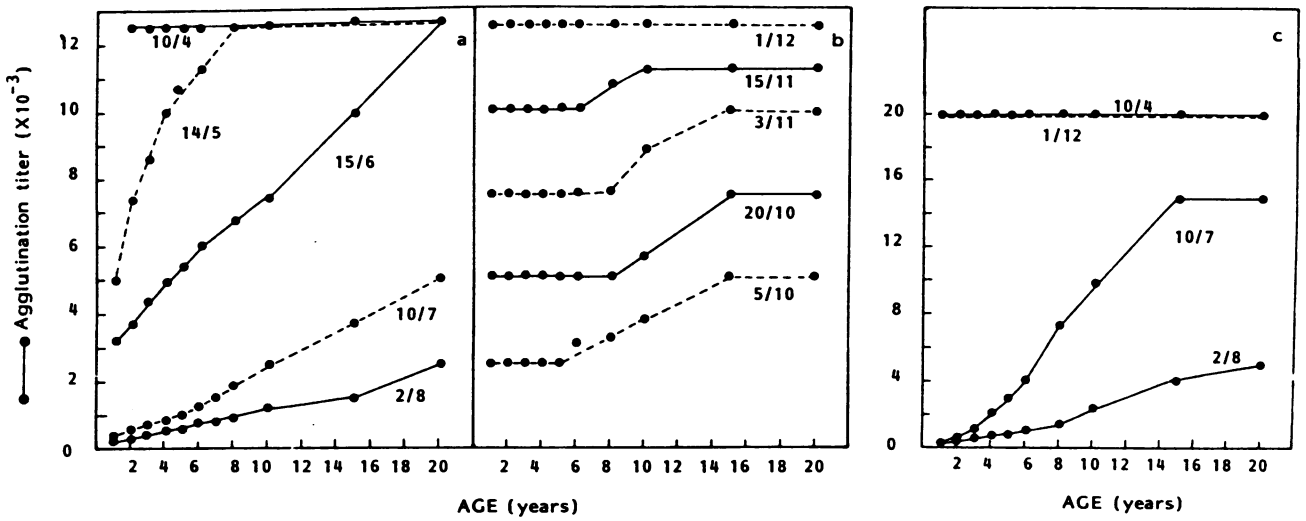

FIG. 2. Seasonal variation of the lectin content of elderberry (a and b) and black locust (c) barks of different ages. At the times indicated (during 1984) bark samples of stems of different ages were analyzed for their lectin content. The different points represent individiual values; as the graphs do not represent time-courses, connecting lines have been drawn to emphasize the seasonal variation of the lectin content.

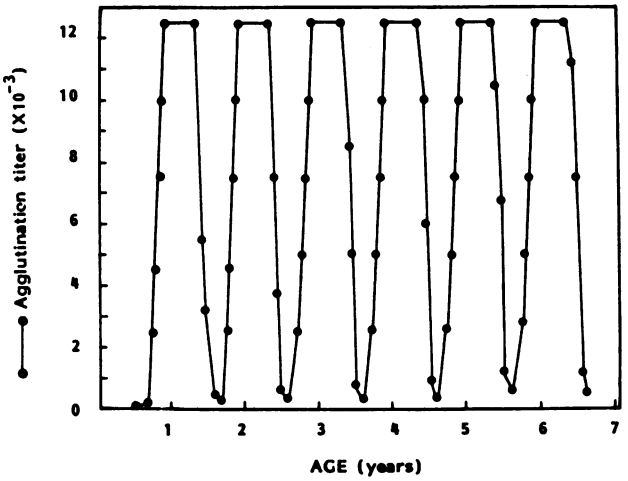

FIG. 3. Seasonal variation of the lectin content of elderberry bark over a period of several years. The data shown in this figure are compiled from those given in Figure 1a and Figure 2, a and b. This means that the fluctuations shown are deduced from experiments done within a time span of 1 year (namely 1984) with bark of different ages. For instance, the points of the 1st and 5th year were obtained in 1984 with bark of shoots sprouted in 1984 and 1980, respectively. Points represent individual values; since the graphs are not time-courses. This figure does not take in account possible differences in the rate of lectin disappearance and/or accumulation as a function of the climatologic circumstances which prevailed during different years. It is, therefore, somewhat artificial.

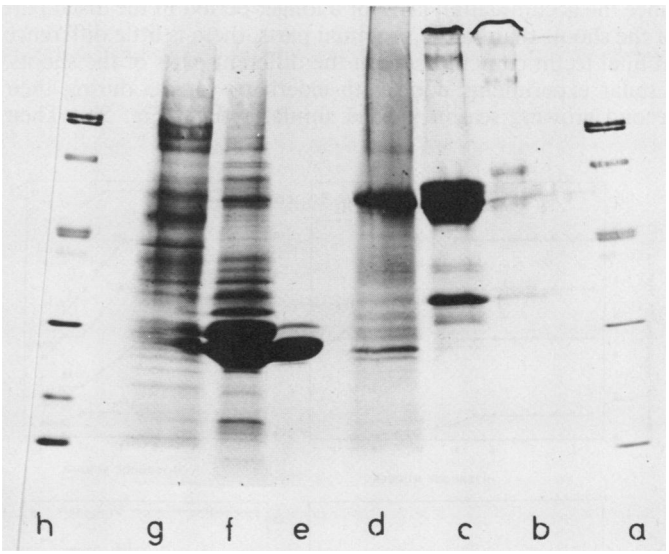

FIG. 4. SDS-PAGE of crude bark extracts and purified bark lectins from elderberry and black locust. Purified SNA (20 μg) and crude extracts (equivalent to 20 mg bark tissue) from 'winter' and 'summer' elderberry bark were loaded on slots b, c and d respectively. Purified RPA (20 μg) and crude extracts (equivalent to 20 mg bark tissue) from winter and summer black locust bark were loaded on slots e, f, and g, respectively. Winter and summer bark were collected in January 1984 and July 1984 respectively. M_r marker proteins were loaded on slots a and h.. They are Cyt *c* (13,000), soya-bean trypsin inhibitor (21,000), carbonic anhydrase (30,000), ovalbumin (45,000), BSA (67,000), and phosphorylase *b* (94,000). Samples (except the marker proteins) were not reduced with mercaptoethanol before applying to the gel. This facilitates the visualization of SNA (3).

tumn and spring, respectively. From the results shown in Figure 2c, a very similar pattern can be deduced with respect to seasonal variations of the lectin content of black locust bark.

Since estimations of the lectin content of bark tissues were based on agglutination assays, control experiments were done to exclude the possibility that the low titers found in bark during the summer months are not due to a possible inhibitory effect of one or another component in the bark during this season. Therefore, we checked the effect of extracts from 'summer' bark on the agglutination activity of both purified lectins and extracts from 'winter' bark. In no case could any inhibitory effect be observed, which indicates that the low titers found during the summer accurately reflect a correspondingly low lectin content during this season. Moreover, SDS-PAGE of crude bark extracts showed that the lectin polypeptides are clearly visible in extracts from winter bark whereas they can not be distinguished among the proteins present in summer bark extracts (Fig. 4).

Lectin Content of Bark Tissue from Different Individuals. To find out whether there are major differences in lectin content between different individual trees, bark samples were collected (during winter) from 20 individuals (of both elderberry and black

locust) and their lectin contents determined. Few, if any, quantitative differences could be observed (at least within the limits of an assay based on the determination of the agglutination titers). Indeed, the agglutination titers varied between 10,000 and 12,500 for elderberry and between 15,000 and 20,000 for black locust.

Proximal and Distal Parts of Shoots Accumulate Bark Lectin at Differential Rate. The results described above were obtained with bark tissue taken from one of the first three proximal internodes of the shoots. This sampling restriction was necessary since preliminary experiments had indicated that there are important differences in lectin content between bark tissue taken from different parts of the shoots. To find out how important these differences are and how the accumulation of lectin changes as a function of the position of bark tissue along the shoot, the lectin content of the bark of each individual internode of the shoots was determined at different times during the period of lectin accumulation. As shown in Figure 5, a and c, the lectin content of the proximal internodes of shoots (which are in their first growing season) is considerably higher than that of the distal internodes, especially during the period of rapid lectin accumulation. It should be noted that both in elderberry and black locust the distal internodes apparently accumulate their respective lectins at a slower rate than the more proximal internodes. However, since the accumulation lasts for a longer period in the distal part of the shoots than in the proximal parts, there is little difference in final lectin content between the different parts of the shoots. Similar experiments done with elderberry shoots during their second growing season yielded similar results (Fig. 5b). Their distal parts also accumulated lectin less rapidly than their proximal parts but continued the accumulation for a longer period so that the final lectin is almost the same throughout the shoots. It is worthwhile to mention that in both elderberry and black locust the yellowing of the leaves and their eventual shedding starts from the proximal internodes and proceeds progressively to the tips of the shoots.

To find out whether proximal and distal parts of the shoots mobilize their lectin pool at differential rates, the lectin content of bark tissue from individual of elderberry shoots was followed during spring and early summer (of the second growing season). Although some slight differences could be observed between the most distal internodes and the rest of the shoots (Fig. 5d) there is apparently little, if any, difference in the rate of lectin disappearance as a function of the position of the bark tissue along the shoot. It should be noted that at the distal end of the shoots, the lectin content is slightly lower than in the rest of the shoots (Fig. 5a).

Distribution of Lectin in *Sambucus ebulus* (Danewort) Plants. Danewort, which is a species closely related to elderberry, contains a lectin that strikingly resembles SNA with respect to its biochemical, physicochemical and carbohydrate-binding properties (M Nsimba-Lubaki, unpublished data). Since this *Sambucus* species is, unlike elderberry (which is a perennial tree), a shrub with perennial roots but annual shoots, it seemed worthwhile to investigate the distribution of the lectin over different tissues, especially in the bark of roots and shoots. As shown in Table III, all tissues of danewort examined in this study, including the bark, contain lectin. However, in contrast to elderberry and black locust, only the bark of the roots accumulates lectin during the autumn and not that of the shoots. Indeed, the bark of the shoots (which eventually die at the end of the autumn) does not contain more lectin at the end of the growing season than during the summer. It appears, therefore, that at least in danewort only the bark of the perennial parts of the plant accumulate lectin.

Seasonal Variation of Lectin Content of Root Bark. Seasonal variations of lectin content occur not only in the bark of shoots but also in that of roots. It can be concluded from Tables I, II and III that the lectin concentration is much higher during the winter than during the summer in roots of elderberry and black locust and in roots of danewort. Although for practical reasons the root system could not be studied in detail, there is evidence that in root bark the lectin content changes seasonally with a circa-annual rhythm. Indeed, when the lectin content of bark of roots of different ages was determined, it was observd that summer bark of roots of all ages (up to 10 years) contained at least two times less lectin than winter bark. This indicates that in the bark of roots, just as in bark of shoots, lectin is accumulated during the autumn and mobilized during spring.

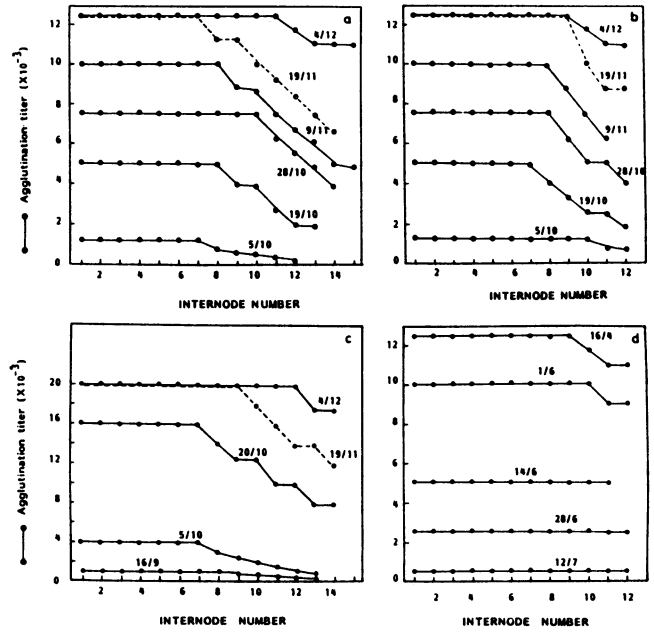

FIG. 5. a and b, Changes of lectin content of bark tissue of different internodes of (a) young (sprouted in 1984) and (b) 1-year-old (sprouted in 1983) elder shoots during the autumn of 1984. c, Changes of lectin content of bark tissue of different internodes of young (sprouted in 1984) black locust shoots during the autumn of 1984. d, Changes of lectin content of bark tissue of different internodes of 1-year-old (sprouted in 1983) elder shoots during spring and early summer of 1984. Extracts were prepared from bark tissue stripped from each individual internode of the shoots and assayed for agglutination activity. Internode 1 corresponds to the most proximal internode whereas the last internode corresponds to the very tip of the shoots. The smaller number of internodes of 1-year-old elder shoots relative to that of young shoots is due to the fact that the tip of the shoots usually die during the winter.

DISCUSSION

Earlier studies on the isolation and characterization of lectins from black locust (6) and elderberry (3) have shown that these two lectins are abundantly present in the bark of both species. Although the results described in this paper confirm these earlier findings it is evident from the present data (1) that both lectins are present in all vegetative organs and (2) that the lectin content of the bark itself is subjected to dramatic seasonal changes. With respect to the first conclusion it should be emphasized that, although present in other vegetative organs and tissues, the lectin concentration of bark exceeds that of the other parts of the trees by 50-fold, at least when winter bark is considered. Similarly, within the bark itself, the lectin concentration varies with a factor of at least 50 as a function of the sampling time. As can be concluded from the results described above, the latter differences have to be ascribed to seasonal variations of the lectin content,

Table III. *Distribution of Lectin in Different Tissues and Organs of Danewort during Different Seasons*

Tissue	Agglutination Titer of Extracts[a]			
	First week of June 1983	Second week of July 1983	First week of September 1983	Last week of November 1983
Leaves				
Young blades	480			
Expanded blades	240		240	
Petioles	40		40	
Flowers				
Peduncles		40		
Petals		160		
Sepals		40		
Styles		120		
Stamens		120		
Pollen grains		0		
Fruits, juice			960	
Seeds			1,920	
Stems				
Pith	20		10	0
Wood	20		5	0
Bark	160		160	120
Roots				
Wood	40		20	40
Bark	240		1,280	7,500
Root tips	2.5		2.5	

[a] All extracts were prepared by homogenizing the tissues in 10 volumes of PBS.

which themselves result apparently from a circa-annual rhythm of lectin accumulation and depletion during autumn and spring, respectively.

Evidently, the lectin accumulation which takes place in the autumn and seems to be accomplished within a period of about 7 to 8 weeks coincides with a period during which the trees switch from an active to a resting state. Conversely, when the trees resume growth during spring their lectin content rapidly decreases. Since bark of trees can be considerd as a type of vegetative storage tissue in which protein and carbohydrate are accumulated during the autumn and mobilized during the spring (7, 8, 12, 13, 17), it follows that the bark lectins behave as typical storage proteins. This, however, does not necessarily mean that the bark lectins are storage proteins.

Besides the typical circa-annual rhythm of accumulation and depletion, there are some other indications that bark lectins are directly or indirectly involved in storage protein metabolism. First, the fact that no lectin accumulation occurs in the bark of the annual shoots of danewort whereas root bark of the same species accumulates large amounts of lectin suggests that accumulation of lectin and other storage materials go hand in hand. Second, those parts of the shoots which first lose their leaves (*i.e.* the proximal nodes) stop to accumulate lectin well before the parts that are still green (*i.e.* the most distal nodes). Third, both SNA and RPA resemble in several aspects (*e.g.* amino acid composition) typical plant storage proteins. Finally, the results of recent immunocytochemical studies which indicated that SNA is localized within the protein bodies (5) strongly support the idea of the involvement of this lectin in protein storage processes in elder bark.

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