Poplar clones differ in their resistance against insects feeding

Hilke Schroeder and Matthias Fladung*

Abstract

Short rotation coppices of poplars are often ecologically and genetically restricted because of the cultivation of only one or few clones. This leads to a suboptimal relation between the plants and the associated insects. This is true for beneficial insects but in particular also for the insect pest species damaging the poplars. In the case of an insect attack in such plantations, the damage is often higher than in forests at ecological equilibrium or in plantations with higher plant-based biodiversity because of different plant species and clones. In the years 2012 to 2016, on that background an assessment of insect pest species and leaf area loss was performed to identify the resistance of different poplar (genus *Populus*: Malpighiales, Salicaceae) clones against insect pests. Due to an assessment once a year in June, only a low number of sawfly and butterfly caterpillars were found. In the first line, beetles belonging to different families have been found on the poplar clones. The main defoliator in the years 2012 to 2015 has been *Chrysomela populi* (Coleoptera, Chrysomelidae). Furthermore, beetles belonging to the genus *Phratora* sp. have been found. In the years 2012 to 2015, the abundance of the willow leaf beetles *Phratora* sp. was low whereas in the year 2016 this beetle was the main defoliator and present in a remarkable higher amount than the poplar leaf beetle *C. populi*. The leaf area loss and the presence of the main defoliating insects have been used to create a ranking of the 20 investigated poplar clones due to their susceptibility or resistance to insects feeding, respectively.

Keywords: *poplar, clones, chrysomelid beetles, resistance, susceptibility*

Zusammenfassung

Pappelklone unterscheiden sich in ihrer Toleranz gegenüber Insektenfraß

Pappel-Energieholzplantagen sind aufgrund des Anbaus nur eines oder weniger Klone häufig in ihrer genetischen und damit auch ökologischen Variation eingeschränkt. Das führt zu einem suboptimalen Verhältnis zwischen den Pflanzen und den mit ihnen assoziierten Insekten. Das gilt sowohl für die Nützlinge als aber vor allem auch für die Schädlinge. Werden diese Pflanzkulturen von Insekten befallen, ist der Schaden häufig größer als bei einem ökologisch ausgeglichenen Wald oder Forst bzw. auch als bei einer Plantage mit höherer Biodiversität auf Seiten der angepflanzten Baumarten und Klone. Vor diesem Hintergrund wurde die Toleranz verschiedener Pappelklone (Gattung *Populus*: Malpighiales, Salicaceae) gegen Schaderreger in den Jahren 2012 bis 2016 anhand von Insekten- und Blattverlust-Bonituren ermittelt. Bedingt durch eine einmalige Aufnahme im Juni jeden Jahres wurden nur wenige Blattwespen- und Schmetterlingslarven gefunden. Überwiegend waren Käfer verschiedener Familien an den Pappeln zu finden. Als Hauptschaderreger in den Jahren 2012 bis 2015 wurde *Chrysomela populi* (Coleoptera, Chrysomelidae) identifiziert. Außerdem wurden Käfer der Gattung *Phratora* (Coleoptera, Chrysomelidae) an den Pappelklonen gefunden. Die Abundanz des Weidenblattkäfers *Phratora* sp. war in den Jahren 2012 bis 2015 eher gering, während im Jahr 2016 der Weidenblattkäfer als Hauptschaderreger in deutlich größerer Anzahl auftrat als der Pappelblattkäfer *C. populi*. Aus dem Blattverlust und der Präsenz der Hauptschaderreger wurde eine Rangfolge der untersuchten 20 Pappelklone bezüglich ihrer Anfälligkeit bzw. Toleranz gegenüber Insektenfraß erstellt.

Schlüsselworte: *Pappel, Klone, Blattkäfer, Toleranz, Anfälligkeit*

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* Johann Heinrich von Thünen Institut, Institut für Forstgenetik, Sieker Landstraße 2, 22927 Großhansdorf

Kontakt: hilke.schroeder@thuenen.de

1 Introduction

The genus *Populus* is one of the world's most important tree genera. High growth rates, particularly of some interspecies hybrids, and a broad range of applicability, in wood and paper industries and for energy production, led to their widespread cultivation in Europe and North America (Dickman and Stuart, 1983; Stettler et al., 1996). Consequently, different poplar species and clones are used for biomass production in short rotation coppices (SRC). Here in par-ticular interspecies-hybrids are well suited because of their superior growth and advanced resistance traits. In general, SRC are composed of only a low number of clones leading to a decrease of ecological and genetic variation. The risk of insects attacks increases with the decrease of biodiversity (Splechtna and Glatzel, 2005). Thus, insect pests and other pathogens cause higher damage in monocultures than in ecologically balanced forests because of the lower genetic and therefore lower ecological diversity in monocultures (Splechtna and Glatzel, 2005; Helbig et al., 2011). Consequently, a few years after establishing of new coppices, a mass reproduction of insect pests causing severe damage is possible. In contrast, high biodiversity, offering a system with a lot of ecological niches, benefits antagonists of the pest species. Furthermore, for some herbaceous and rice plants even an increase of biomass could be observed when growing in genotype mixture instead of monoculture. For example, tall goldenrod (*Solidago altissima*) produced 46 % more biomass than predicted when grown in mixtures than when grown in monocultures (Crutsinger et al., 2008). Zhu et al. (2000) found that rice yield increased with genotypic diversity explained with a reduction of disease infection in diverse mixtures compared to monocultures.

Thus, possible solutions for minimizing the danger of defoliation by pest insects are breeding of poplar species or clones resistant against insects feeding and planting of clone mixtures enhancing the genetic diversity of poplar SRCs. For this purpose, the knowledge of resistance levels of poplar clones or species against insects feeding is necessary. Following this task, one aspect of the FNR funded project FastWOOD deals with the assessment of resistance levels of different poplar clones against herbivorous insects.

2 Material and Methods

2.1 Study sites and poplar clones

Twenty poplar clones grown on nine different study sites in Germany (Table 1) have been chosen for the evaluation of the resistance level of different poplar clones. The study sites have been setup during the first and second phase of the FastWOOD project in the years 2010 to 2014 (Table 1). The poplar clones are comprised of four different *Populus* species (*P. trichocarpa, P. maximowiczii, P. deltoides*, and *P. nigra*) in different crosses within section as well as between sections. Most of them are registered clones, only the four named with "NW" are newly bred ones by the NW-FVA (Nordwestdeutsche Forstliche Versuchsanstalt, Hann. Münden, Germany). The number of different clones varied from four to 15 clones per study site (Table 2).

At all study sites, the clones were arranged in plots. Every plot contained 24 to 52 trees of the same clone depending on the study sites. Every study site was composed of four to six repeats for each clone.

Table 1
Characteristics of the study sites used for assessment of foliage damage and herbivorous insects. Two dates in the columns "Harvest" means December/January.

Study site	Location	Setup	Harvest 1	Harvest 2
1 Lehmbach (SPF)	Bavaria	April 2010	2012/2013	2015/2016
2 Thammenhain	Saxony	2010	2012/2013	Feb. 2016
3 Trenthorst	Schleswig-Holstein	April 2010	2014/2015	
4 Stiedenrode'11	Hesse	2011	2013/2014	
5 Stiedenrode'13	Hesse	2013	2015/2016	
6 Stölzingen	Hesse	2010	2012/2013	2015/2016
7 Seeburg	Hesse	2014		
8 Wallstawe'10	Saxony-Anhalt	2010	2012/2013	2015/2016
9 Wallstawe'11	Saxony-Anhalt	2011	2013/2014	2016/2017

2.2 Assessment of foliage damage and presence of insects

In the years 2012 until 2015, thirty trees of each clone at each site where the clone is present have been assessed for defoliation level and presence of insects once per year in June. In the year 2016, only four study sites with eight clones in total have been examined.

To guarantee a standardized assessment, a scheme was developed and given to all persons involved in the assessment. First of all, five trees per plot were selected in the first year, and the same five trees were assessed in the following years as far as possible. The five trees were located in the middle of each plot to avoid border effects. For the assessment of foliage damage and insects, the whole tree was inspected. The foliage damage was assessed in 10 % steps, where 0 % was no damage and 100 % complied with total defoliation. Furthermore, species have been determined and number of insects has been documented in four steps of (1) no insects observed ("none"), (2) up to ten insects ("low"), (3) more than ten and up to 29 ("medium"), and (4) equal or more than 30 insects ("high") per tree. As far as possible, the insects were determined directly at the study sites. For this purpose, a leaflet with short descriptions and photos of the most common and therefore expected insect species (beetles and their larvae as well as caterpillars) was devel-oped and given to the observers. In rare cases insects or photos of insects were sent to us for a more detailed determination.

Table 2

Detailed information for occurrence of each clone at the different study sites in alphabetical order and the years of assessment. "X" = the clone is nonexistent at the specific site or has not been assessed. Numbers of study sites are as given in Table 1.

Clone	Study site								
	1	2	3	4	5	6	7	8	9
AF2	2012-2014	2012-14, 2016	2012-2016	2013-2015	2015	2014, 2016	X	2014, 2015	2013-2016
Androscoggin	2012-2014	2012-2014	X	X	X	X	2015	X	X
Hybride275	2012-2014	2012-14, 2016	2012-2016	2013-2015	2015	2014, 2016	2015	2014, 2015	2013-2016
Koreana	X	X	2012-2016	X	X	X	X	X	X
Matrix11	2012-2014	2012-2014	X	X	X	X	2015	X	X
Matrix49	2012-2014	2012-2014	X	X	X	X	2015	X	X
Max1	2012-2014	2012-14, 2016	X	2013-2015	2015	2014, 2016	X	2014, 2015	2013-2016
Max3	2012-2014	X	X	X	X	X	X	X	X
Max4	X	X	2012-2016	X	X	X	X	X	X
Muhle-Larsen	2012-2014	2012-14, 2016	X	2013-2015	2015	2014, 2016	2015	2014, 2015	2013-2016
NW7-177T	2012-2014	2012-14, 2016	X	X	X	2014, 2016	X	2014, 2015	X
NW7-17C	2012-2014	2012-14, 2016	X	X	X	2014, 2016	X	2014, 2015	X
NW7-197S	2012-2014	2012-14, 2016	X	X	X	2014, 2016	X	2014, 2015	X
NW7-204A	2012-2014	2012-14, 2016	X	X	X	2014, 2016	X	2014, 2015	X
OP367	X	X	2012-2016	X	X	X	X	X	X
Robusta	X	X	X	X	X	X	2015	X	X
Rochester	X	X	2012-2016	X	X	X	X	X	X
Trichobel	2012-2014	2012-2014	X	X	X	X	2015	X	X
Weser4	2012-2014	2012-2014	X	X	X	X	2015	X	X
Weser6	2012-2014	2012-2014	X	X	X	X	X	X	X

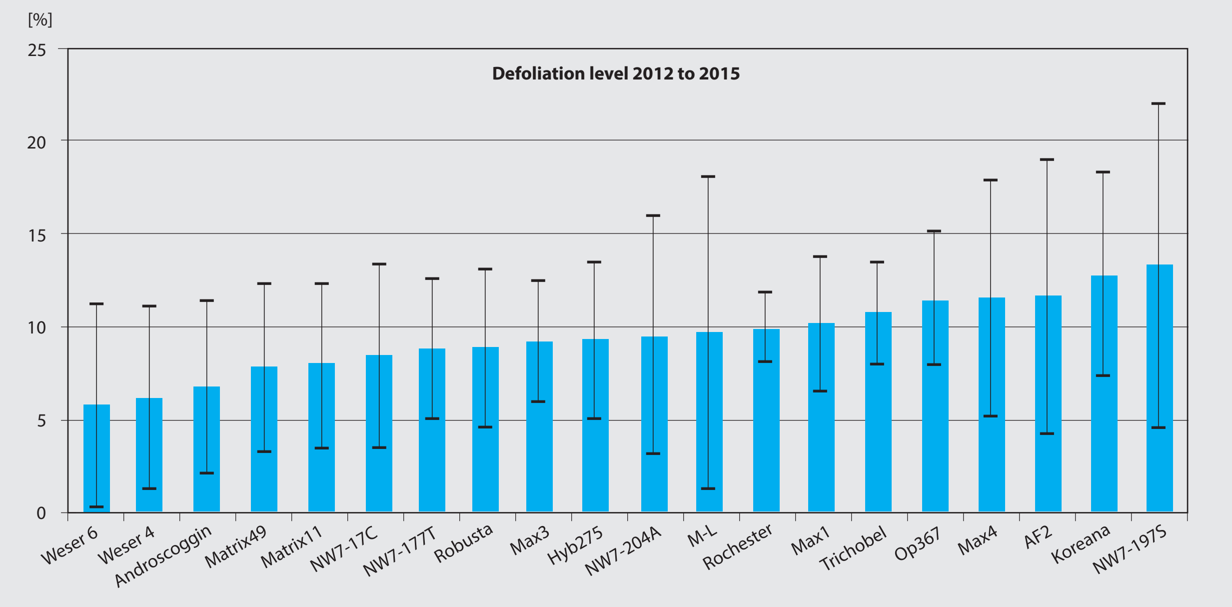

Figure1

Mean level of defoliation [% leaf area loss] and standard deviation for all investigated study sites and 20 clones for the years 2012 to 2015. M-L = Muhle-Larsen, Hyb275 = Hybride 275.

2.3 Calculation of a clone ranking

The defoliation level and the presence of the three insect species (*Chrysomela populi*, *Phratora* sp., and *Byctiscus populi*) were used to calculate a ranking of the clones due to their "resistance level". For this purpose, the position of each clone in the Figures 1, 3 and 4 and an additional Figure for *Byctiscus populi* (not shown here) were used for the calculation of a sum score (e.g. in Figure 1, the clone 'Weser6' got the value 1 and 'NW7-197S' the value 20. In Figure 3 'Rochester' and 'OP367' both got the value 1 and 'NW7-197S' the value 19). Thus, each clone got four values and this sum was used to perform a ranking from high resistance to susceptibility. Again the calculations for the years 2012 to 2015 and the year 2016 were performed separately.

3 Results

3.1 Defoliation level

Although defoliation can have other reasons than feeding by insects, it is an indication for the overall fitness of trees and, when visible, often an indication for the presence of insects. We calculated the mean defoliation level together for all study sites and all clones because we wanted to analyse the defoliation level for each clone independent of field effects. The overall loss of leaves was low for the four years 2012 to 2015. It was between 5.8 % ('Weser6') and 13.4 % ('NW7-197S') (Figure 1). In the year 2016, the defoliation level was slightly higher for all eight in this year investigated clones resulting in the lowest level of 10.7 % for 'NW7-17C' (8.5 % in 2012 to 2015) and the highest level of 14.9 % for the clone 'NW7-197S' (Figure 2).

3.2 Main defoliating insects

Besides a small number of sawflies and some individuals of *Cerura vinula* (Lepidoptera, Notodontidae), mainly beetles have been found. As expected, the most often found insect in the years 2012 to 2015 was the poplar leaf beetle, *Chrysomela populi*. This species has been detected on all but two clones ('Rochester', 'OP367') with an amount of up to 35 % of all trees for single clones, and mostly in a low to medium number of up to ten to lower than 30 beetles per tree (Figure 3). The two species of the genus *Phratora* (*P. vitellinae*, *P. vulgatissima*) have been combined because they are undistinguishable in the field. They were present at 19 clones, just at the clone 'Rochester' no insects could be observed. *Phratora* sp. has been found mainly in a low number of less than ten beetles per tree (Figure 4). The third observed coleopteran was *Byctiscus populi*, only once *B. betulae* has been found. Thus, these two weevil species also have been combined in the analysis. They were found at only thirteen clones in a low number below ten insects per tree to an amount between 2 % and 12 % (data not shown).

In 2016, overall much more insects were observed at the trees but with an even greater difference between the clones. In Table 2, the presence of the two insect species *Chrysomela populi* and *Phratora* sp. is shown for the years 2012 to 2015 compared to 2016 for the eight clones observed in 2016. For *Chrysomela populi,* the presence increased from 17 % in the years 2012 to 2015 to over 60 % in 2016 for the clone 'NW7-177T'. The lowest number in 2016 was observed for the clone 'Hybride 275' with 18 % (Figure 5). That was slightly lower than the average of the years before (20 %) (Figure 3). For *Phratora* sp., the increase in 2016 was extreme with a presence of insects between 48 % and over

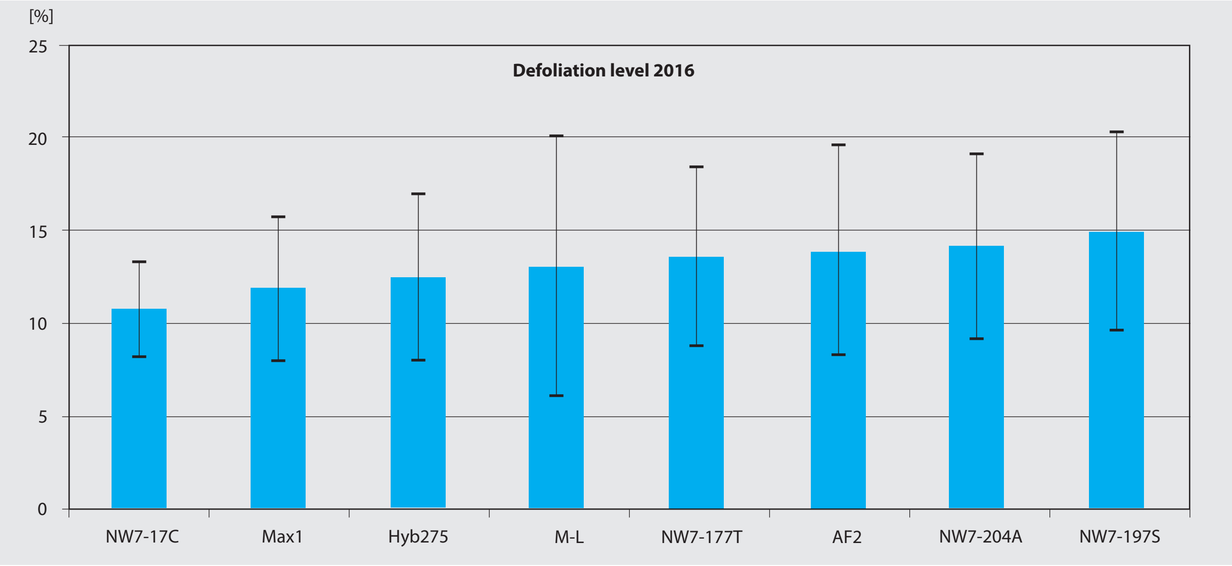

Figure2

Mean defoliation level [% leaf area loss] and standard deviation for the four study sites and eight clones investigated in 2016. M-L = Muhle-Larsen, Hyb275 = Hybride 275.

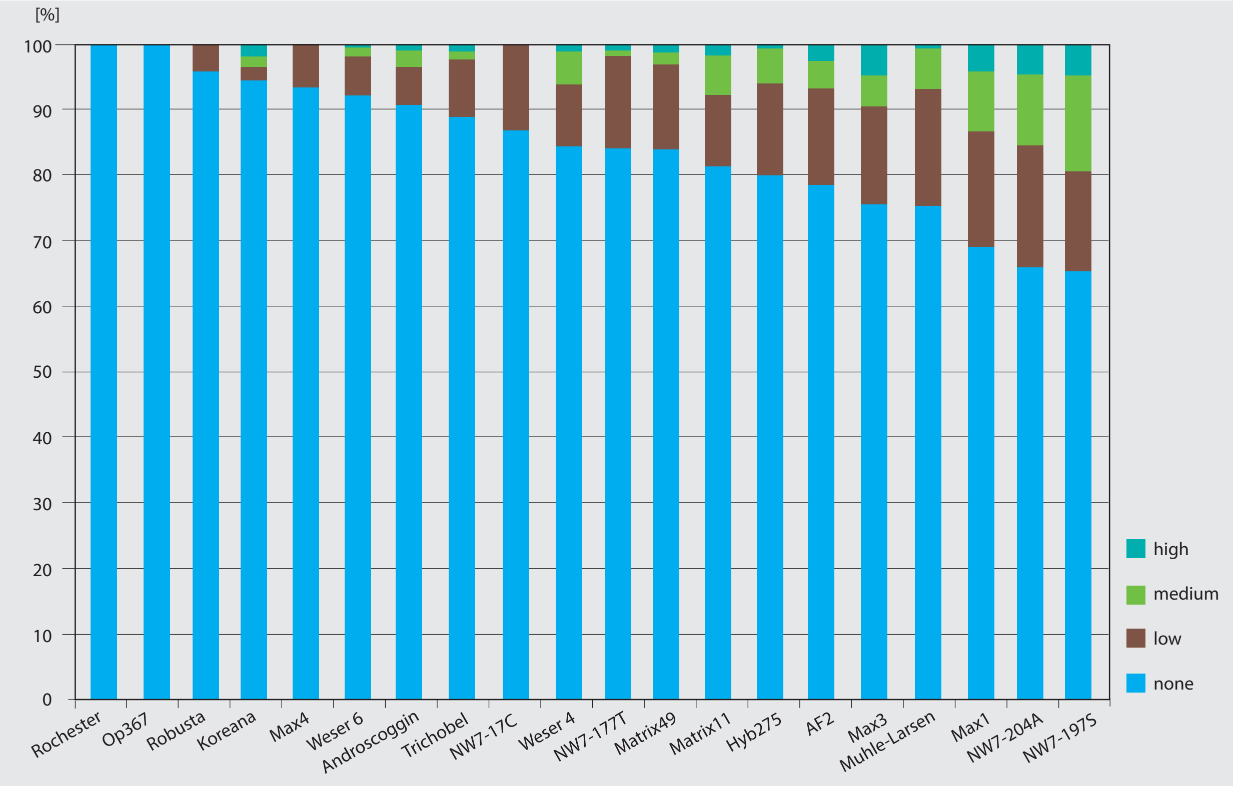

Figure 3

Distribution of abundance classes [percent of all investigated trees per clone] of *Chrysomela populi* found on the 20 investigated clones on average for the years 2012 to 2015. Hyb275 = Hybride 275.

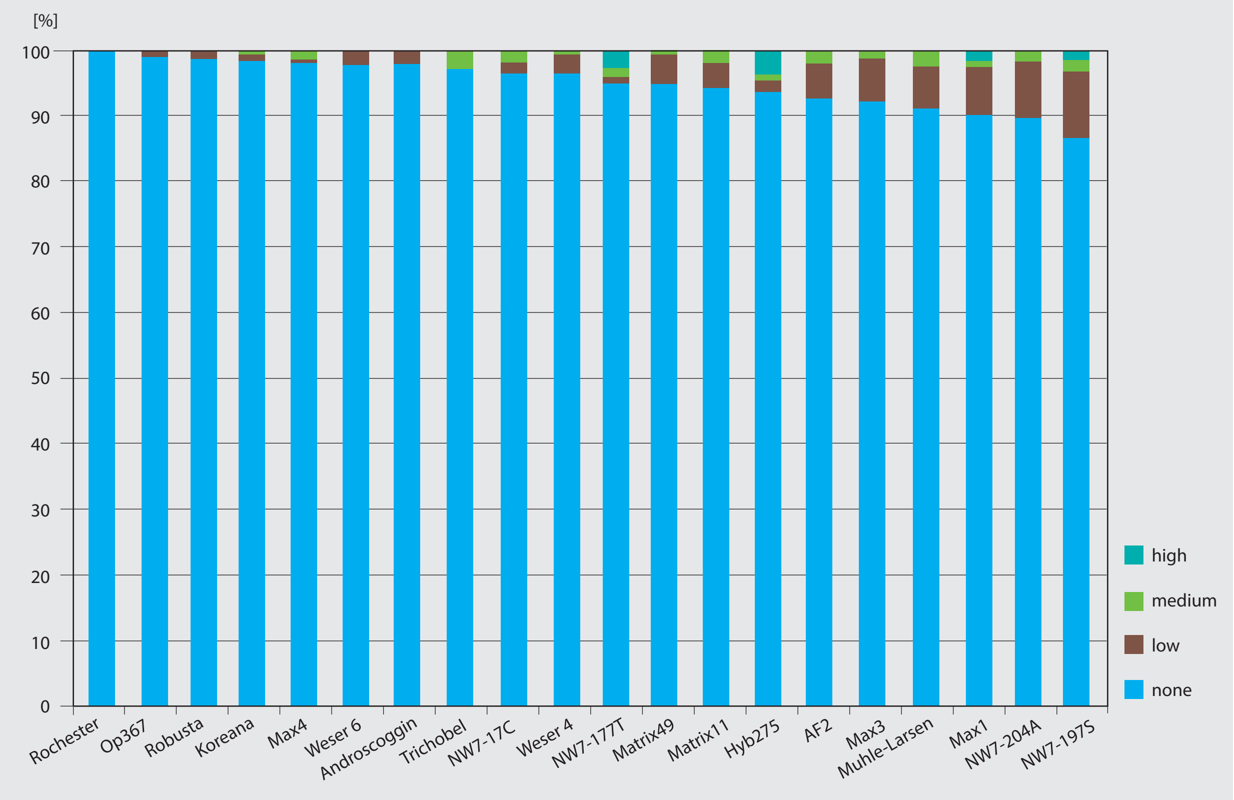

Figure 4

Distribution of abundance classes [percent of all investigated trees per clone] of *Phratora* sp. found on the 20 investigated clones on average for the years 2012 to 2015. Hyb275 = Hybride 275.

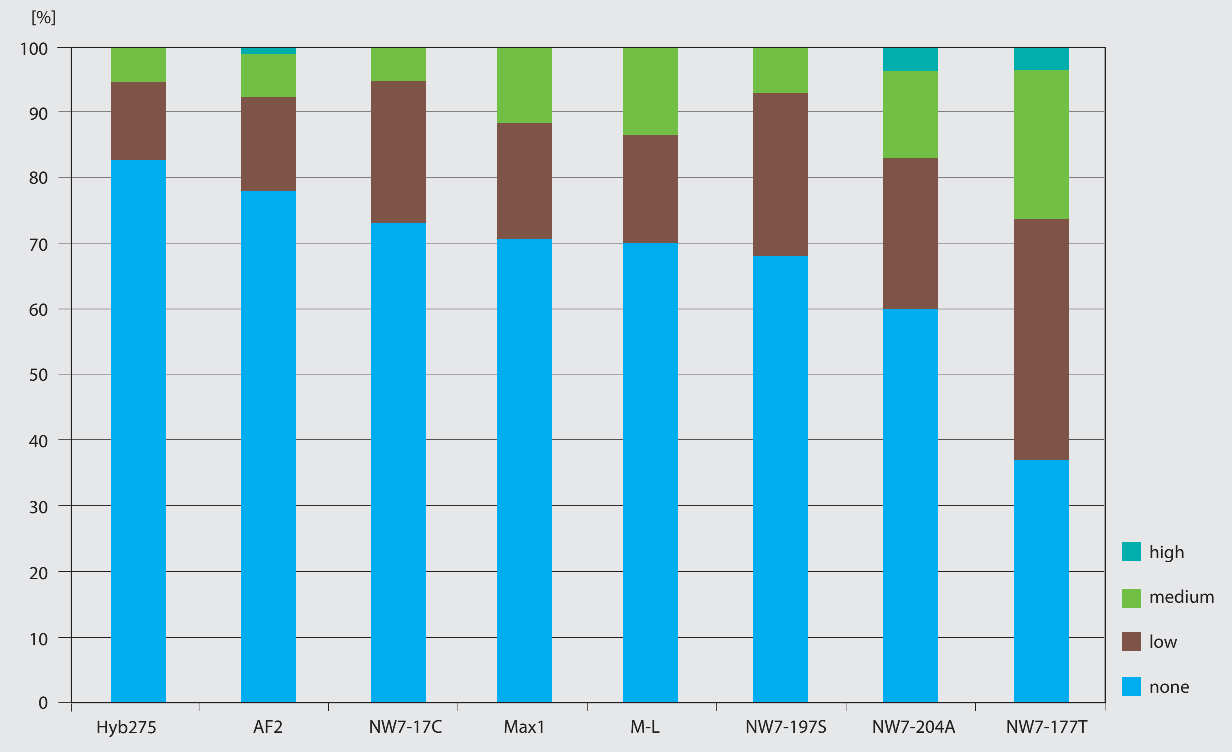

Figure 5
Distribution of abundance classes [percent of all investigated trees per clone] of *Chrysomela populi* found on the eight investigated clones for the year 2016. M-L = Muhle-Larsen, Hyb275 = Hybride 275.

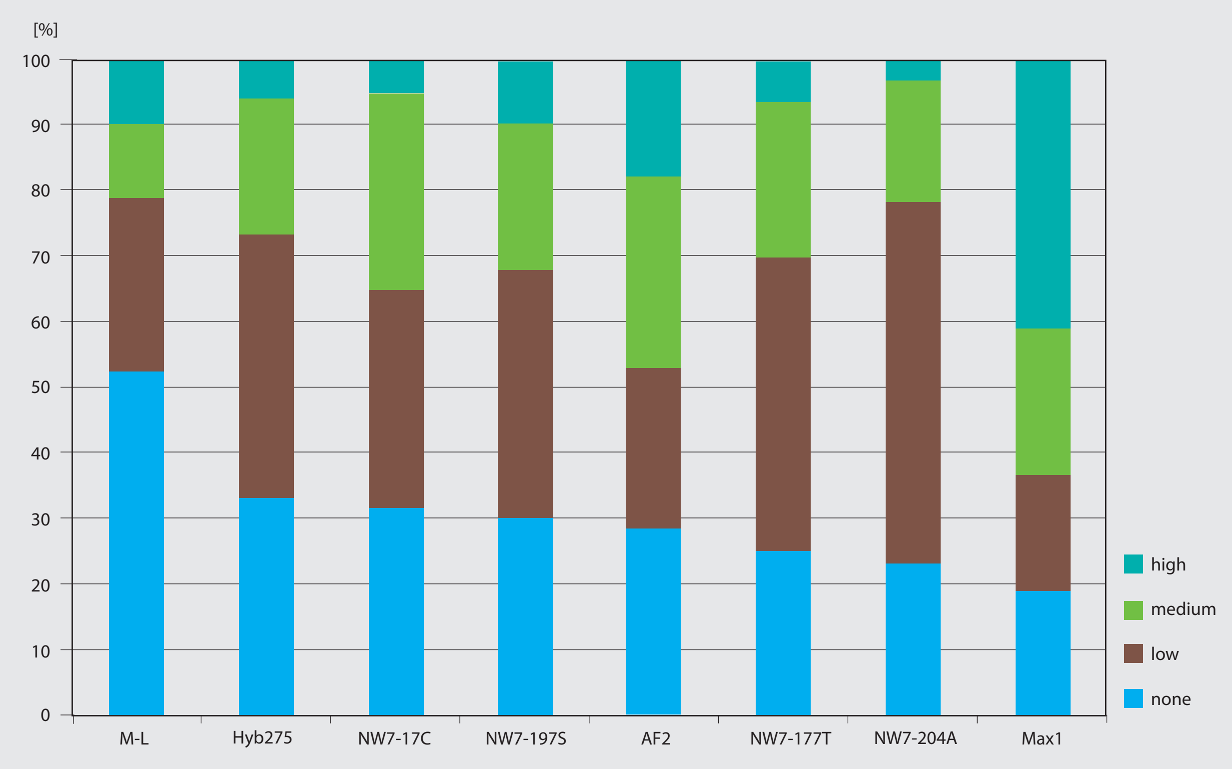

Figure 6
Distribution of abundance classes [percent of all investigated trees per clone] of *Phratora* sp. found on the eight investigated clones for the year 2016. M-L = Muhle-Larsen, Hyb275 = Hybride 275.

80 % (Figure 6, Table 3). In 2016, two further observations have been performed in August and in September. The defoliation level later in the year was clearly higher than in June and was then in accordance with the high number of insects (data not shown).

Table 3

Comparison of the presence [%] of Chrysomela populi and Phratora sp. for the years 2012-2015 and 2016 for eight clones. M-L = Muhle-Larsen.

	Chrysomela populi		
Clone	**2012 to 2015**	**2016**	**difference**
Hybride 275	20 %	18 %	-2 %
AF 2	21 %	21 %	0 %
NW7-17C	13 %	26 %	+13 %
Max 1	31 %	29 %	-2 %
M-L	24 %	30 %	+3 %
NW7-197S	34 %	31 %	-3 %
NW7-204A	32 %	40 %	+8 %
NW7-177T	17 %	62 %	+45 %
	Phratora sp.		
Hybride 275	5 %	66 %	+61 %
AF 2	9 %	71 %	+62 %
NW7-17C	2 %	69 %	+67 %
Max 1	13 %	81 %	+68 %
M-L	10 %	48 %	+38 %
NW7-197S	8 %	70 %	+62 %
NW7-204A	3 %	76 %	+73 %
NW7-177T	6 %	75 %	+69 %

3.3 *Ranking of the poplar clones*

Ranking of the clones is based on the sum score averaged for the years 2012 to 2015 calculated as described in 2.3 and separately for 2016 (Table 4).

Some of the eight clones used in 2016 changed their position to each other when comparing the two rankings (Figure 7).

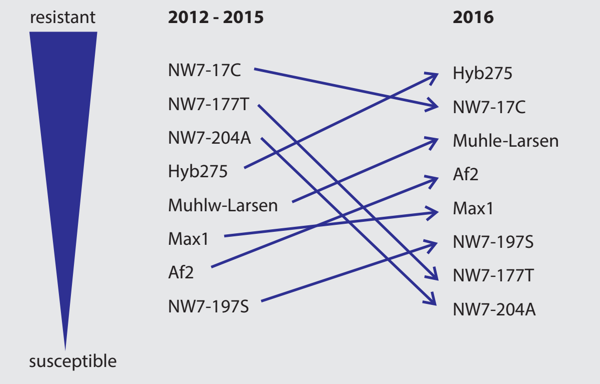

Figure 7

Comparison of the ranking of eight poplar clones for the years 2012 to 2015 and 2016. Hyb 275 = Hybride 275.

Table 4

Ranking of the 20 (eight) clones and the belonging calculated values.

	2012 to 2015		2016	
Clone	**Sum score**	**rank**	**Sum score**	**rank**
Weser6	12	1		
Rochester	16	2		
Androscoggin	16	3		
Weser4	19	4		
NW7-17C	23	5	7	2
OP367	23	6		
Max4	24	7		
Koreana	25	8		
Robusta	26	9		
Matrix11	28	10		
NW7-177T	30	11	19	7
NW7-204A	36	12	21	8
Hybride275	35	13	6	1
Matrix49	37	14		
Trichobel	38	15		
Max3	39	16		
Muhle-Larsen	51	17	10	3
Max1	51	17	14	5
AF2	52	18	13	4
NW7-197S	55	19	18	6

4 Discussion

Chrysomela populi is frequent all over Europe causing severe damage on several species of the family Salicaceae and is therefore one of the most important species from the viewpoint of forestry (Gruppe et al., 1999; Sage et al., 1999; Urban, 2006 and citations therein; Ye, 2011; Schroeder and Fladung, 2015). Following Helbig and Landgraf (2009), *C. populi* is even the most important pest species in short rotation coppices in Germany which confirmed our finding that *C. populi* is the most often occuring beetle on poplars. The poplar leaf beetle can consume a high amount of leaf mass. Urban (2006) observed a quantity of up to 200 cm^2 in about six weeks fed by a single *C. populi* individual. In comparison *Byctiscus populi*, sometimes also described as potential pest in poplar SRCs, is mentioned with about 17 cm^2 fed by a single beetle during its whole lifetime (Urban, 2013).

Also *Phratora vulgatissima* is well known and described as poplar damaging insect (Peacock et al., 1999; Sage et al., 1999), a species that we indeed didn't find in high numbers but frequently at most clones in the years 2012 to 2015. In 2016, the dominance changed dramatically. We found much more *Phratora* sp. than *C. populi* feeding at our poplar clones. Thus, we also can confirm that *Phratora* sp. is a severe poplar damaging beetle.

There are some conspicuous clones, e.g. the clone 'Rochester', where no insects could be found although a defoliation degree of 10 % was estimated. Assessment of the insects has been done only once a year. Thus, this is only a snapshot of the activity of all insects possibly feeding on the poplar clones. The clone ’Rochester therefore may have been lost some leaves due to earlier feeding insects as caterpillars. There are some more prominent clones with a low degree of leaf area loss and also a small amount of observed insects (‘Weser6’ and ‘Weser4’, ‘Androscoggin’ and ‘NW7-17C’). The combination of the results of defoliation level and presence of insects lead to a reasonable statement about the resistance of the clones. So, overall there is a ranking of clones due to their resistance against insects feeding, where long established clones occupy the first four places followed by a newly bred one based on the data of 2012 to 2015. This ranking has been used to select resistant and susceptible clones for a transcriptomic analysis comparing the RNA sequencing results of resistant and susceptible clones after feeding of *Phratora* sp. and also unfed control plants. The results of the transcriptomics analysis will be published elsewhere.

When looking only at the eight clones used in 2016 and comparing their position with that in the years before something has changed. So, the clone ‘NW7-17C’ withstand as resistant, also the susceptible clones ‘Max1’ and ‘NW7-197S’ didn’t change their position dramatically. More prominent is the change of the in 2012 to 2015 as susceptible ranked clone ‘Muhle-Larsen’. This clone changed to a more or less resistant one in 2016. Thus, we can conclude that the data of five years can differ so much that a clear statement for a clone if it is susceptible or resistant is difficult to draw and depends very much on the overall amount of insects feeding in the different years. A further parameter for feeding of the insects seems to be the number of harvests (rotations). We observed a higher defoliation level in the third rotation. This is only a tendency and the data until now are not sufficient for a reliable analysis. More years and more assessments per year would be important to obtain a view on the dynamics of the insect’s presence on different clones.

Nevertheless, until now there are only initial studies about preferences of the herbivorous insects for different poplar clones (Schulz et al., 2009 and citations therein). Thus, the here presented study is a first contribution to a deeper understanding of the necessity of using special clone mixtures in SRCs to increase the ecological variation. In this study, the resistance of poplar against insects was determined on already existing clones. We deeply encourage poplar breeders to include the trait “insect resistance” as goal in future poplar breeding programmes.

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