Young *Chrysomela* larvae prefer lower phenolics in their diet (Coleoptera, Chrysomelidae)

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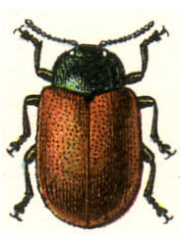
Svensk sammanfattning: I denna studie jämförde vi hur larver och vuxna av bladbaggen *Chrysomela tremula* väljer när de får välja mellan bitar av aspblad med högt och lågt innehåll av fenoler. Fenoler är antioxidanter som finns i riklig mängd i lövträd av släktena *Betula*, *Populus* och *Salix*. Vi använde fältinformation från SwAsp-samlingen av asp (Albrectsen 2006) och identifierade två genotyper: klon 10 som ofta uppvisar höga skadenivåer och innehåller lite bladfenoler och klon 36 som sällan angrips i fält och som innehåller höga koncentrationer av bladfenoler. Våra testplantor hade odlats i växthus där fenolskillnaderna mellan kloner inte blir så tydlig som i fält. Vi satte upp preferensförsök för att observera hur bladbaggarna valde mellan klonerna i petriskålar. Vi kunde konstatera att unga larver (stadium 1) valde som de gjort i fält, dvs klon 10 valdes oftast, men detta var signifikant endast för äldre blad. Ingen skillnad förelåg när något större larver (stadium 2) och vuxna skalbaggar fick välja mellan yngre blad. Bladbaggar är kända för att kunna uppta och använda vissa fenoler för sitt eget försvar. Vi kunde även konkludera att den totala mängden fenoler inte kunde användas som ett mått på bladens innehåll av prefererade salicylater.



In 2003, staff at Umeå Plant Science Centre selected 120 wild aspens (*Populus tremula*) from all over Sweden. They took root cuttings so that genetically identical copies could be made of these trees, and the young plants were grown together in one garden. Now, six years later, we call this group of young trees the Swedish Aspen Collection, or SwAsp (Albrectsen 2005). Although they belong to the same species and all originate in Sweden, we have found out that the individual SwAsp trees vary quite a lot. The main differences are the physical leaf forms and chemical makeup of the leaves. It is well known that aspen supports a specialist fauna of arthropod herbivores, many of which can tolerate, and even benefit from, the chemicals the aspen produce to protect themselves from herbivores. We are studying SwAsp to understand how the trees differ in their leaf chemicals, and if this explains why we see different levels of feeding by arthropod herbivores.

We found two particularly interesting SwAsp trees in our garden, Tree 10 (originally from Simlångsdalen, Halland) and Tree 36 (initially from Vårgårda, Västergötland). We have noticed in our garden that Tree 10 suffers high levels of attack from various chrysomelid beetles, eriophyd galling mites, tenthrenidinid wasp larvae, and cecidomyiid galling midges, to name a few herbivores. We have looked at the chemical composition of Tree 10's

Table 1. Results from choice test with *Chrysomela tremula* larvae and adults feeding on aspen leaf discs representing the two different genotypes Tree 10 and Tree 36.

Instar	Time (h)	Leaf age	Replicates	Feeding	Tree 10	X^2	Sign.
Larva 1	22	Young	30	28	15	0.34	No
Larva 1	22	Old	30	27	21	8.63	Yes
Larva 2	6	Young	27	19	12	2.40	No
Adult	4	Young	20	11	7	0.90	No

leaves, and we noticed there are low levels of phenolic compounds. Higher levels of phenolics in *Populus* are widely understood to be a defence against herbivores, so we got to work to find out more. We noticed that Tree 36 has less herbivore attack and higher levels of leaf phenolics in the garden. We decided to see if our observations in the field were true in the more controlled conditions of the laboratory.

We grew plants of Tree 10 and Tree 36 in the greenhouse from root cuttings until they were about 30 cm tall. For the experiment, a circular leaf disc (about 1 cm^2) was punched out from each leaf. We collected *Chrysomela tremula* adults from the wild and raised several generations in the laboratory on a varied diet of leaves from different aspen trees. When we had enough instar 1 and 2 larvae, we set up a choice test, in which we offered one leaf disc of each aspen genotype to a singular larva or adult at the time.

Each choice test was replicated 20-30 times, depending on the available number of specimens for each *Chrysomela* group. We controlled the light conditions and ensured that the beetles were hydrated. Then we waited to see which tree's leaves they preferred to feed on. Only young leaves were offered to instar 2 larvae and adult beetles, whereas instar 1 larvae were offered young leaves (from the top of the plants) in one test, and older leaves (from the middle of the plants) in another test. Because the different instars of *C. tremula* ate the leaf discs at different speeds, we tested the differences in feeding at different times. We tested the adults for up to four hours, the instar 2 larvae for up to six hours, and the instar 1 larvae for up to 22 hours. We could not run the tests longer because the animals would be hungry and preferences would not be seen. At these times, we noticed the maximum differences between the amounts of Tree 10 and Tree 36 leaves that had been eaten (Tab. 1).

To be sure that our conclusions were fair, we tested our counts of preferred leaf discs using a X^2 test statistic and Yates' correction. This is a way of testing whether the number of preferred leaf discs in Tree 10 leaves was different from the outcome if the test animals had eaten at random.

In all cases, more than half of the leaf discs eaten came from Tree 10 leaves, but the rule is that if the calculated X^2 value is smaller than 3.84 (for 30 replicates), we must conclude that there is no difference between what we see and a random result, and any difference we see is not significant. However, if the X^2 value is higher than 3.84, we can be 95 % sure that there is a difference in preference between the two leaf discs, and if X^2 exceeds 6.63 we can be 99 % sure.

The results of the test showed that for young leaves, neither larvae nor adults showed any feeding preference for leaf type. However, in the older leaves there was a significant difference in the leaf type when fed to instar 1 larvae; Tree 10 leaves were eaten much more frequently than Tree 36 leaves. We can be 99 % sure of this result because our X^2 of 8.63 exceeds the 6.63 threshold for this experiment.

Our tests have shown that young *C. tremula* larvae have a preference for older Tree 10 leaves over Tree 36 leaves. Tree 10 has consistently lower levels of phenolics in its leaf constituents. Phenolics are powerful antioxidants that can harm insect digestion and survival, which has wider impacts on ecology and conservation. This first study has opened up a lot more questions about the diversity of phenolics in aspen trees, their impact on insect feeding, and differences in leaf age. We know from previous studies of *Chrysomela* that they sequester some phenolics of the salicylate type for their own defence, and another conclusion for this study was that we could not use levels of total phenolics as a clue for the presence of specific preferred salicylates in the aspen clones. The lack of difference in adult choice between phenolic levels was also supported by studies of the *Chrysomela* oviposition culture described in a companion article (Albrectsen 2009).

References

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En dikt av Osip Mandelstam [1891-1938]

Till glädje tag emot ur mina händer
en smula solsken och en smula honung
så som Perséfones små bin befallt oss.

Man lösgör ej den oförtöjda ekan
och ljudlös är en skinnskodd skugga
och livets fasa tätnar obesegrad.

Vad annat återstår oss då än kyssar,
små honungskyssar, ludna som en bisvärm
som måste dö när den har lämnat kupan.

De surrar genom nattens klara vildmark.
Taýgetos är deras täta hemskog
och lungört, tid och mynta deras föda.

Till glädje tag emot min vilda gåva,
ett halsband, glanslöst och förtorkat,
av döda bin som skapat sol av honung.

[November 1920 ur diktsamlingen Tristia,
till svenska av Hans Björkegren]