The Effect of Pollutant Emission on the Resistance and Tolerance of *Artemisia vulgaris* to Herbivores

Dissertation

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III Abbreviations

CEC	Cation Exchange Capacity
DCA	Detrended Correspondance Analysis
DIN	Deutsche Industrie Norm
EV	Eigenvalue
FSU	Friedrich-Schiller-Universität
GDR	German Democratic Republic
HPLC	High Performance Liquid Chromatography
JA	Jasmonic Acid
lg	length of gradient
LUFA	Landwirtschaftliche Untersuchungs- und Forschungsanstalt
MeJA	Methyl Jasmonate
MPI	Max-Planck-Institute
PCA	Principle Component Analysis
PSH	Plant Stress Hypothesis
PVH	Plant Vigor Hypothesis
RDA	Redundancy Analysis
SE	Standard Error
TGL	Technische Güte- und Lieferbedingungen
Xcal	plant available concentration
Xtot	total concentration
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

General Introduction

Herbivory may affect the morphology, physiology, growth and reproduction of plants and as a consequence it is likely to be an important factor in the evolutionary ecology of plants (Rosenthal & Berenbaum 1991; Bernays & Chapman 1998, 2000; Edwards & Crawley 1999; van Oene et al. 1999; Baldwin 2001). From a plant's point of view, herbivores constitute an important biotic source of stress. Stress in this context can be defined as an environmental factor that causes a potentially injurious change in a biological system (Hoffmann & Parsons 1993). However, plants have developed effective mechanisms helping them to escape from or defend themselves against herbivores or to tolerate the effects of herbivore feeding. Plant defenses are physical (silica, thorns, trichomes) or chemical (primary and secondary metabolites) traits that affect herbivore performance (Simms & Fritz 1990; Rausher 1992; Berenbaum 1995; Duffey & Stout 1996; Halitschke et al. 2000; van Dam et al 2000; Kessler & Baldwin 2001).

Both plants and herbivores encounter abiotic conditions that have potential stressing effects (Hunter & Price 1992; Paine et al. 1993). In addition to direct consequences of abiotic stress (Andrzejewska et al. 1990; Crawford et al. 1996), herbivores can also be influenced by indirect effects, mediated by stress-induced changes of the host-plants metabolism. This kind of herbivore reaction is valid for many factors with a negative influence on plants, since in many plant species different types of abiotic stress have been shown to cause similar responses (White 1984). This may be due to the elicitation of similar hormonal responses that slow plant growth (Chapin et al. 1987; Chapin 1991) or to the induced expression of similar genes (Chen et al. 2002). For example, plant responses to anthropogenic pollution stress often resemble those that result from naturally occurring abiotic and biotic stresses (Culliney & Pimental 1986), and both types of stress frequently result in reduced growth or a change in the accumulation of defense compounds (Waterman & Mole 1989; Kozlowski & Pallardy 2002).

A rich literature considers the interactions between abiotic and biotic stress in plants (White 1984; Coley et al. 1985; Jones & Coleman 1991; Herms & Mattson 1992;

Baldwin & Ohnmeiss 1994a 1994b; Mole & Joern 1994; Bi & Felton 1995; Fineblum & Rausher 1995; Preszler & Price 1995; English-Loeb et al. 1997; Koricheva et al. 1998a, 1998b; Björkmann 2000) For herbivores, plant quality is determined by both food value and host plant defenses, each in turn being influenced by a multitude of nutrients and secondary metabolites that vary in both quality and quantity. Based on these complex connections, two competing hypotheses describing the relation between plant stress and herbivore performance have been formulated: White (1984, 1998) argues that nitrogen is the nutrient that usually limits the reproductive performance of herbivores and that plant stress caused by drought or pollution tends to increase available nitrogen e. g. soluble amino acids in the plant phloem (Trumble & Hare 1989; Letourneau & Fox 1989; Clancy 1992; Trumble et al. 1993; Hunter & McNeil 1997; Grundel et al. 1998; Obermaier & Zwolfer 1999; but see Fischer & Fiedler 2000). Furthermore, the dysfunctional metabolism of stressed plants could result in weakened defenses (Katoh et al. 1989; Bolsinger et al. 1992). This ‘Plant Stress Hypothesis’ (PSH) has been confirmed in numerous studies showing beneficial effects of host-plant stress on herbivore performance (e. g. Trumble & Hare 1989; Paine et al. 1993; Larsson & Björkman 1983; Trumble et al. 1993; Redak et al. 1997; for an overview see Koricheva et al. 1998).

In forestry, however, the opposing observation has been made: most attacks by insect herbivores occur on young and fast growing trees. A possible explanation of this phenomenon is that plants face fundamental problems in defending rapidly growing modules against herbivores (Price 1991). Additionally, environmental stress can often significantly increase the production of secondary compounds so that an otherwise palatable species becomes unpalatable to the herbivore (Lindroth et al. 2000). Integrating such observations, the Plant Vigor Hypothesis (PVH) predicts that herbivore performance will be better on rapidly growing tissues compared to stressed tissues (Kimberling et al. 1990; Price 1991; Prada et al. 1995; Preszler & Price 1995; Vieira et al. 1996; Cobb et al. 1997; Larson & Whitham 1997; Sumerford et al. 2000). The term vigor in this context means any plant in a plant population or any module that grows rapidly and ultimately reaches a large size relatively to the mean growth rate and size. Both the PSH and the PVH have received substantial empirical support and represent two opposing ends of the spectrum of responses to stress. In any particular system the effect of stress may depend on parameters such as plant life form, herbivore feeding guild, host-plant specialization, developmental stage of plant and/or herbivore and stress type (Larsson 1989; Price 1991; for an overview see Koricheva et al. 1998).

Both the PSH and the PVH regard altered levels of secondary metabolites in plants suffering from abiotic stress as a possible explanation for changes in herbivore performance patterns. Secondary constituents accumulate as the end products of relatively long biosynthetic pathways. These chemicals have a slow turnover within the plant compared to primary metabolites, and their synthesis may continue throughout the life of the plant. Secondary plant compounds often inhibit the growth and development of insects and can cause herbivore mortality (Todd et al. 1971; Isman & Duffey 1982, 1983; Manuwoto & Scriber 1986; Bryant et al. 1987; Lindroth et al. 2000; Kelly & Curry 1991; Matsuki & MacLean 1994; Hemming & Lindroth 1995; Ayres et al. 1997; Heath et al. 1997; Dearing et al. 2002; Halitschke et al. 2000; Baldwin 2001). Secondary plant substances also frequently affect insect behavior by acting as deterrents and feeding inhibitors (Kraft & Denno 1982; Matsuda & Senbo 1986; Kelly & Curry 1991; Mori et al. 1992; Gross & Hilker 1995; Van Dam et al. 1995, 2000; Bernays & Chapman 2000; Pass & Foley 2000; Baldwin 1999). In addition, secondary compounds may act indirectly by attracting predators of the herbivores (Rowell-Rahier & Pasteels 1992; Dicke 1994; Takabayashi & Dicke 1996; Turlings & Benrey 1998; Halitschke et al. 2000; Kahl et al. 2000; Kessler & Baldwin 2001).

The three main classes of secondary products are the terpenoids, nitrogen containing substances and phenolics. Phenolic compounds are aromatic structures bearing one or more hydroxyl groups. They are often used as model compounds in the context of plant defense and their levels are known to respond to changing abiotic conditions (Katoh et al. 1989). A remarkable feature of secondary plant metabolites is that increases in their concentration may also be induced dynamically in response to external chemical and mechanical signals or herbivore feeding (Karban & Baldwin 1997; Grace et al. 1998; Reymond et al. 2000; Renwick et al. 2001). However, few studies have examined how abiotic stresses influence induced resistance to herbivores and those that have, tended to focus on the effect of nutrient deprivation on secondary metabolite production (Baldwin & Ohnmeiss 1994a, 1994b; Bolter et al. 1998; van

Dam & Baldwin 2001). Induced herbivore resistance, particularly to chewing insects, is mediated by the jasmonate cascade, based on jasmonic acid (JA) (McConn et al. 1997; Baldwin 1998, 1999; Farmer et al. 1998; Thaler 1999). This compound arises from the octadecanoid pathway, starting-from the common plant fatty acid lineolenic acid (Mueller 1997). Even the exogenous addition of jasmonic acid or its methyl ester methyl jasmonate (MeJA) has been found to induce a variety of wound- and herbivory-induced chemical defenses (Farmer & Ryan 1990; Karban & Baldwin 1997).

In experiments studying plant-herbivore interactions, MeJA-induction may be a suitable alternative to natural herbivore feeding, eliciting similar responses in the plant, but avoiding side effects like the transmission of diseases (Fowler & Lawton 1985). Despite the widespread use of jasmonates to elicit plant defense reactions, little is known about the effect of MeJA-induced defenses on sucking insects; most of the induced-resistance studies have used chewers as model herbivores. In addition to elicitation of plant defenses, the jasmonate cascade is also known to mediate a variety of reactions in the context of the plants response on abiotic stress. Jasmonic acid is involved in the signaling of drought and pollution stress (Sembdner & Parthier 1993; Gross & Parthier 1994; Creelman & Mullet 1995, 1997) and plays a possible role in heavy metal tolerance (Rakwal et al. 1996; Xiang & Oliver 1998; Maksymiec & Krupa 2002). The jasmonate pathway could therefore provide a linkage between the plant’s responses to biotic and abiotic stress.

In this thesis, I aim to examine how abiotic stress affects a host-plant-herbivore system using fitness measures, herbivore reaction and plant secondary chemistry. To study this, I utilized *Artemisia vulgaris*, a common ruderal plant that colonizes a disturbed grassland ecosystem along a well-characterized gradient in soil pollution that resulted from the dust emissions of a former fertilizer factory in Steudnitz, Germany (Metzner et al. 1997). The fertilizer dust contained high amounts of phosphate and heavy metals and caused a strong degradation of the surrounding soil. Since 1979, the influence of pollution impact at Steudnitz was investigated on different levels of chemistry and biology. Studies of mosses and higher plants (Heinrich 1984; Marstaller 1987) as well as of arthropods (Köhler 1984; Sander 1984; Bährmann 1985, 1988, 1989; Müller 1985; Voigt 1985, 1987; Schäller et al. 1987;

Perner et al. 1996; Eggers 1997; Wegener 1998; Metzner 2000) have been conducted on this site. The influence of soil degradation on plant-herbivore interactions, however, has not been investigated yet.

The here presented study investigates plant-herbivore interactions on different functional scales along the pollution gradient:
To estimate soil quality and uncover the importance and the spatial distribution of particular pollutants, soil analyses were performed (Chapter 2).
Preliminary experiments were conducted (partly published in Köhler & Held 1998) to choose a suitable model system containing one plant (*Artemisia vulgaris*) and three herbivore species from different feeding guilds (sucking, chewing and gall forming) that are likely to vary in their response to host-plant stress (Chapter 3).
In field and laboratory trials, the fitness and herbivore resistance of *A. vulgaris* was tested (Chapter 4). Plant fitness was measured in the field based on germination, growth and reproduction of single genotypes of *A. vulgaris* transplanted to three differently polluted field sites. At all field sites, jasmonate-induction was used to elicit defense reactions and insecticide treatments were used to partly eliminate the effects of natural herbivory. Herbivore performance experiments were carried out in the field and preference experiments with plant material harvested at the differently polluted field sites were conducted in laboratory.

To test if secondary chemical variation provides a possible mechanism that could explain the observed reaction patterns in plants and herbivores, a chemical analysis was performed. High Performance Liquid Chromatography (HPLC) was used to explore low-molecular weight phenolics in leaf extracts of *A. vulgaris* plants harvested at the differently polluted field sites. A multitude of measured soil factors, experimental treatments and the multiple responses in the plants chemical profile were combined in a multivariate statistical analysis to reveal correlations among the different functional scales (Chapter 5).

This study is the first providing a phytocentric analysis of soil degradation affecting plant fitness, induced resistance to herbivores from different feeding guilds, and secondary chemistry along a pollution gradient in the field. The main questions asked are:

How does soil pollution act on fitness and herbivore-defense of a model plant?
Do herbivores of different feeding guilds react differently to host-plant stress?
Can the Plant Stress Hypothesis or the Plant Vigor Hypothesis explain the observed reaction patterns in the plant-herbivore system?
Can the expression of particular soil parameters be linked with secondary metabolite patterns?
Do soil characteristics and leaf chemistry correlate with patterns of plant resistance?
What role–does the jasmonate cascade play in plant response to abiotic and biotic stress?

This thesis is part of an interdisciplinary graduate research working group of the Friedrich-Schiller-University of Jena “Analysis of the function and regeneration of degraded ecosystems”.

History and Characterization of the study area

Climate and geography

The study area near Dorndorf Steudnitz is part of the Thuringian region “Mittleres Saaletal” and is located about 10 km north of Jena, Thuringia, Germany (51°01’N, 11°41’E, 140 m). The geology of the Saale valley is primarily determined by the triassic calcareous grounds (Schramm 1993). The soil of the lower slope where the study area was located is a shallow limestone rendzina that largely consists of loamy sand (Metzner 2000).

The climate of the Saale valley is dry-warm (temperate) with an annual mean temperature of 9.5 °C (reference period 1966-1995). Rainfall is rather sparse; the mean annual precipitation for the reference period is 603.8 mm (data from Jena weather station). The highest portion of the yearly rain falls during the vegetation period. This rain maximum in summer indicates a highly continental influence on the climate (see Table 2.1). The mean wind strength is 1.8 Beaufort with the main wind direction of 51% south-southwest (Langer 2000).

During 1999 and 2000, the first two years of the present study, the weather was warmer and drier than the mean of 1966 -1995. Especially in spring and early summer of 1999, the rainfall was unusually low. In 2000, weather conditions were similar with higher temperatures and lower rainfall especially in early summer. In contrast, the year 2001 was only slightly warmer and had a higher precipitation than the reference period (Table 2.1).

Table 2.1 Climatic data measured at the weather station Jena. Presented are the monthly and annual mean values for air temperature and precipitation for a reference period (1966 –1995) and during the presented study (1999 –2001).

	1966 -1995	**1999**	**2000**	**2001**
Air temperature (°C)				
January	1.0	4.0	2.0	1.6
February	1.5	1.6	5.3	3.5
March	5.0	6.3	6.6	5.2
April	8.5	9.6	11.7	8.6
May	13.6	15.1	15.9	15.3
June	16.6	16.3	18.4	15.5
July	18.6	19.9	16.8	19.7
August	17.8	17.5	19.1	19.8
September	14.1	17.3	15.0	13.0
October	9.8	9.7	11.8	13.0
November	5.0	4.5	6.8	5.0
December	2.2	3.5	3.8	0.7
Annual mean	9.5	10.4	11.1	1.6
Precipitation (mm)				
January	36.9	25.9	35.8	19.9
February	33.5	46.4	47.9	26.2
March	45.7	41.2	87.2	90.8
April	54.4	29.9	34.3	30.6
May	60.0	54.7	33.7	45.7
June	76.3	66.5	36.9	60.3
July	59.2	108.9	59.7	127.1
August	65.5	67.3	70.2	27.0
September	43.9	15.1	56.1	48.5
October	41.4	15.5	29.4	33.2
November	42.3	71.4	28.1	72.0
December	44.6	39.4	21.4	59.6
Annual amount	603.8	582.2	540.7	640.9

History of the study area

The Steudnitz field site is influenced by almost 50 years of anthropogenic pollution originated from a neighboring manufacturing plant (Figs. 2.1, 2.3, 2.4). Starting before World War II a factory located in Steudnitz produced cement until 1957 when it was converted in a P-fertilizer factory (Anke et al. 1991; Seifert et al. 1999). From

1970 on a mixture of raw phosphate, soda and sand was used to burn calcine phosphate. In the 1980s about 100,000 t of phosphate fertilizer were produced which was more than 30% of the total fertilizer production of the GDR (Petschow et al. 1990). Since the end of the 1960s, the surrounding was polluted with large amounts of dust. Especially the transport of the raw materials to the oven and the draw off of the end product both caused high emissions of more than 50 g/m^2 in 30 days (Vogler & Gebauer 1981). Single values exceeded 100 g /m^2 in 30 days. For the year 1979, Vogler & Gebauer (1981) calculated a total annual amount of dust emission of 3,146 tons. The primary wind direction south-southwest was the reason for the highest pollution existing at the lower slope north of the factory (Figs. 2.1, 2.4).

The dust and gaseous emissions caused significant changes of element concentrations in the soil. Immediately after the shutdown of the factory strong increases in Cd, Na, Ni, Pb and As contents and decreases in Mg, Fe, Mn and Zn contents were measured in the surrounding soil. A slight increase was shown for Mo, P and Ca content (Anke et al. 1991). The emissions also caused conspicuous changes in the patterns of presence and abundance of plant and animal species (Heinrich 1984). Damaged plants showed a changed morphology, necrosis and partly were killed. During highest emission levels in the 1970s and 1980s a large part of the area was unvegetated due to the thick dust layer and high concentrations of toxic compounds.

Since the end of the emission in 1990, the vegetation on the affected soil is in a process of regeneration. Starting with single salt tolerant species (e. g. *Puccinellia distans* Parl.) a fast succession took place and today a ruderal plant community consisting among other species primarily of *Atriplex nitens*, *Agropyron repens* and *Artemisia vulgaris* characterizes the lower slope near the former factory (Heinrich 2001).

Figure 2.1 View on Steudnitz field sites during fertilizer production in 1979 (photograph anonymus)

Figure 2.2 View on naturally occurring *Artemisia vulgaris* growing near the former fertilizer factory in Steudnitz during the present study in 2000 (photograph M. Held)

The study area is located northwest of the former fertilizer factory at the western lower slope of the Saale valley and therefore was exposed to high emission levels. The three field sites are located on the lower slope, at increasing distance from the factory and are called sites 1, 2 and 3, respectively (Table 2.2, Fig. 2.3, 2.4). From site 1 to site 3, with increasing distance from the factory, soil pollution levels decrease. The original soil composition before the onset of pollution probably was very similar (Langer 2000).

Table 2.2 Characterization of the 3 field sites along a gradient of increasing distance from the pollution source. Mean ±SE values of exposition and inclination (N = 10).

	Site 1	**Site 2**	**Site 3**
Distance from pollution source (m)	50	200	800
Inclination (°)	39.42 ±0.28	34.88 ±1.12	41.21 ±0.64
Exposition (°, 180 means south)	182.37 ±0.93	186.33 ±1.14	193.73 ±1.49

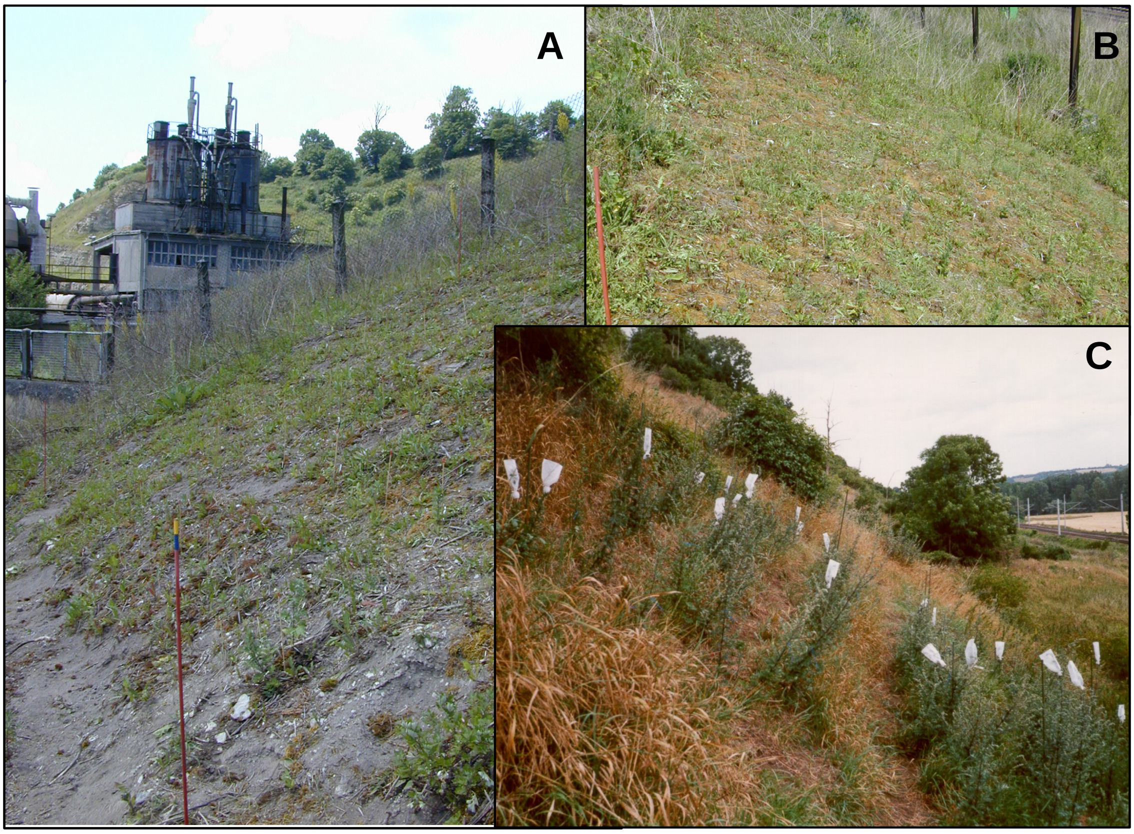

Figure 2.3 A) Heavily polluted site 1 near the former factory B) medium polluted site 2 C) relatively unpolluted site 3 with transplanted *A. vulgaris* plants covered with aphid enclosures (see Chapter 4) (photographs M. Held).

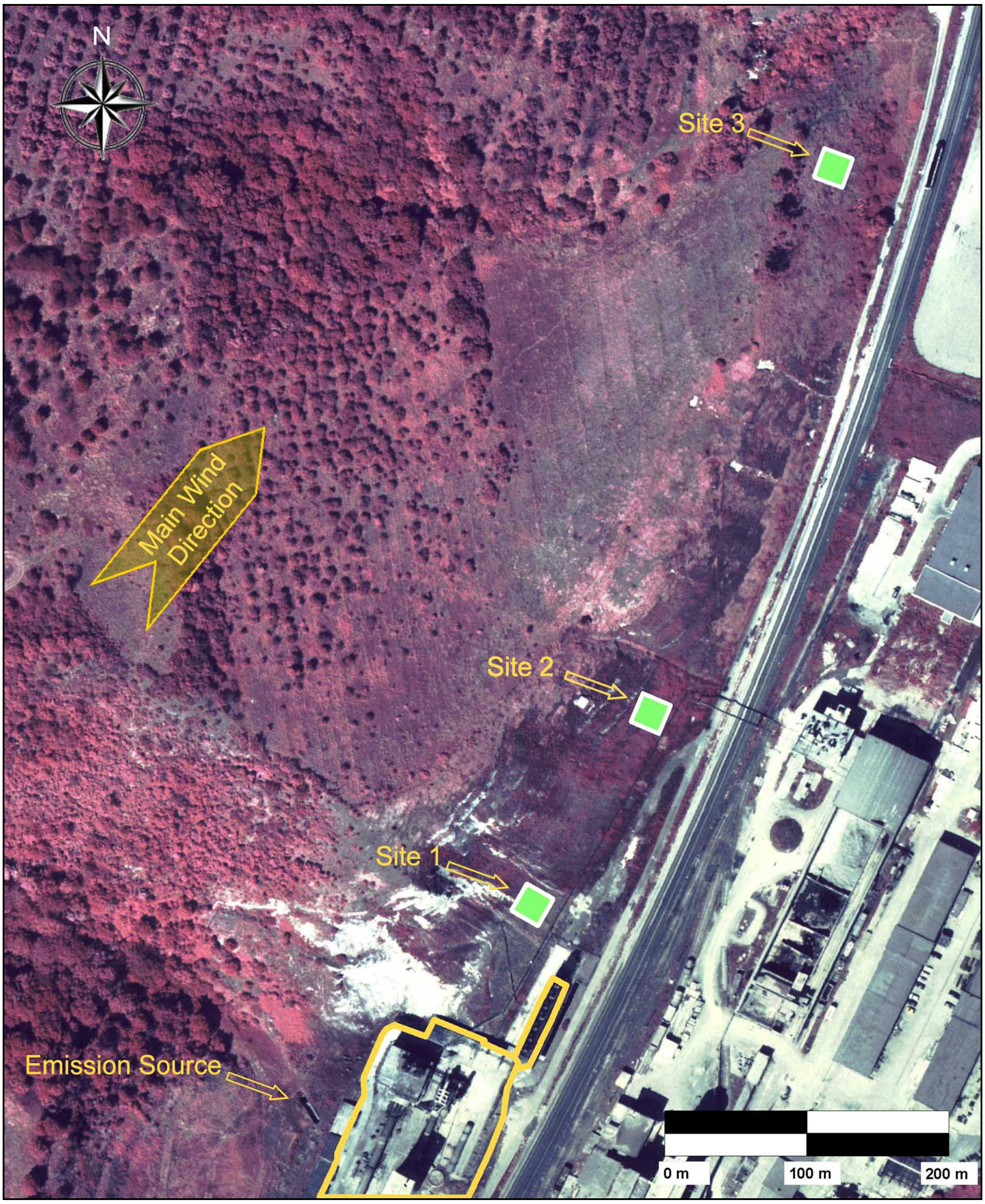

Figure 2.4 Aerial photograph of the study area in Steudnitz with the 3 study sites, the position of the former factory, and the main wind direction displayed (Thüringer Landesverwaltungsamtamt 1993).

Soil characterization:

Introduction

The soil pollution in the Steudnitz research area is well studied. Multiple soil analyses during the past 20 years allow a detailed description of temporal and spatial patterns of soil quality. Earlier measurements found a decreasing gradient of ph, heavy metals, sodium, calcium and fluor in the soil (Heinrich 1984; Metzner et al. 1997; Metzner 2000) with increasing distance from the pollution source. However, the soil situation changed rapidly during the last years. Furthermore, previous soil analysis as well as the large variation in natural vegetation suggests, apart from the pollution gradient, a small-scale patchiness of soil quality. Therefore, earlier results cannot be applied on the present study, and own measurements of soil parameters were carried out in order to evaluate spatial patterns of soil quality within the study plots.

Material and methods

Relative differences in soil moisture were measured with a Hydra Logger equipped with a Hydra soil moisture probe (VITEL inc., Chantilly, VA, USA) in autumn 2000 after 6 days without precipitation. In each of the five compartments at each plot 10 measurements to a depth of 10 cm were performed.

To record and quantify the soil contamination in the study area, soil samples were taken and analyzed. In 1999 on each of the three study sites, five compartments within the experimental plots were chosen for the collection of samples. In each compartment, three circles of 1 m diameter were selected and 10 soil cores (0 – 10 cm depth) were sampled per circle, the 10 cores of a circle being pooled (Fig. 2.5). This method allows an estimation of the spatial distribution of soil parameters between the study sites as well as the estimation of patchiness within each site.

The samples were air dried and analyzed according to DIN- and TGL-instructions (VDLUFA 1991) in the Institute of Geography of the FSU Jena.

Element contents and soil acidity (pH) were measured to assess the chemical quality. An aqua regia exposure of the soil was performed to obtain total element concentrations. Humus quality was determined by the soil contents of nitrogen (N %), plant available phosphorus (Pcal) and potassium (Kcal).

In addition, the cation exchange capacity (CEC) was determined, which is a measure of the soil's ability to adsorb and exchange cations (Tan 1993). CEC is the sum of exchangeable bound cations in the soil. These Na, K, Mg and Ca ions are the so-called exchangeable bases, because with increasing content of these ions the soil pH increases (Scheffer & Schachtschabel 1992). The salt used to remove the cations was unbuffered $BaCl^2$. The amount of $BaCl^2$ released to the solution was measured and represents the total CEC.

To estimate possible toxic effects of measured soil parameters on plants, the obtained values were compared with soil contamination thresholds given by Scheffer & Schachtschabel (1992), the Deutsches Umweltbundesamt (1986) and the Landwirtschaftliche Untersuchungs- und Forschungsanstalt Thüringen (LUFA, Kerschberger 1993).

Statistics

To test for significant correlations between different environmental parameters Pearson’s correlation coefficient was used with normally distributed data and Spearman’s Rank correlation coefficient when normality was not fulfilled (Bühl & Zoefel 1995). Normal distribution was tested with the Kolmogorov-Smirnov test, homogeneity of variances with the Levene test. Soil quality at the sample plots and sub plots were compared by one-way ANOVA followed by a Student-Newman-Keuls post hoc test or in case of non normal distribution by a Kruskal Wallis ANOVA on ranks followed by a Dunn post hoc test. SPSS 9.0 and Microcal Origin 6.0 software were used for the statistical analysis.

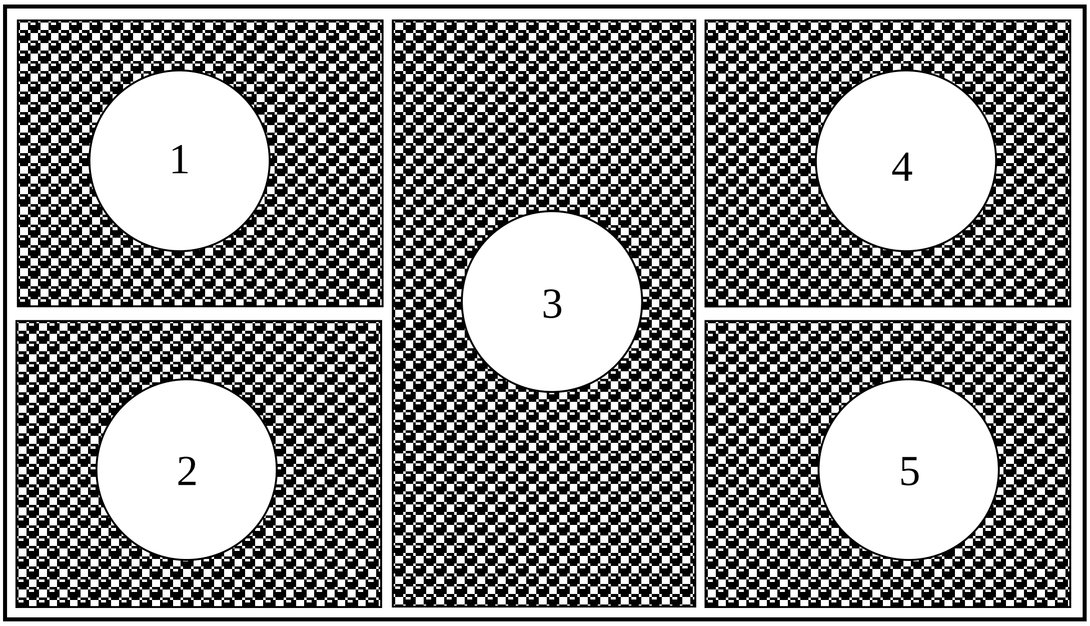

Figure 2.5 Schematic drawing of the soil sampling design at one of the study sites. The rectangle represents one of the study plots. Dotted rectangles refer to a subplot with a group of transplants (compare Chapter 4). The circles within each subplot represent the corresponding area of soil sampling with numbers indicating samples.

Results

Between site differences

The soil analyses revealed different distribution patterns among the analyzed soil compounds. A gradient of directional changes along all three sites was only verified for a few elements, whereas others showed a more diverse distribution or no difference in concentrations between the sites.

The latter was true for calcium carbonate concentrations, which decreased slightly with increasing distance from the pollution source, the differences between the sites, however, not being significant (Fig. A1 appendix, Kruskal-Wallis ANOVA on ranks, $H_2 = 0.267$ $P = 0.875$). Similarly, the copper concentration slightly decreased from site 1 to site 3 but there was no significant difference between the sites (Fig. 2.6, one-way ANOVA, $F_{2,43} = 0.84$, $P = 0.439$).

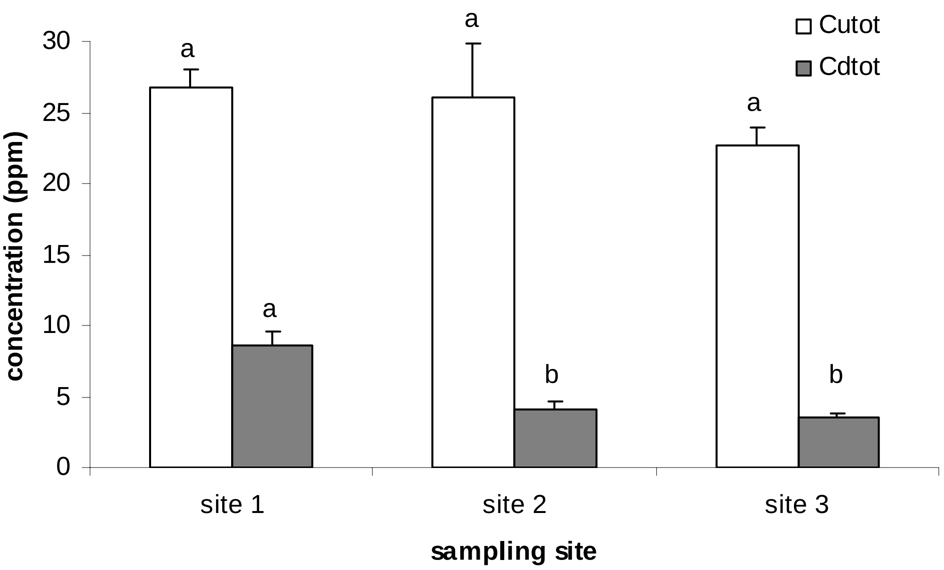

Figure 2.6 Mean (+SE) total concentrations of copper (Cutot) and cadmium (Cdtot) in the topsoil along three sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences for each group at P <0.05.

In contrast, the concentrations gradients of the other heavy metals zinc, lead (Fig. 2.7) cadmium (Fig. 2.6) and nickel (Fig. 2.8) were significant. The first three had significantly higher concentrations at site one, compared to sites 2 and 3 (Kruskal-Wallis ANOVAS on ranks, zinc: $H_2 = 9.717$, $P = 0.008$, lead: $H_2 = 29.086$ $P < 0.001$, cadmium: $H_2 = 16.688$, $P < 0.001$), whereas nickel significantly increased with decreasing soil pollution (Fig. 2.8, Kruskal–Wallis ANOVA on ranks, $H_2 = 7.044$, $P = 0.030$). Total magnesium concentrations did not differ significantly between the sites (Fig. 2.8, one-way ANOVA, $F_{2, 43} = 1.113$, $P = 0.338$).

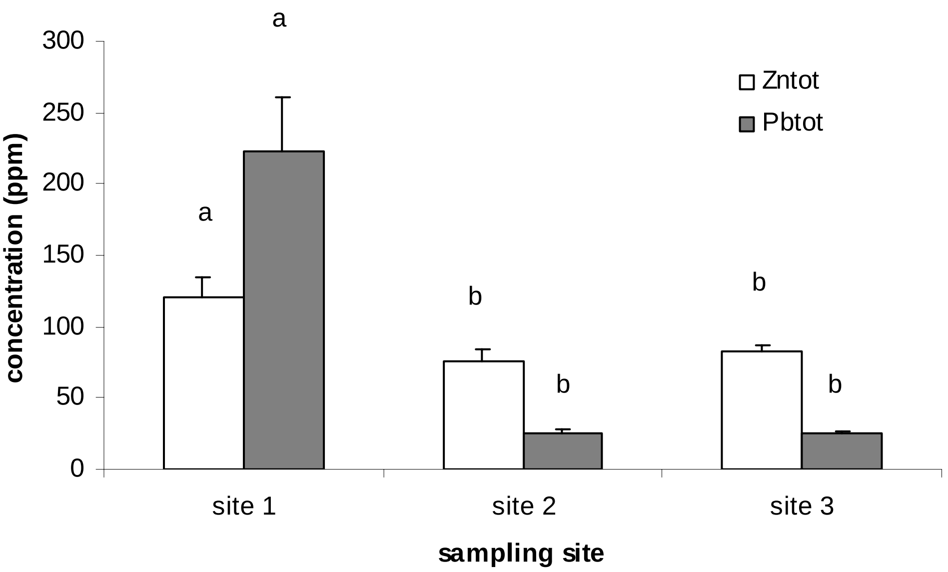

Figure 2.7 Mean (+SE) total concentrations of zinc (Zntot) and lead (Pbtot) in the topsoil along three sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences for each group at *P* <0.05.

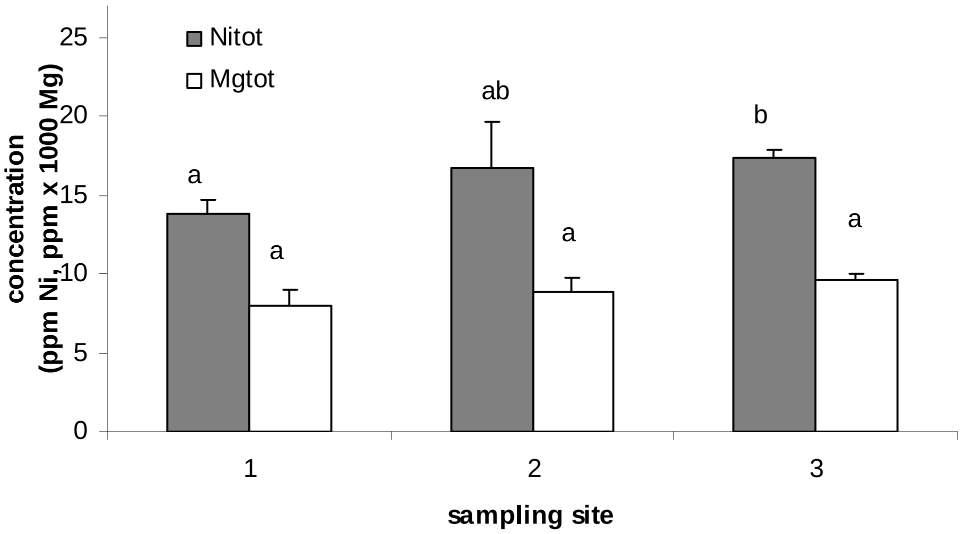

Figure 2.8 Mean (+SE) concentrations of the total content in nickel (Nitot) and magnesium (Mgtot) in the topsoil along three sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences for each group at *P* <0.05.

In contrast to the distribution of most heavy metals, a further group of soil parameters did not display directional changes along the pollution gradient, although partly showed significant differences between the three sample sites. The total content of potassium in the soil was significantly lower at site 1, compared to the two other sites (Fig. 2.9, Kruskal–Wallis ANOVA on ranks, H_2 = 31.406, *P* <0.001). Soil pH was highest at site 1 and 3 and lowest at site 2. The difference between site 2 and 3 was significant (Fig. 2.9, Kruskal–Wallis ANOVA on ranks, H_2 = 12.079, *P* = 0.002). Total soil contents of calcium and sodium did not differ between the sites (Fig. 2.9 one-way ANOVAS, all *P*s <0.251).

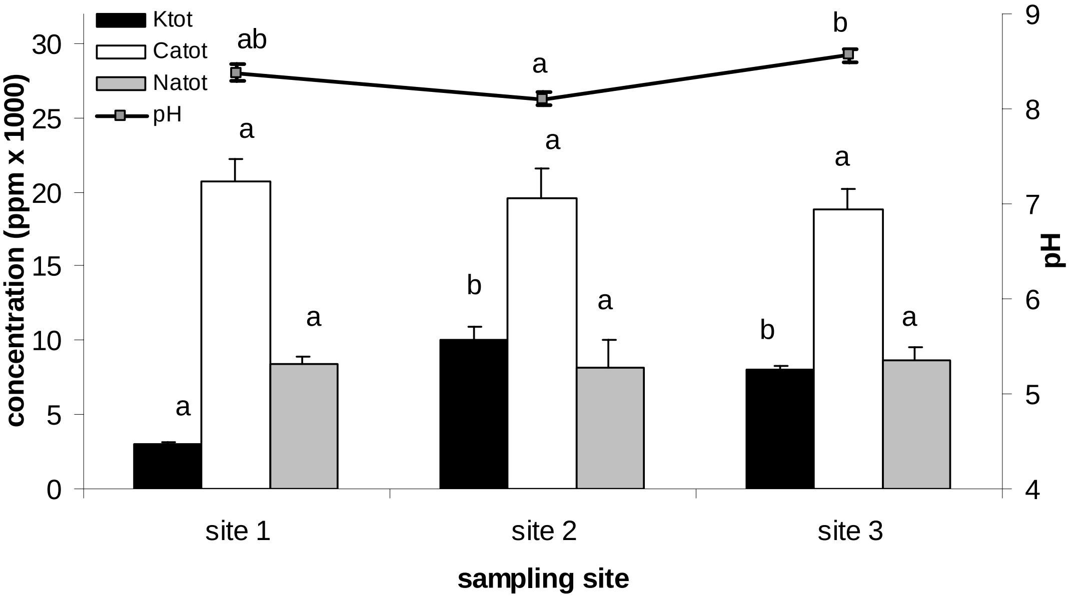

Figure 2.9 Mean (+SE) concentrations of the total potassium (Ktot), calcium (Catot) and sodium (Natot) content as well as pH in the topsoil along three sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences for each group at *P* <0.05.

The group of elements representing the humus quality differed in their distribution patterns. Total nitrogen concentrations showed a clear gradient, with concentrations being significantly lower near the former pollution source than at the most distant site 3 (Fig. 2.10, Kruskal-Wallis ANOVAS on ranks, H_2 = 24.364, *P* <0,001). In contrast, plant available potassium tended to gradually decrease with increasing distance from the former factory (Fig. 2.10, One way ANOVA, F2,43 = 2.702, *P* = 0.079).

The concentration of phosphate was, as expected, highest at site 1, however a significant difference existed only with site 2, but not with site 3, which had intermediate phosphate levels (Fig. 2.10, One way ANOVA, $F_{2,38}$ = 5,087, *P* = 0.011).

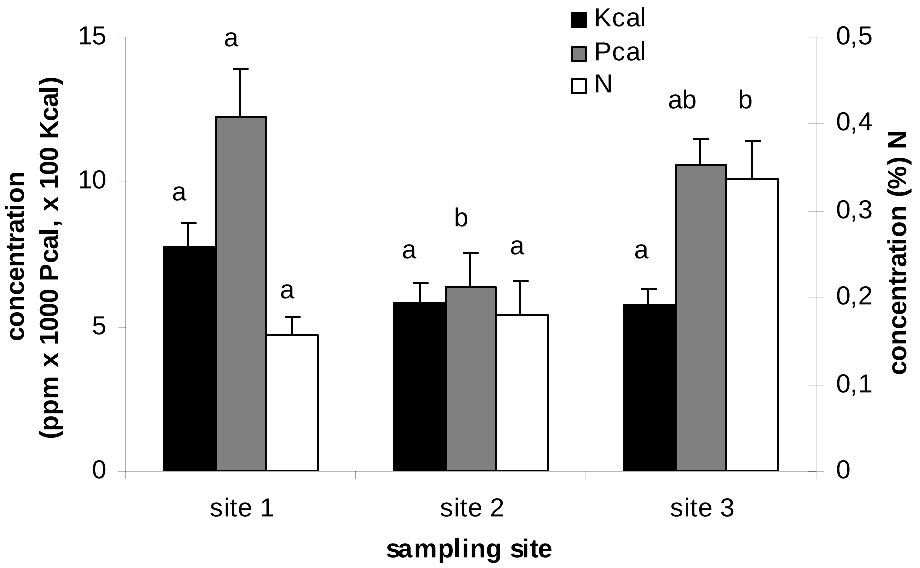

Figure 2.10 Mean (+SE) concentrations of plant available potassium (Kcal) plant available phosphate (Pcal) and nitrogen (N) in the topsoil along three sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences for each group at *P* <0.05.

The barium extracted total cation exchange capacity (CEC) decreased from site 1 to site 3. CEC ranged from a mean of six cmol/z/kg at site 1 to more than 21 cmol/z/kg at site 3 (see Fig. A2 appendix) and these differences were significant (Kruskal–Wallis ANOVA on ranks, H_2 = 28.311, P = <0.001).

Although this general directional distribution of CEC, single exchanged base cations showed a more diverse pattern (Fig. 2.11). The difference of concentrations between the sites was significant for magnesium (Kruskal-Wallis ANOVAS on ranks, H_2 = 7.905, *P* = 0,019) and potassium ions (Kruskal-Wallis ANOVAS on ranks, H_2 = 29.402, *P* <0.001), which both increased from site 1 to site 3 (Fig. 2.11).
Similarly, sodium concentration was significantly higher (Kruskal–Wallis ANOVA on ranks, H_2 = 29.427, *P* <0.001) at the unpolluted site 3 than at the medium polluted site 2 and the heavily polluted site 1 (Fig. 2.11). In contrast, calcium concentration did not differ significantly between the sites (One-way ANOVA, $F_{2,43}$ = 0.352, *P* = 0.705).

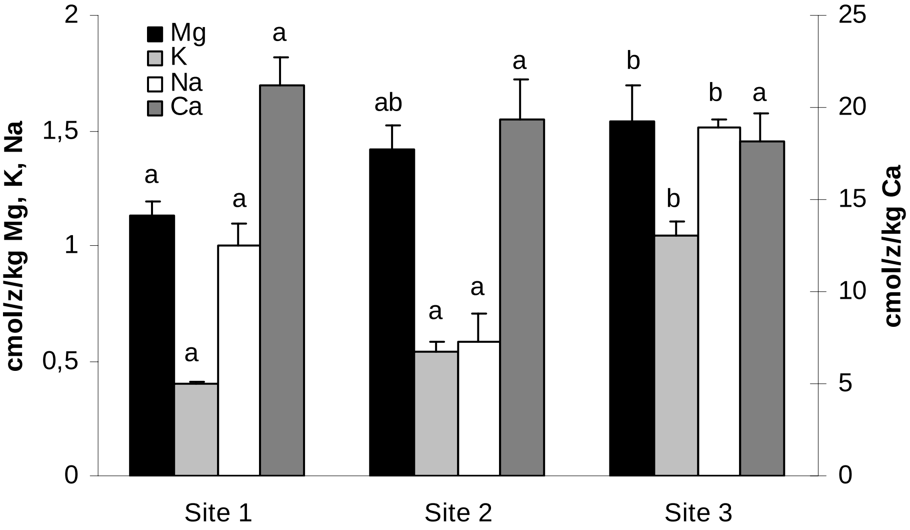

Figure 2.11 Mean (+SE) exchange capacity of alkaline cations (Mg, Na, K, Ca) in the topsoil along three sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences for each group at $P < 0.05$.

Total and plant available element content

Calcium and potassium concentrations were measured twice, one time as plant available concentration and one time as total soil content. Interestingly, for potassium, the relation between the two measurements was not constant along the pollution sites but a higher portion was available at the relatively unpolluted site 3 than at both other sites (Figs. 2.9, 2.10, 2.11). In contrast, calcium did not show any differences of ratios between plant available and total concentration along the gradient (Figs. 2.9, 2.11, A1 appendix).

Correlations between single soil parameters

The test for correlations between different soil parameters showed a complex pattern of significant interactions (Table A1, appendix): The content of $CaCO_3$ correlated positively with Ca, and Mg and negatively with Cu. Nitrogen correlated positively with CEC as well as plant available potassium. Plant available potassium correlated positively only with CEC and negatively with Pb. CEC correlated positively with N and Ni, K, and Ni, and negatively with Pb and Cd. Plant available phosphate showed a positive correlation with Cu and Na and a negative one with Ni, K and Mg. Total concentrations in Zn correlated positively with Pb, Cu, and Cd and negatively with Mg. Pb concentrations correlated positively with K, Zn and Cd and negatively with

CEC. Cu concentrations correlated negatively with $CaCO_3$ and Mg and positively with phosphate and zinc. A positive correlation existed also between Ni and CEC, whereas the Ni concentration correlated negatively with P, Cd and Na.

Within site variance

Apart from the differences occurring between the three study sites, variance also was high within sites. For almost all analyzed parameters, significant differences between the five samples within one study site could be shown (Table A2 appendix).

This high variance within each plot is evidence for a small-scale patchiness of soil quality that overlays the large-scale gradient. Especially at the medium polluted site 2, sub-samples of some elements show a five-fold ($CaCO_3$, N, P) 10-fold (Catot) or even 20-fold (Na) difference to other sub samples (Table A2 appendix).

Comparison with previous measurements

Besides the presented spatial gradients, temporal changes in soil quality can be demonstrated by comparison with earlier soil measurements. Since the end of the fertilizer production in 1990, pH and element contents continuously decreased (Table 2.3). An exception is the calcium concentration, which seemed to increase between 1990 and 1997. Some elements (e.g. sodium) show a strong temporal decrease whereas others (e.g. Cd) decrease only slightly.

Table 2.3 Minimum and maximum values of different soil parameters (pH and element contents (ppm)) measured at the lower slope in Steudnitz (1979 - 1997) and at study site 1 and 2 (2000). Threshold values for Cd, Pb, Cu, Zn, according to Deutsche Klärschlammverordnung (Umweltbundesamt 1986) and EU directive 86/278/EEC (2001), critical values for pH, Na, Mg, K, P, Ca according to Scheffer & Schachtschabel (1992). Reference numbers (Ref.) mean the following authors: 1. Heinrich (1984), 2. Schaeller (1987), 3. Metzner et al. (1997), 4. Soldt (1997), 5. Held & Baldwin (2002). Parts of the table are according to Metzner et al. (1997).

Year	1979	1981	1987	1990	1991	1993	1996	1997	2000	Critical value
Ref.	1	1	2	3	3	4	3	3	5	
PH	7.0 - 8.3	8.2 - 9.3	7.0 - 8.4	7.6 - 10.1	7.4 - 8.3	6.3 - 9.4	7.15 - 8.17	7.1 - 8.6	7.8 - 9	6.0 - 7.0
Na	330 - 3830	4120 - 6550		5900 - 24500	3900 - 13900	100 - 8250	900 - 17800	930 - 14900	2620 - 3760	1000 - 10000
Mg	47 - 155	40 - 90		280 - 6200	2700 - 7900	1460 - 50100	3000 - 10110	2800 - 7200	4460 - 16580	500 - 5000
K	150 - 960	560 - 760		700 - 18000	1700 - 14200	2490 - 12700	2600 - 6400	3200 - 7200	1970 - 19080	2000 - 33000
P	350 - 4000	3750 - 4750		600 - 79400	2500 - 120000			980 - 119000	4073 - 21027	200 - 800
Ca		39200 - 48000		16000 - 234000	69000 - 288000	20 - 279000	101200 - 300000	6200 - 318000	22000 - 254100	1000 - 12000
Cd			0.15 - 15	0.02 - 0.86	2 - 30	0.08 - 54.6	1 - 11.5	0.16 - 11	1.49 - 14.13	1.5
Pb			13 - 50			23.2 - 169	19.8 - 41.6	14 - 33	11.94 - 557.71	100
Cu			8.7 - 31			9.16 - 44.5	8.6 - 21.9	5.8 - 39	8.23 - 63.25	60
Zn			36 - 82			24.6 - 245	37.4 - 70	48 - 83	26.83 - 287.37	200

Soil moisture

Tthe measurements of soil moisture did not indicate significant differences between the sites (Figure A3 appendix, One-way ANOVA, $F_{2,147} = 1.672$, $P = 0.191$).

Discussion

Steudnitz soil is heavily polluted

Certain types of human-generated stress can have a major effect on terrestrial ecosystems, on soil chemistry, microbial activity and plants. For instance, salts are capable of inducing drought stress in plants and high heavy metal concentrations can have toxic effects on the plant (Alloway 1990).

Even 10 years after the closure of a fertilizer factory in Steudnitz, the soil is still strongly contaminated. Dust emissions of the former factory altered soil texture resulting in high sand content accompanied by atypical element contents and pH. The present analyses revealed a general contamination pattern along the pollution gradient, that is similar to the one described by Metzner et al. (1997), characterized by relatively low concentrations of nitrogen and nickel but extremely high levels of calcium and heavy metals near the factory.

Heavy metal concentrations show a strong gradient

The heavy metal cadmium is described as a typical pollutant occurring near fertilizer and cement industry (Scheffer & Schachtschabel 1992; Wild 1995). Together with other heavy metals (copper, zinc and lead), to which it showed a significant correlation, Cd gradually increases in direction to the factory and reached significantly higher concentrations at study site 1. However, the actual toxicity of heavy metals in Steudnitz is difficult to estimate, as these are able to form complexes with calcium (Alloway 1990). The high calcium concentrations measured at all field sites in Steudnitz could therefore prevent heavy metal availability for and toxicity to plants.

Some soil parameters show a non-linear distribution

Apart from the heavy metals showing a linear distribution along the gradient, some other soil parameters did not respond gradually but showed a more complex distribution, reaching extreme values at a particular site. Soil pH for instance was very high at all sites even for soils on limestone. However, pH values were significantly higher at site 1 and 3 compared to site 2. A very similar pattern was evident for the concentration of plant available phosphate. Phosphate content results from the apatite dust emissions of raw substrate during fertilizer production, which is also responsible for the high sand content at sites 1 and 2 (Metzner 2000). The phosphate pattern is remarkable, because this element was expected to be a main pollutant, showing a clear gradient. However, this non-linear distribution can be explained by the slightly higher calcium concentrations near the former factory and the fact that phosphate availability can be negatively influenced by Ca-concentration in the soil by formation of calcium phosphate (Scheffer & Schachtschabel 1992).

Humus quality and nutrient content

In addition to phosphate, nitrogen is the element most commonly determining plant performance (Chapin 1987, 1991; Wedin & Tilman 1990). Virtually all nitrogen in the soil is present as organic N in soil organic matter. However, the sandy soils at site 1 have low humus contents due to sparse vegetation and litter production near the factory. Such soils display low cation exchange capacity and contain smaller quantities of nutrients than do heavier soils (Lamersdorf & Meyer 1993). Although the measured values of plant available potassium did not differ between the sites, nitrogen concentration and total CEC mirror this circumstance: both show a significant positive correlation and significantly increased with increasing distance form the factory. Exchange capability of the cations Mg, K and Na is responsible for this increase of total CEC, whereas Ca ion exchange did not differ between the sites.

The presumption of a possible nitrogen deficiency in Steudnitz is also supported by the findings of Wagner (unpublished results), who detected a relatively low production of dry biomass (100 - 150 g/m^2) near the former factory, that could be doubled by the addition of nitrogen fertilizer or in a similar extend by addition of a complete N-P-K fertilizer. However, for all described measurements it must be taken into account that season and soil depth at the moment of sampling influence the contents in plant available phosphate, potassium and total nitrogen (Cain et al. 1999). Therefore, seasonal differences in the concentrations of these values are possible (Ward et al. 1979; Scheffer & Schachtschabel 1992).

Elements show different availability between the sites

Comparisons between total and plant-available contents of potassium and sodium showed higher portions of the elements to be available at the relatively unpolluted site 3, what also can be explained by the higher CEC at this site.

Sand content, obviously having its origin in the dust emission of the former factory, continuously decreases with increasing distance (Metzner et al. 1997). The sandy structure of the soil at site 1 also has an effect on element distribution and soil quality. Loamy sand like that at site 1 is characterized by a poor water holding capacity. Together with the sparse vegetation near the former factory this is responsible for leaching possibly resulting in a loss of ions. This leaching and a translocation of elements along the slope and into deeper soil layers were already described by Metzner (2001) and could be responsible for the lower nitrogen and potassium contents near the factory. However, own measurements of soil water content did not reveal clear differences between the sites. To explain that it must be taken into account, that the measurement of soil water content at one particular date is probably not sufficient to estimate differences in soil water capacity between the sites and that a measurement during a drought period would possibly have revealed differences.

Smale-scale patchiness of soil quality

In addition to the described large-scale spatial gradients, also non-directional small-scale differences within the study plot were found. Almost all analyzed soil parameters showed a high variance within each plot, proving the assumed small-scale patchiness of soil quality. The incorporation of the concentrations of pollutants measured during the past 20 years supports this fact, as all studies found a large range of values among the analyzed compounds.

This condition confirms the usefulness of the performed own soil analysis and was relevant for the experimental design of further field test in Steudnitz, concerning the influence of soil quality on plant-herbivore interactions (Chapter 4). The experiments performed on this scale had to be adapted to the soil patchiness. However, the complex distribution of soil elements along the gradient was also advantageous, because it offered the possibility to differentiate between the effects of particular soil parameters (Chapter 5).

Element level partly reach plant-toxic values

Comparisons of own analysis results with previous measurements (Table 2.3) revealed, regardless of the high variance in all datasets, a general decrease of extreme values for most pollutants since 1990, some (e. g. calcium) however seemed to increase. This observation of some elements reaching higher concentrations in 2000 than in most analyses before can be explained by the position of study site 1, which is closer to the factory than the sampling sites of earlier studies and by the observed smale scale spatial patterns of soil quality. Additionally, different methods of measurement were used in the different studies. Therefore, one must be careful, when comparing maximum values between studies with threshold values. Nevertheless the general pattern of a temporal increase of soil quality in Steudnitz is obvious. Despite this fact, the occurrence of extreme values measured at site 1 and 2 (Table 2.3) shows that even 10 years after the closing of the fertilizer factory, the soil in Steudnitz is highly contaminated, with some parameters still exceeding official critical values. Even at site 3, the concentration of some elements reaches values that are higher than these thresholds. The thresholds are not levels of toxicity for plants but arbitrarily set limits for agricultural soils. However, one group of pollutants, the heavy metals, can damage root systems which results in a reduction of water and nutrient uptake and adversely influences the performance of the whole plant (Kahle 1993; Daniel et al. 1997). The measured concentrations of heavy metals in Steudnitz are obviously beyond the range of toxicity for plants. In an exemplary study, McGrath et al. (1995) found a 50% reduction of CO^2 fixation in Cyanobacteria for concentrations of 114 ppm for Zn, 33 ppm for Cu, 2.9 ppm for Cd and 40 ppm for Pb. These toxic limits are exceeded 2-fold (Zn, Cu), 5-fold (Cd) or even 13-fold (Pb) by the maximum values measured at site 1. However, an ecological consequence of the measured high within-site variance could be that even at the heavily polluted site 1 there are small patches available for the plants where the concentration of polluting elements allow germination and growth similar to that on unpolluted soil.

Apart from direct toxic effect of soil pollution, there are supplementary mechanisms that could lead to plant stress in Steudnitz. For instance, the mycorrhizae associated with plants are also susceptible to soil pollution (Cairney & Meharg 1999; Egerton-Warburton & Allen 2000). The loss or poor vigor of the fungal microbiont could further reduce P and N availability to the plant (Hendrickson et al. 1991). However, Blancke (unpublished results) found a five-fold increase for levels of arbuscular mycorrhiza at the heavily polluted site 1 compared to the relatively unpolluted site 3. This finding indicates an advantage of additional mycorrhiza-mediated nutrient supply for plants growing near the former factory and hence supports the possible importance of nitrogen deficiencies.

The soil analyses found many possible pollutants and complex interactions between these. Overall, the results suggest high concentrations of phosphate, heavy metals and a possible nitrogen deficiency as well as a degraded water availability to be main pollutant factors in Steudnitz. Deducing from the general gradient revealed by the soil measurements, site 1 is termed “heavily polluted”, site 2 “medium polluted” and site 3 “relatively unpolluted”.

Study Organisms and Preliminary Experiments

Artemisia vulgaris

Artemisia species occur in dry habitats and mountainous regions, mainly in Europe and North America; very few species are known from South America and Southern Africa (Valant-Vetschera & Wollenweber 1995).

Common mugwort (*Artemisia vulgaris* L.) is distributed in 82% of the European territories and most of the temperate regions of the northern hemisphere (Grime et al. 1988). It is a secondary wind pollinated plant with a height of 0.60-2.50 m. It invades ruderal sites, where it can spread clonally, although it primarily reproduces sexually, by the production of seeds (Inderjit & Foy 1999). At such sites, it can predominate for many years (Grime et al. 1988). New plants germinate and ramets emerge from overwintering rhizomes in April. The shoots grow vegetatively until August, when the inflorescence begins to form. The flowers bloom in September (Fig. 3.1) and the wind dispersed seeds mature through September and October. A large individual can have a high reproductive output with up to 500,000 flowers and about 10,000,000 blossoms, which produce 3-5 million fruits (Garnockjones 1986). *Artemisia vulgaris* contains a multitude of secondary metabolites and has toxic effects to livestock (Inderjit & Foy 1999).

Figure 3.1 Upper part of a flowering *Artemisia vulgaris* plant from Steudnitz study site 2 (photograph M. Held)

A. vulgaris appeared at the polluted field sites in 1992 making it one of the earliest successional species. It rapidly reached high densities, and until 1994 it was a dominant species on the lower slope. Since then its abundance has decreased, but it still occurs along the entire pollution gradient (Heinrich et al. 2001). Today in 2002, *A. vulgaris* is still common in the study area but especially at the less polluted site it becomes increasingly replaced by grasses and other perennial species.

Model herbivores

As model herbivores three species from different feeding guilds (chewing, sucking and gall forming) were chosen. In view of the plant model and the questions under consideration (see Chapter 1), three herbivore species were deemed particularly suitable for study, the monophagous aphid *Macrosiphoniella artemisiae* Boyer de

Fonsc. (Hemiptera: Aphididae), the gall forming monophagous aphid *Cryptosiphum artemisiae* Buckton (Hemiptera: Aphididae) and the grasshopper *Chorthippus mollis* Charpentier (Orthoptera: Acrididae).

Macrosiphoniella artemisiae

Aphids are able to affect plant fitness and are frequently used for studying plant-herbivore interactions. The choice of *Macrosiphoniella artemisiae* as the model sucking herbivore species for the plant resistance experiments was obvious as only one herbivore species of this type is common on *A. vulgaris* in the study area. All members of the genus *Macrosiphoniella* feed on Asteraceae and the majority is highly specialized, living and feeding on only one genus or even a single plant species. *Macrosiphoniella artemisiae* is a relatively big-sized phloem sucking herbivore. It lives on the upper stems and leaves of *A. vulgaris*, especially between the inflorescences, frequently in large colonies (Schmitz 1999).

Figure 3.2 Two individuals of *Macrosiphoniella artemisiae* on an inflorescence of *Artemisia vulgaris* in Steudnitz (photograph M. Held)

The life cycle of *M. artemisiae* is simpler than in other aphids as host alternation does not take place. All generations live and feed on *A. vulgaris* as host plant (Börner

1952), reproduce parthenogenetically until summer, when sexuals are produced, too. Like in all *Macrosiphoniella* species, the eggs overwinter on lower parts of the host plant or on withered leaves (Heie 1995). *Macrosiphoniella artemisiae* is a very mobile species. When disturbed it lets itself fall to the ground and reclimbs the host plant or surrounding plants sometime later.

M. artemisiae occurred for several years in high densities in the study area (personal observation). The species lives monophagous on *A. vulgaris* but has occasionally been found on *Artemisia absinthium*, which, however, is uncommon in the study area. Due to the characteristics described (monophagous feeding type, large size, occurrence in Steudnitz, no host change) *M. artemisiae* can easily be maintained in enclosures on plants in the field and is highly suitable for the planned experiments

Cryptosiphum artemisiae

Like all hemiptera, *Cryptosiphum artemisia* is also a sucking insect but with a very different habit from *M. artemisiae.* Only nine species of the genus *Cryptosiphum* are known in the world and *C. artemisia* is the only species found in Europe, however, very little is known about its phenology. The aphids are monophagous, living inside reddish solid galls on *A. vulgaris* leaves and act as phloem parasite. In some years *C. artemisiae* host infestion rates can be high with up to 20 % of plants showing galls (personal observation).

Figure 3.3 *Artemisia vulgaris* plant in Steudnitz infested with galls of *Cryptosiphum artemisiae* (arrows) (photograph M. Held).

Chorthippus mollis

Grasshoppers are typical chewing herbivores that are often used for lab and field experiments on food choice. However, most such experiments are performed with grasses or using herb-feeding grasshopper species. There is no herb-feeding grasshopper that feeds on *A. vulgaris* known in the region, but the grass-feeding species *Chorthippus mollis* (Charpentier, 1825) is a Acridid species known to be less particular in food choice than other species, feeding partly on herbs, and is common around Jena (Schäller 1980). As a xerothermophilic species, *Chorthippus mollis* occurs in semi-arid and arid grassland (Thorens 1988, 1989, 1993). The species is active from the hatching of the nymphs, at the end of May, until the middle of November, when the last adults die. The grasshopper is univoltine with a single hibernation period for the eggs. The eggs are laid in clutches with a mean of 9.7 eggs/female (Thorens 1989). Male grasshoppers have 4 - 6 and females have 5 - 6 larval instars (Thorens 1993).

The species of the genus *Chorthippus* are generally grass-feeders with mandibles of the graminivorous type (Kaufmann 1965; Aguirre et al. 1987; Gangwere & Spiller 1995). *C. mollis* does not accept all grass species equally but prefers some like *Festuca* and *Dactylis* (Kaufmann 1965; Köhler & Schäller 1981). The species can even be reared on a synthetic dry diet with *Dactylis* powder added (Schäller 1980). The only demonstrated case of *C. mollis* feeding on herbs instead of grass in the field was an observation in southern Germany where in 35 out of 50 cases *C. mollis* chose *Medicago*, *Melilotus* and *Genista* species as a food source (Gottschalk 1993; Brandt & Gottschalk 1998).

Figure 3.4 *Chorthippus mollis* (photograph F. Julich)

Preliminary experiments

Introduction

The model system consists of one host plant species and three herbivore species. In the following preliminary experiments described here the suitability of the host plant and two of the three herbivores for the main experiments planned (Chapter 4) was tested.

Artemisia vulgaris seed viability

Genetic adaptation to soil quality and other environmental factors can lead to substantial intraspecific variation in plants and genotypes with different nutrient requirements. *Artemisia vulgaris* is known to express a high genotypic and phenotypic variation in different environments (Grime et al. 1988). In the preliminary examination of seed viability the evidence for local adaptation along the pollution gradient or differences in germination capabilities due to maternal effects were tested. Angiosperm seeds remain attached to the maternal parent throughout most of the development and can therefore be influenced by effects like the supply of resources to the parent during ripening. In Asteraceae seeds (achaena) that do not contain endosperm, the embryo itself is the storage site and its provisioning with resources influences its size and viability, which in turn could affect germination. Both the impact of abiotic stress as well as the induction of plant defenses with the plant hormone methyl jasmonate (see chapter 4) could through such maternal effects influence the fitness of the next generation. Maternal effects might be an important factor in natural plant populations and have potential implications for ecological studies (Schmitt & Antonovics 1986; Schmitt et al. 1992). A way to test maternal effects regardless of their origin (genetic or phenotypic) is the performance of reciprocal germination tests (Thompson et al. 1991), which was done in the present study.

Suitability of A. vulgaris as the sole diet for C. mollis

Previous observations suggested that the grasshopper *C. mollis* is capable of feeding on herbaceous plants, however fitness relevant parameters such as mortality, life span, body weight and the number of produced eggs were influenced by the plant species that were chosen as food source (Kaufmann 1965; Schäller & Köhler 1981).

This was the justification for preliminary trials to check if *C. mollis* can be exclusively reared on *A. vulgaris* and was therefore suitable as a model herbivore of the chewing type for further experiments relating to plant stress (Köhler & Held 2000).

Material and Methods

Seed viability

Artemisia vulgaris seeds from one randomly chosen induced (details of induction see Chapter 5) and one control plant were collected from each the polluted site 1 and the relatively unpolluted site 3. Seed size was measured for 50 seeds from each of the four seed sources.

Viability and germination of seeds were determined in laboratory assays. The percentage of germinating seeds was determined using topsoil collected at the three field sites and sterile sea sand (Merck, Darmstadt, Germany). Ten replicates with 20 seeds in each cup were examined daily for 10 days.

Chorthippus mollis rearing

In July 1999, 32 imagines of *C. mollis* (16 male, 16 female) were collected on a slope in the Leutratal near Jena, Germany and reared in cages in the laboratory on *A. vulgaris* branches. These were cut every three days in a ruderal area near the unpolluted site 3 in Steudnitz. Two weeks later (end of July 1999) sand boxes were provided for oviposition. These boxes were checked for egg packets in November. The egg clutches were counted and stored in sand in the fridge until April 2000, when the boxes were put at room temperature and the hatching larvae were counted. These hatchlings were divided into two groups, one provided with *A. vulgaris* and the other with a mixture of grass species (*Dactylis glomerata, Festucca pratensis* and *Bromus erectus*.) as a food source. In July 2000, after no further hatches could be expected, all egg clutches were dug out, checked for remaining eggs and measured in length.

Results

Artemisia vulgaris seed viability

Seeds from the polluted site 1 were slightly smaller than seeds harvested at site 3 but this difference was not significant. (Fig. 3.5, one-way ANOVA, F = 2.097, *P* = 0.102).

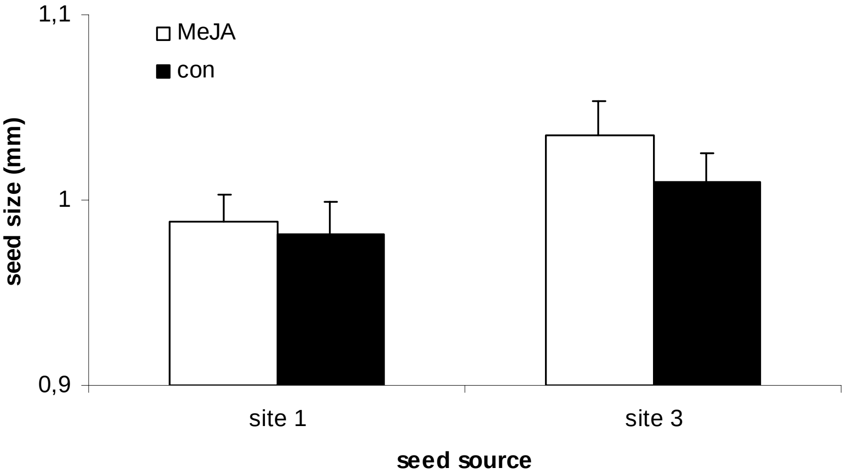

Figure 3.5 Mean (+SE) size of *Artemisia vulgaris* seeds (n = 50) harvested from induced (MeJA) and control (con) *Artemisia* plants at two differently polluted field sites (polluted site 1, unpolluted site 3).

Similarly, the laboratory germination trial revealed no significant effect of seed sources (parental soil and parental induction), but only differences between germination rates on the different soil types (Fig. 3.6, three-way ANOVA, soil type: F = 126.108, *P* <0.001, parental soil: F = 0.386, *P* = 0.536, parental induction: F = 0.638, *P* = 0.426). Neither the fact that parental plants grew on polluted soil nor their induction with methyl jasmonate had any effect on germination rates of seeds produced by these plants.

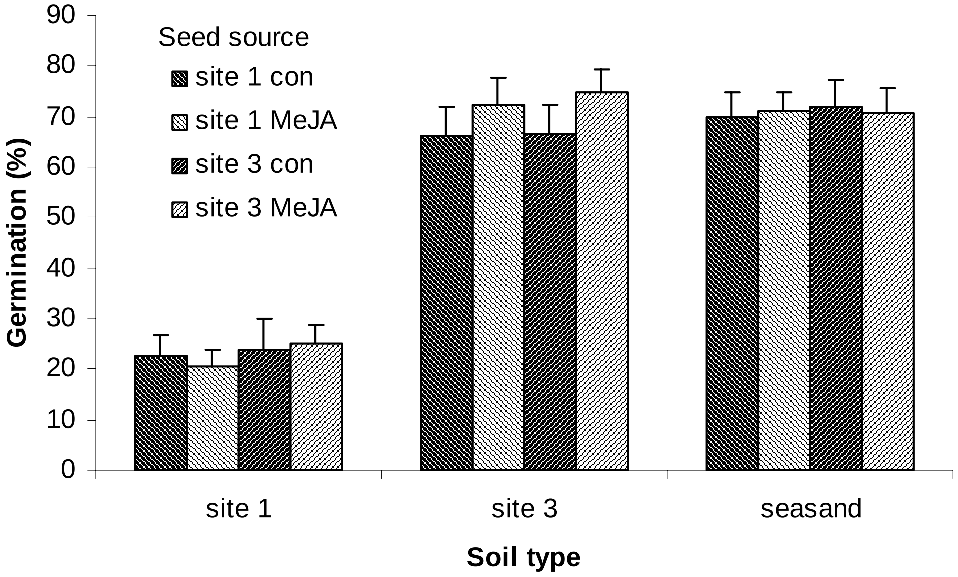

Figure 3.6 Mean (+SE) germination rates of *Artemisia vulgaris* seeds (n = 10) harvested from induced (MeJA) and control (con) *Artemisia* plants at two differently polluted field sites (polluted site 1, unpolluted site 3) in laboratory experiments on soil collected at both field sites and on neutral substrate (sea sand).

Chorthippus rearing on A. vulgaris

The larvae reared on *A. vulgaris* exhibited similar development and mortality as known from other *Gomphocerinae* species reared on grass (Schäller & Köhler 1981). About half of the animals reached an age of 2 months, which is comparably high, and the last individual died at beginning of November 1999, at an imaginal age of about 14 weeks. This phenology is similar to the one described for naturally occurring *C. mollis* populations in the area around Jena, where sometimes individuals can be found until beginning of November (Köhler 1998).

Because of cage limitations the preliminary breeding experiments were performed without replicates of the treatment groups. Therefore, only tendency of reactions, but no statistical analysis can be presented. The reared *C. mollis* population produced a quantity of 51 egg packets; 31 in a first and 20 in a second period. Therefore, most females produced 2 - 4 egg packets. The size of the egg packets (length/diameter) in the first period was between 10.1/4.8 mm and in the second period between 9.5/4.8 and 16.3/4.7 mm. The number of unhatched eggs in the egg packets was low except for three completely unhatched packets. In 35 egg packets all eggs hatched and in the other packets only 1 - 2 possibly infertile eggs remained. Altogether, 167 eggs were laid, of which 39 remained unhatched. From this the low number of 3.3 eggs per egg packet can be calculated. After a more than 4-week post-diapause period, 128 larvae hatched. The larvae, which were reared on *A. vulgaris* and grass as food showed no higher mortality during larval development but developed relatively slow.

Discussion

A. vulgaris is a suitable model plant

A. vulgaris was chosen as the focal plant of this study because it has a high tolerance for soil degradation and occurs in relatively high densities along the entire gradient in the study area. Furthermore, it is an important part of the local plant community in Steudnitz (see Chapter 2). Fitness related studies are easy to perform with this plant as it is characterized by high growth rates and produces many seeds (Grime et al. 1988). Due to its high frequency of occurrence in central Europe, numerous herbivores live and feed on *A. vulgaris*. For instance, Schmitz (1996, 1999) found an herbivore complex consisting of 181 species closely linked to this plant species, a number, which approaches that of many shrubs or trees (Kennedy & Southwood 1984).

The lack of differences in germination due to different seed source allowed me to conclude that *A. vulgaris* does not exhibit strong genetic differences or local adaptations in the 10 years since the factory had closed. This is not surprising, because of the high mobility of the wind drifted *Artemisia* pollen and seeds and the consequent fact that all plants in Steudnitz belong to a population with a continuously mixed gene pool. However, this lack of evolved adaptations is only true for germination. Other traits were not investigated.

Methyl jasmonate induction did also not directly affect seed viability. Therefore, *A. vulgaris* is a suitable model plant to investigate plant herbivore interactions in Steudnitz. I used seeds from a single plant growing at the unpolluted site for all remaining experiments.

Chorthippus mollis is a suitable model herbivore

Chorthippus mollis has a mandibular structure of the graminivorous type with a lamella formed chewing area (Ingrisch & Köhler 1998) and is therefore particularly adapted to crush grass diet that is rich in silicates. The use of *Artemisia* species (*A. tridentata*) as additional food source has been demonstrated in experiments for a

North American Catantopinea grasshopper (*Melanoplus sanguinipes*) (Johnson & Lincoln 1991; Messina et al. 1996). However, the breeding of *C. mollis* on *A. vulgaris* as shown here is new, as it contrasts with the results of earlier food choice experiments on other *Chorthippus* species, in which *A. vulgaris* was not eaten, whereas all grass species were (Kaufmann 1965). My own results suggest the following:

1. The graminivorous type of feeding in *C. mollis* is a result of high preference for grass species but not a result of high rejection of herb species. If only herbs are available then this food source can be used alternatively.
2. *C. mollis* seems to be an exception among the Gomphocerinae, as in its normal habitat grass vegetation is rare and the grasshopper is sometimes reliant upon herbs as food source. Most importantly, although the use of *A. vulgaris* as an exclusive food source resulted in a lower fitness of the female *C. mollis* (lower number of egg packets and eggs), the grasshopper could be reared on *A. vulgaris* leafs in the laboratory. It was therefore useful for further choice test experiments investigating the defense capabilities of *A. vulgaris.*

Plant stress, plant fitness and herbivore reaction

Introduction

Abiotic and biotic stress affect plants

The term stress frequently is defined as an environmental factor that causes a potentially injurious change in a biological system (Hoffmann and Parsons 1993). Numerous stress factors affect plants during their life cycle. Plant ecologists use the term abiotic stress when referring to habitats where certain resources necessary for plant growth are scarce (Grime 1977, 1989). This terminology has been adopted by herbivore ecologists working on plant-herbivore interactions (e. g. Coley et al. 1985). It is essential to distinguish this meaning of stress from short-term changes, such as weather, disturbance, or wounding, in which stress is a temporary or transitory state. In abiotic stress, this is a normal situation, to which plants may have responded by evolving certain specific adaptations. A general phenomenon in stressed plants is slow cell growth; this in turn leads to smaller structures, such as buds, leaves and shoots (Kozlowski 2000; Kozlowski & Pallardy 2002). Therefore, here I follow the definition of Price (1991) and regard plants as stressed, when for any reason, performance in module growth is reduced below that achieved under optimal conditions.

In addition, herbivory can be regarded as a main biotic stress that is capable of severely reducing plant fitness either directly or indirectly by reducing plant competitive ability (Bernays & Chapman 2000). Climatic conditions and the supply of water and nutrients are probably the main abiotic factors influencing plant performance but a huge number of studies (Culliney & Pimentel 1986; Rebele et al. 1993; Masters & McNeill 1996; Redak et al. 1997; Zvereva et al. 1997a, 1997b; Kozlowski 2000; Zvereva & Kozlov 2000; Heinrich et al. 2001) have increasingly demonstrated the importance of anthropogenic soil and air pollution, which also affects plant fitness in many systems. Previous work has shown that both types of stressing agents, biotic and abiotic, often interact.

The Plant Stress Hypothesis

Nevertheless, the effects of abiotic and biotic stressing agents on plants had generally been studied independently until White (1984), Rhoades (1985) and Mattson & Haack (1987) generated the idea of the plant stress hypothesis (PSH). According to this hypothesis plants affected by abiotic stress show decreased resistance against herbivores. The general idea of the mechanism was that plant stress causes a mobilization of nitrogen in soluble form thereby increasing total available nitrogen (e. g. free amino acids) in the plant. For primarily nitrogen limited herbivores this facilitates an increased growth rate, leading to higher fitness (White 1984). Rhoades (1985) and Mattson & Haack (1987) complemented this argument by noting the likelihood that stressed plants are less able to synthesize defensive chemicals, as important primary and secondary pathways in the plant may be imbalanced. For instance, air pollution can disturb the phenolic synthesis in plants (Katoh et al. 1989; Kainulainen et al. 1993). Lower levels of foliar phenolics and tannins were observed in Japanese cedars (*Cryptomeria japonica*) fumigated with SO_2 (Katoh et al. 1989).

The Plant Vigor Hypothesis

In other studies, however, the resistance was highest in fast growing vigorous plants and much lower on stressed plants. Whitham (1980, 1987) for instance showed a preference of a galling aphid (*Pemphigus betae*) for large young leaves of *Populus augustifolia.* To explain these results, Price (1991) formulated the plant vigor hypothesis, which postulates that plant resistance is lower in vigorous plants. The term “vigor” in this context means any plant or part of a plant, such as a shoot or leaf, that grows rapidly and ultimately reaches a large size relative to the mean growth rate and size achieved in the population of plants or parts.

Pattern in insect responses on stressed host plants

Several attempts have been made to integrate both hypotheses (Larsson 1989; Koricheva et al. 1998). Those studies emphasize a lack of a single overriding effect on insect performance. Instead, there seems to exist a continuum of insect response to plant stress. However, Koricheva et al. (1998) extracted in a Meta analysis some patterns of herbivore responses to plant stress. In their study, feeding guild to which the herbivorous insect belongs seemed to be the most important determinant of insect response. Sucking insects especially benefit from plant stress whereas gall makers and chewing insects are negatively affected.

The type of stress applied to plants did not significantly modify general herbivore responses. That confirms the observation that different types of abiotic stress induce similar responses in plants. For instance, the response to insufficient nutrient supply is similar to the response to other types of environmental stress like osmotic or water stress (Chapin 1991). However, stress type had an influence on the guild of sucking insects; water stress tended to decrease population growth of suckers, whereas pollution increased it and heavy metals had more pronounced effect than other pollution sources (Koricheva et al. 1998).

There are numerous studies on the effect of pollution on constitutive defenses, but little is known about the interaction between induced response reactions and pollution stress. Induced plant responses include physical, nutritional and allelochemical traits that change in response to herbivory (Karban & Myers 1989; Wold & Marquis 1997). These induced responses, which may lead to induced resistance against herbivores, are important in many plant-herbivore systems (Karban & Myers 1989; McGurl et al. 1992; Orozco-Cardenas et al. 1993; Baldwin 1998; Farmer et al. 1998) and often can be elicited by the ubiquitous wound hormone jasmonic acid (JA). Increases in phenolic biosynthesis, gene expression or enzyme activity, and accumulation of the products of these enzymes, are commonly associated with JA treatment or herbivory (Karban & Baldwin 1997). JA-treatment is therefore a suitable tool to simulate herbivory. Although most work on induced defenses has been carried out using chewing herbivores, studies with sucking herbivores have also shown induced molecular responses in plant and connections to the jasmonate cascade (Moran & Thompson 2001). For example, aphid feeding can increase lipoxygenase mRNA in tomato (Fidantsef et al. 1999), suggesting that plant sensitivity to phloem feeding could involve jasmonate synthesis and signaling activity.

Studies investigating the influence of plant stress on plant herbivore interaction usually show some gaps. The effect of stress on a plants ability to defend against herbivores is non-linear (Larsson et al. 1989), what means that even herbivores that are able to benefit from plant stress show a decreased performance when the stress level increases above a certain threshold. However, only a few studies have tested multiple levels of stress, thereby allowing detection of any non-linear relations.

Furthermore, most studies test insect responses only to short-term stress episodes (shorter than the life span of the host plant), which is in contrast to the general definition of stress among herbivore ecologists.

Additionally, variation due to plant genotype is not taken into account in most of the studies, despite the fact that plant genotypes by environment interactions are quite common and the magnitude of insect responses to plant stress may often depend on plant genotype (Coleman & Jones 1988; Talhouk et al. 1990; Herms & Mattson 1992; Pilson 1992; Marino et al. 1993; Preszler & Price 1995; Masters & McNeill 1996; Cronin & Abrahamson 1999; Havill & Raffa 1999; Ballabeni & Rahier 2000; Underwood et al. 2000). The here presented experiments are designed to take into account such factors, and to characterize the interactions between pollution stress and simulated herbivory on the resistance of *A. vulgaris* to herbivores from different feeding guilds.

Material and methods

Plant culture

Artemisia vulgaris plants were grown from seeds of one plant (for details on seed source see Chapter 3) for three weeks in walk-in growth chambers (York International GmbH, Mannheim, Germany) with a 14:10 h light:dark photoperiod at 20 °C : 18 °C and 65 % relative humidity and ~1000 µM m^{-2} s^{-1} photosynthetically active radiation.

In April 2000, 360 plants of most similar size and morphology were transferred to field plots that had been cleared of competing ruderal plants. Smaller plants were not removed and the soil was disturbed as little as possible. Plants were randomly assigned to seven treatment groups of eight replicate plant pairs (Fig. 4.2) for each of the three field plots (Fig. 4.1, Table 4.1). Due to exceptionally low rainfall in early summer of 2000, (see Chapter 2) the plants were watered during the first 3 weeks after transplanting.

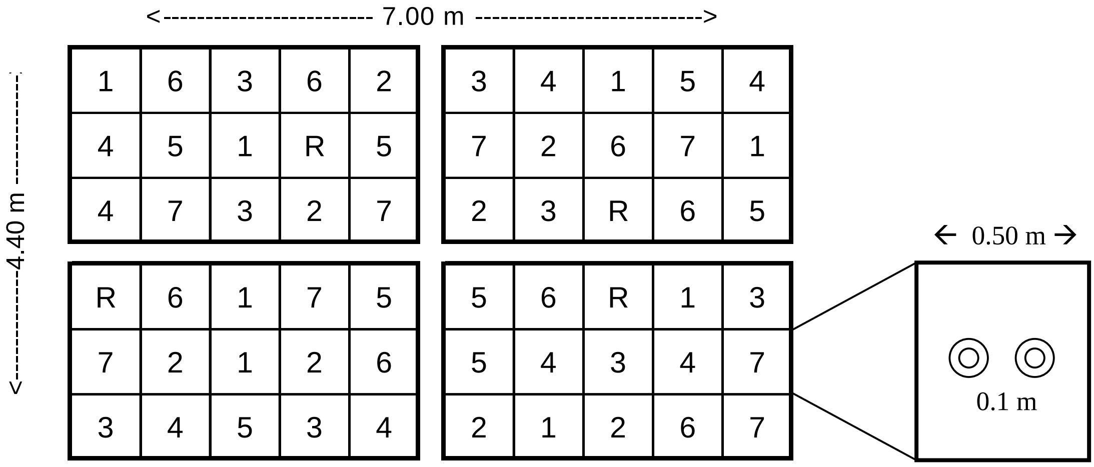

Figure 4.1 Experimental design of one of the three field plots. The numbers (1-7) refer to the different treatment pairs (see Table 4.1, R = reserve pairs). The distance between the two plants in a pair was 10 cm, the distance between pairs and the width of the edge zones was 50 cm.

Figure 4.2 Treatment pair of *Artemisia vulgaris* plants (see Figure 4.1, Table 4.1) growing at Steudnitz field site 1 (photograph M. Held).

Methyl Jasmonate treatment

Plants were either treated with the plant hormone methyl jasmonate (MeJA, Fig. 4.3) (Bedoukian Research, Danbury, Conn. USA) to induce a plant defense response or left untreated. MeJA (250 µg per plant), close to its thermodynamic equilibrium for the two naturally occurring epimers – 90.1 % 1R, 2R MeJA and 8.3 % 1R, 2S MeJA – was applied in 20 µl of lanolin paste to the upper and lower surfaces of two fully expanded leaves of each plant (Baldwin et al. 1996). Control plants received the same amount of pure lanolin. This procedure delivers a quantitatively controlled dose of jasmonate to plants at a level that is within the physiological range of endogenous jasmonate dynamics of plants (Zhang & Baldwin 1997).

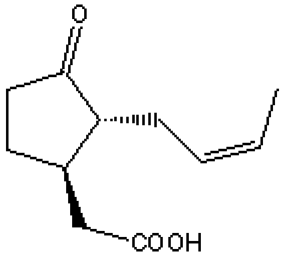

Figure 4.3 Chemical structure of jasmonic acid. In methyl jasmonate (MeJA) the H of the carboxyl group is replaced by a CH_3 group.

Insecticide treatment

To remove naturally occurring herbivores, plants were sprayed with a 0.1 % solution of a pyrethrine-piperonylbutoxide mix (Spruzit, Neudorff GmbH KG, Germany) in distilled water, which has been proved to be suitable for the control of native herbivores (Pfrommer & Mendgen 1992; Maklakov et al. 2001). Control plants were sprayed with the same quantities of distilled water. During spraying, the treated plants were enclosed with plastic shields to avoid contamination of neighboring plants. The MeJA and insecticide treatments were repeated twice a week from May until September, when seeds were harvested.

Plant survival, growth and reproduction

Different combinations of Insecticide treatment and MeJA induction were performed to evaluate different aspects of the fitness and herbivore-defense capabilities of *A. vulgaris* (Table 4.1). The following fitness-correlated parameters were recorded for each plant: seedling survival, stem length (measured twice a month), time of flowering and seed production.

Table 4.1 Experimental pairs with 7 treatment combinations were established to answer the following questions: (a) untreated controls; b) MeJA treated; c) herbivore removal; d) aphid performance bioassay; e) cage without aphids)

	a	b	c	d	Corresponding question
a		X			1. How does MeJA elicitation affect plant performance?
a			X		2. Does natural herbivory influence plant performance?
a		X	X		3. Is MeJA induced growth inhibition compensated by insecticide treatment?
b		X	X		4. Does the absence of herbivores alter the costs and benefits of induction?
b			X		5. How does the benefit of induction compare to that of herbivore removal?
d		X		X	6. Is induced resistance detectable?
e	X				7. Do the enclosures affect plant performance?

As they were reared from seed, the plants initially grew as a single stem. During the experiments, buds at the base of the main stem began to develop so that the plant consisted of a main central stem surrounded by several lateral stems. The growth measurements recorded the length of the main stem. Growth rates were measured as the change in branch length between May and August, the period of intensive growth (Fig. A4 appendix). To compare growth rates of field-grown plants with plants grown under optimal conditions, 50 seedlings were transferred to 2 l pots in soil [60 l peat moss, 30 l Vermiculite, 30 l Perlite, 250 ml ground limestone, 250 ml bone meal and 400 ml Osmocote 14:14:14 pellets (N:P:K; 2 - 3 month release rate)] and grown in the glasshouse at 26 - 30 ºC under 16 h supplemental light from Philips Sun-T Agro 400 Na lights.

Flowering phenology was recorded when the first inflorescences began developing. When the plant switches from vegetative to reproductive growth, the apical bud stops producing leaves and buds that will form the branches of the inflorescence will appear. Plants that had inflorescence branches with at least one open blossom were scored as flowering, plants that had no open blossoms were scored as not flowering. Flowers are formed in heads, which turn brown after pollination while the seeds (achenes) in the head ripen. In late autumn the head opens so that the seeds are exposed to be carried away by the wind. To avoid this, plants were harvested before the capsules opened and seed production was estimated by measuring the cumulative length of the flower buds of all branches of each plant. This was then multiplied by an empirically determined factor for each plant, which related flower length into seed number.

Germination tests

Viability and germination of seeds were determined in both laboratory and field assays. In the laboratory assays, the percentage of germinating seeds was determined on upper soil collected at the three field sites and on sterile sea sand (Merck, Darmstadt, Germany). Ten replicates with 20 seeds in each cup were examined daily for 10 days at 24 °C with a 16:8 h light:dark photoperiod and ~250 µM m^{-2} s^{-1} photosynthetically active radiation. The design of the field bioassays was similar; on each of the field plots ten squares of 10 x 10 cm were cleared from vegetation, 20 seeds were sowed in each square and examined for 15 days.

Aphid performance

To estimate natural herbivore densities in the field along the pollution gradient the distribution of the gall-forming aphid species *Cryptosiphum artemisiae* was examined. Starting at the polluted site (1), every 40 m gall frequency was measured on 20 randomly chosen *A. vulgaris* plants. These galls were harvested and in the laboratory the gall size and the number of unwinged and winged aphids per gall was determined. Gall size classes were determined according to diameter as class 1 (0 – 2 mm), class 2 (2 – 5 mm), class 3 (5 – 10 mm) and class 4 (>10 mm).

Herbivore performance and plant resistance in the field were measured by examining the fecundity of the aphid species *Macrosiphoniella artemisiae*. Only apterous adults of *M. artemisiae* were used for the experiment, in which 10 x 10 x 20 cm polyester enclosures covered the upper branch and developing flower buds of each *A. vulgaris* plant. Personal observations proved that ladybird (Coleoptera: Coccinellidae) and hoverfly larvae (Diptera: Syrphidae), which were the main predators of aphids in Steudnitz, were excluded by these enclosures. Two newly hatched adult female aphids from a laboratory culture of *M. artemisiae* from Steudnitz were introduced into each enclosure in July 2000. On each of the three plots, eight plant pairs were caged and one plant of each pair was treated with MeJA. Control plants were caged without adding aphids (cage control). Asexual reproduction over 12 days was measured by counting the number of nymphs produced from the original adults on each enclosed branch. Additionally the number of alatae (winged aphids) was measured.

Grasshopper performance

Chorthippus mollis was reared in the laboratory on control plant material of *A. vulgaris* harvested from the polluted site 1 and on induced and control material from the unpolluted site 3, where MeJA-induced resistance against aphids was demonstrated. To assess the effect of this induced resistance on grasshopper fitness, eight female grasshoppers were raised in individual cages from eggs to adult on induced or control foliage. They were mated and provided with boxes of sand for oviposition. Fitness was measured by the number and viability of eggs produced. Viability was measured by examining the eggs after 4 months of diapause to check for living embryos. In a second experiment, food choice tests were performed, to measure how much plant material of each type was consumed, in conjunction with observations of grasshopper feeding behavior. In these choice tests, third-instar nymphs and imagines that had received no food for 24 hours were provided with MeJA-induced and control plant material from the unpolluted site 3 or, in another test control material from site 3 and control material from site 1 (8 replicates each). The plant material had been collected immediately before the experiment and transported on ice to the lab. Single branches from each of the two test plants were placed in water flasks and offered to single caged grasshoppers. The grasshoppers were allowed to feed for 48 hours, the first 60 minutes of which were monitored. The attractiveness of both food types was determined according to:

$$A = 60 - T \qquad \text{(A: relative attractiveness)}$$
$$\text{(T: time to first contact in min)}$$

Additionally the absolute time feeding on the two different plant materials was measured. The plant material was weighed before and after to estimate consumption.

Statistical analysis

Differences between the treatment groups were tested for significance by one-way ANOVA followed by *post hoc* tests (Student-Newman-Keuls) or Kruskal–Wallis

ANOVA on ranks followed by a Dunn *post hoc* test when the prerequisite for normal distribution and homogeneity was not fulfilled. To compare groups, additional *t*-tests were used: paired for the mugwort field experiment; unpaired for the germination bioassay, the food choice tests and the grasshopper reproduction experiment. SPSS 9.0 and Microcal Origin 6.0 software were used for the statistical analysis. Linear regression was used to compare plant growth and the number of seeds produced.

Results

Germination tests

Soil pollution dramatically decreased seed germination in both field and laboratory trials (one-way ANOVA, $F_{6,69} = 81.73$, $P < 0.001$; Fig. 4.4). Compared to site 3, only 50% of the seeds germinated at site 2 and only 25% of the seeds germinated at site 1. Similar results were obtained in both laboratory and field trials. The germination rate on sea sand was not different from germination on soil from the unpolluted site 3 in field and laboratory trials (one-way ANOVA, $F_{2,29} = 1.4873$, $P = 0.244$, Fig. 4.4).

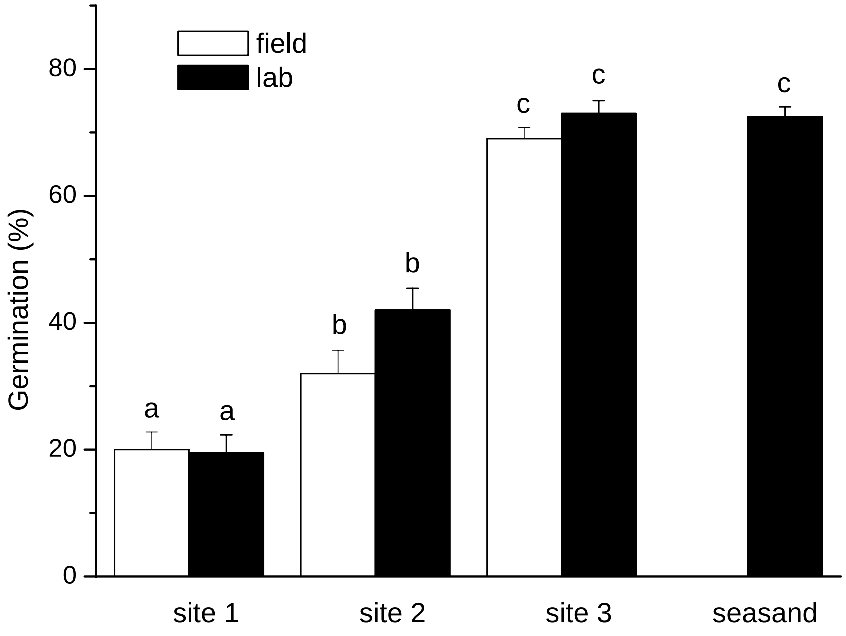

Figure 4.4 Mean (+SE) germination rates of *Artemisia vulgaris* (n = 10) in the field, in laboratory experiments on soil collected at the three field sites of decreasing soil pollution (1 – 3) and on neutral substrate (seasand). Different letters reflect significant differences at $P < 0.05$.

Plant survival and growth

Survival rates of transplanted seedlings were not affected by soil pollution. Almost all transplanted *A. vulgaris* plants survived: only eight of the 360 plants died during the experiment (3 at site 1, 2 at site 2, 3 at site 3). Plant growth rates, however, decreased as soil pollution increased (one-way ANOVA, $F_{8,95} = 189.74$, $P < 0.001$; Fig. 4.5).

Plants grown in the greenhouse reached heights of up to 2.8 m, which corresponded to a growth rate of 19.5. The growth rates, however, were not significantly different from those reached by field plants at site 3 (*t*-test, $t_{94} = -1.311$, $P = 0.193$).

Neither herbivore removal with insecticide sprays (experimental pair two, see Table 4.1), nor enclosing the plants (experimental pair 7) had any significant effects on plant growth (all P values >0.081). Herbivore removal did not affect growth rates at sites 1 and 2 but there was a trend towards increased growth among the fast-growing plants at site 3 (paired *t*-test, $t_8 = 2.036$, $P = 0.081$).

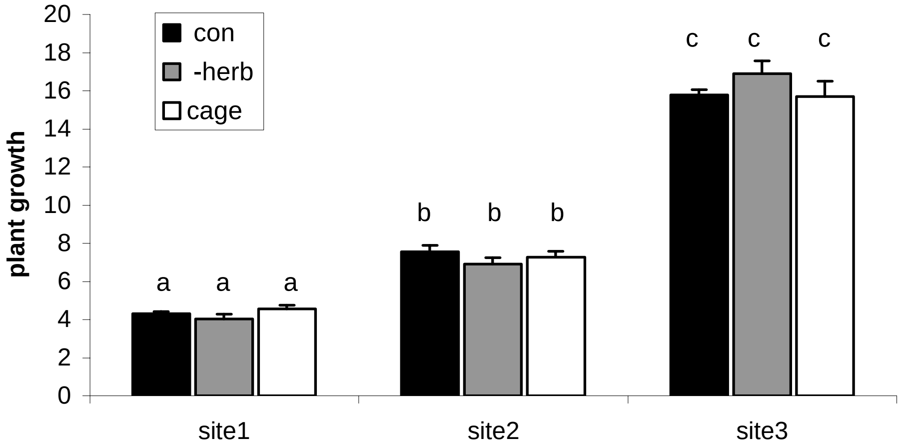

Figure 4.5 Mean (+SE) growth rate of *A. vulgaris* treated with insecticide (-herb, n = 8) or being encaged (cage, n = 8) at the three field sites of decreasing soil pollution (1 - 3). Control plants (con, n = 16) were combined in one bar. Different letters reflect significant differences at $P < 0.05$.

MeJA elicitation (experimental pair 1), on the other hand, significantly decreased the growth (Fig. 4.6) at site 2 by 40 % (paired *t*-test, $t_8 = -3.143$, $P = 0.017$) and site 3 by 15 % ($t_8 = -7.339$, $P < 0.001$) but not at site 1 (paired *t*-test, $t_8 = -1.59$, $P = 0.133$).

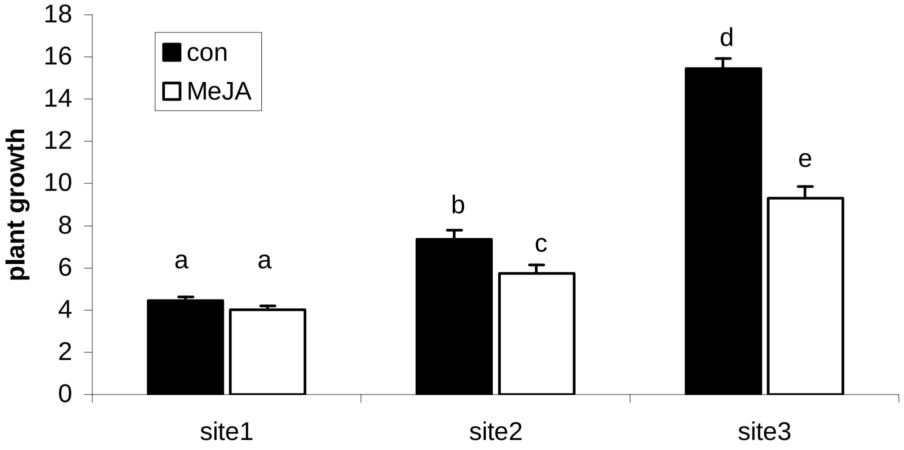

Figure 4.6 Mean (+SE) growth rate of *Artemisia vulgaris* control plants (con) and plants treated with methyl jasmonate (MeJA) at the three field sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences at P <0.05.

All combined treatments of MeJA induction and herbivory gave similar results than the pure MeJA-treatments. Independent of which combinations of treatments were compared, the MeJA treated plants always showed the same pattern of growth rates (Fig. 4.7), but no additional effects could be detected at site 1 (one-way ANOVA, $F_{4,36} = 0.218$, $P = 0.927$), site 2 (one-way ANOVA, $F_{4,36} = 0.184$, $P = 0.945$) or site 3 (one-way ANOVA, $F_{4,36} = 0.292$, $P = 0.881$). In contrast all plants in experimental pairs 3-5, that were not MeJA-induced had the same performance as control plants, independently from enclosing or insecticide treatments or site (site 1: one-way ANOVA, $F_{6,54} = 0.841$, $P = 0.545$, site 2: one-way ANOVA, $F_{6,54} = 1.131$, $P = 0.359$, site 3: one-way ANOVA, $F_{6,54} = 0.614$, $P = 0.718$).

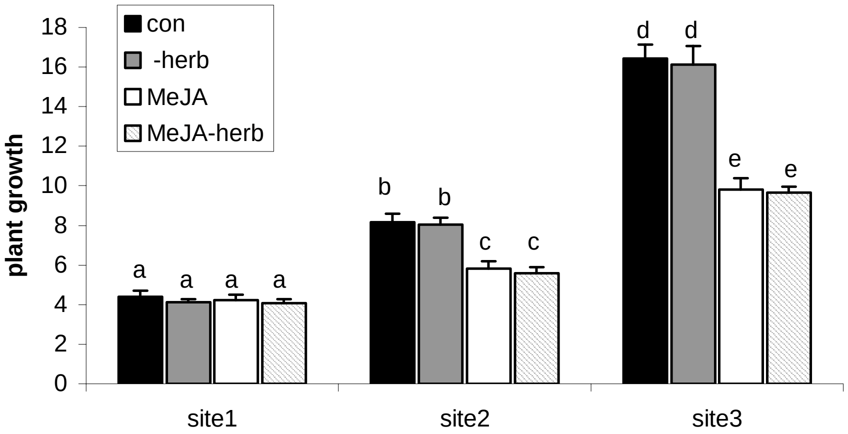

Figure 4.7 Mean (+SE) growth rate of *Artemisia vulgaris* control plants (con), plants treated with insecticide (-herb), with methyl jasmonate (MeJA) and with combined insecticide and MeJA-treatment (MeJA-herb) at the three field sites of decreasing soil pollution (1 - 3). Data for the bars MeJA-herb and MeJA are combined from experiments 3, 4 and 5 (see Table 4.1). Different letters reflect significant differences at *P* <0.05.

Plant reproduction

The first flowering plant was recorded on August 28. Within 12 days, all remaining study plants started flowering. Induced plants showed at all sites a delayed flowering relative to control plants (Fig. 4.8, Two way ANOVA, F = 8.749, *P* = 0.03). However, there was no significant difference in flowering time across the differently polluted field sites (Two way ANOVA, F = 1.364, *P* = 0.257) and no interaction between site and induction (F = 0.503, *P* = 0.605).

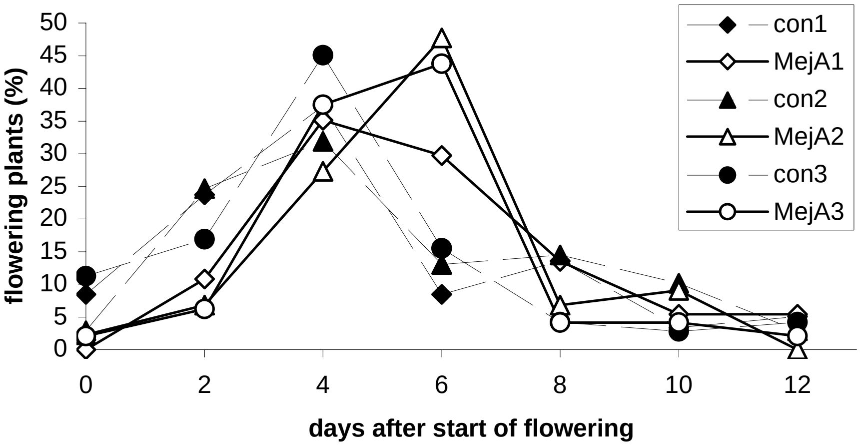

Figure 4.8 Cumulative percentage of flowering control (con) and MeJA-treated plants at three field sites of increasing soil pollution (1 - 3) during 12 days of examination starting at August 28.

With increasing soil pollution the number of produced seeds decreased rapidly (Kruskal-Wallis ANOVA on Ranks, $H_2 = 173.979$, $P < 0.01$). Plants at site 1 produced a mean seed number of 5,400 (SE = 20.1), plants at site 2 produced 11,500 (SE = 16.5) and plants at site 3 produced 31,400 seeds (SE = 18.5, Figure A5 appendix). Seed production was positively linearly correlated with branch length; this relationship held for the pooled dataset (Fig. 4.9, $R^2 = 0.808$, $P < 0.001$) as well as for the single treatments (R^2s ranging from 0.56 to 0.86, all *P* values <0.001) (Table A3 appendix). At all 3 sites, MeJA-induced plants produced less seeds than control plants (mean 13.2% lower at site 1, 24.17% lower at site 2 and 31.4% lower at site 3 (see Figure A5 appendix). The effects of site and induction on seed production were significant (two-way-ANOVA, site: $F_{2,327} = 150.52$, $P < 0.001$, induction: $F_{2,327} = 33.45$, $P < 0.001$).

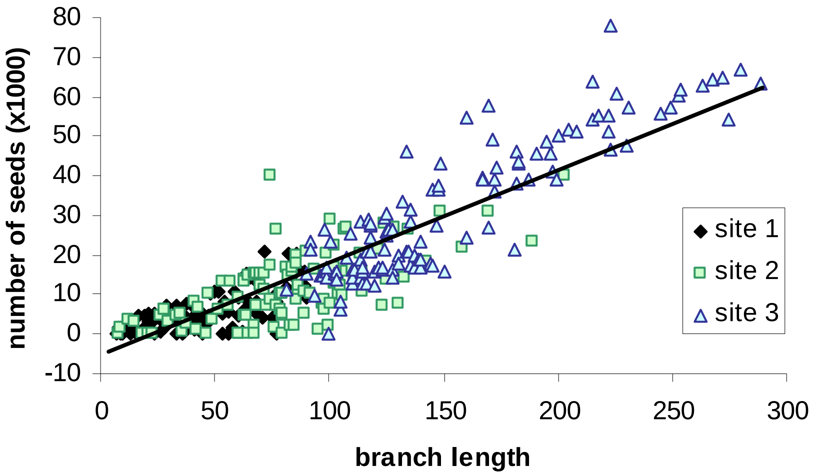

Figure 4.9 Branch length of the experimental plants (n = 328) along 3 sites of decreasing soil pollution (1 - 3) is linearly related to the number of seeds produced at the end of the growing season ($R^2 = 0.808$, $P < 0.01$).

Aphid performance

At the investigated sites along the pollution gradient 153 of 400 examined *Artemisia* plants (38%) were infested with 576 *Cryptosiphum* galls. The mean gall number per plant was 0.91 (± 0.11). The distribution of galls along the pollution gradient showed an increase of the infestation rate for both, the percentage of infested plants and the mean gall number per plant (Fig. 4.10). However, only the correlation between the distance from the pollution source and the mean gall number/plant was significant ($R^2 = 0.244$, $P = 0.027$) but the correlation between distance and the number of infested plants was not, although a clear trend was apparent ($R^2 = 0.18$, $P = 0.062$). The ratio between both parameters remained constant along the whole gradient.

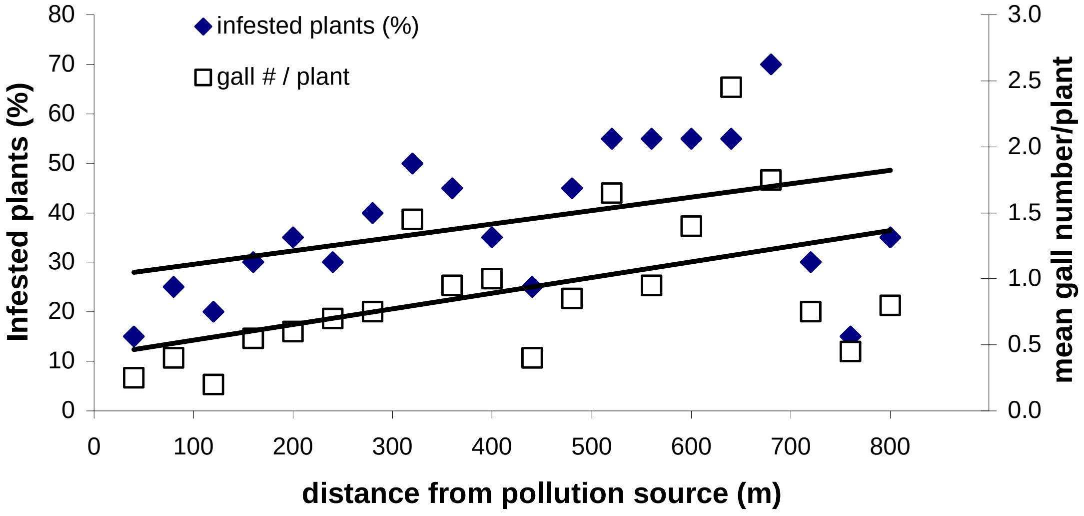

Figure 4.10 Linear regression of the mean percentage of *Artemisia vulgaris* plants infested with galls of the species *Cryptosiphum artemisiae* and the mean number of galls/plant along a gradient of decreasing soil pollution.

All size classes were existent and showed a similar distribution along the gradient. (Fig. 4.11, Kruskal-Wallis ANOVA on Ranks, $H = 10.762$, $P = 0.932$). The gall size at all sample sites was normally distributed with galls sized between 5 and 10 mm (size class 3) being the most abundant class (Kolmogorov-Smirnov test, all P-values >0.08).

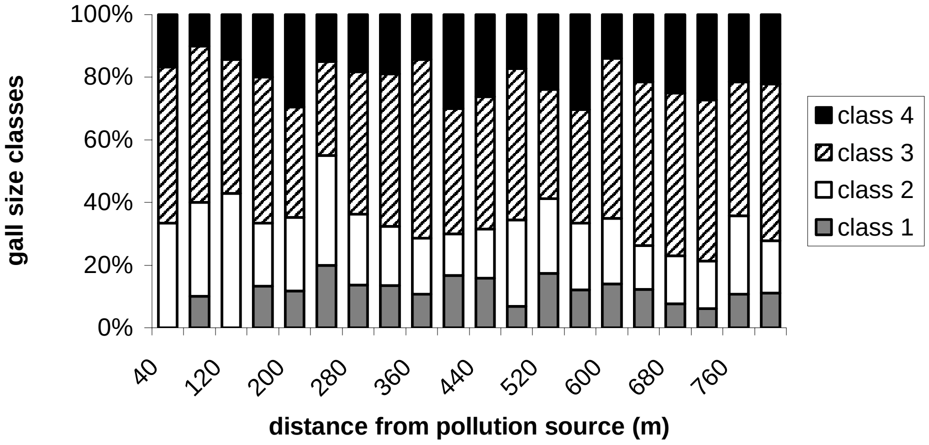

Figure 4.11 Distribution of *Cryptosiphum artemisiae* gall size on *A. vulgaris* plants along a gradient of decreasing soil pollution, size classes: 1: 0 - 2 mm, 2: 2 - 5 mm, 3: 5 - 10 mm, 4: >10 mm.

Altogether 5409 individuals of *C. artemisiae* were counted in the galls giving a mean of 9.37 aphids/gall. The individual numbers did not depend on the distance from the pollution source (Kruskal-Wallis ANOVA on Ranks, H_{19} = 11.075, P = 0.921), however there was a trend of more aphids present in galls of larger size (Kruskal-Wallis ANOVA on Ranks, H_3 = 7.615, P = 0.055), although slightly. Single winged individuals were only found in two of the 576 examined galls.

Macrosiphoniella artemisiae exhibited high rates of asexual reproduction in the field enclosures on *A. vulgaris*. The two experimentally introduced adults produced as many as 150 individuals within 12 days in the aphid enclosures. Among the offspring in the enclosures no winged aphids (alatae) were recorded.

The aphids' performance decreased on induced plants as well as on plants on polluted soil (Fig. 4.12). Resistance differed significantly (paired *t*-test, t_8 = -7.339, P <0.001) between induced and control plants at the unpolluted site 3. Soil pollution (site 1) was associated with a 50% decrease in aphid performance compared to control plants. MeJA elicitation decreased the performance by a similar amount (60%), but the induced difference waned as soil pollution increased and no induced difference was found at site 1 (paired *t*-test, t_8 = -1.022, P = 0.34) and site 2 (paired *t*-test, t_7 = -1.657, P = 0.141). Furthermore, the resistance of control plants tended to decrease when soil pollution increased (Fig. 4.12; one-way ANOVA, $F_{5,47}$ = 2.87, P = 0.026) whereas there was no measurable between site difference for the induced plants (one-way ANOVA, $F_{2,23} = 0.363$, $P = 0.7$)

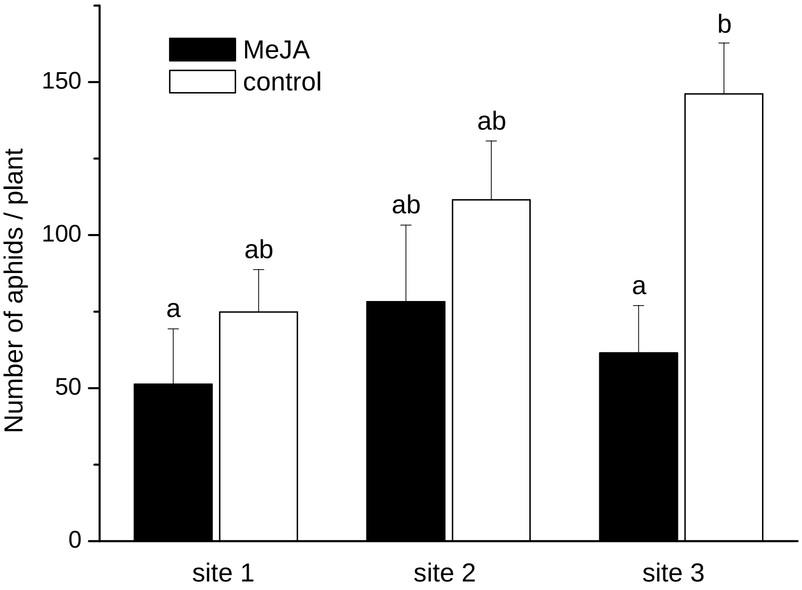

Figure 4.12 Mean (+SE) rates of reproduction of two apterous adults of *Macrosiphoniella artemisiae* experimentally infested on an enclosed branch of methyl jasmonate (MeJA) treated and untreated (control) *Artemisia vulgaris* at three field sites of decreasing soil pollution (1 - 3). Different letters reflect significant differences at $P < 0.05$.

Grasshoppers performance

The first individuals of *Chorthippus mollis* hatched at the beginning of June and the last died at the end of November. Survival and developmental time of the grasshoppers were not influenced by the different food sources. All individuals on both food types survived and developed within 10 weeks from eggs to imagines. The total number of eggs laid per female was similar between the treatment groups, but the number of viable eggs produced decreased significantly when the grasshoppers fed on induced plant material (t-test, $t_{14} = 2.266$, $P = 0.04$) compared to control material from the unpolluted site (Fig. 4.13). No significant difference could be seen between the total number of eggs from grasshoppers reared on control material from the polluted site 1 and the unpolluted site 3 (t-test, $t_{14} = -1.404$, $P = 0.182$) however there was a trend that the number of viable eggs decreased for grasshoppers reared on material from site 1 (t-test, $t_{14} = -1.868$, $P = 0.083$).

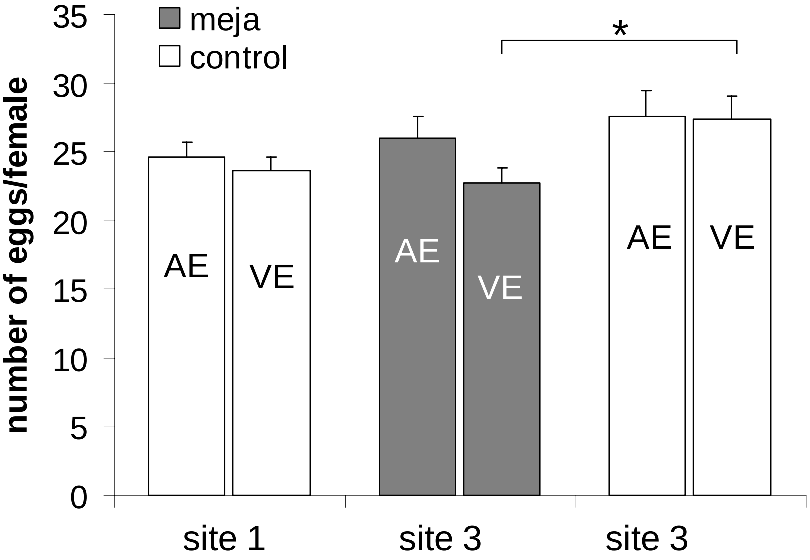

Figure 4.13 Reproduction of *Chorthippus mollis* on untreated (control) *Artemisia vulgaris* material collected at the polluted field site 1 and methyl jasmonate (MeJA) treated and untreated (control) *A. vulgaris* material collected the unpolluted field site 3. The bars depict the number of all laid eggs (AE) and of viable eggs (VE) produced per female (mean +SE, n = 8). Asterisk reflects significant differences at $P < 0.05$.

Grasshopper food choice

C. mollis showed a similar feeding behavior when feeding on both, *Artemisia* leaves and grass. The feeding began at the edges of the leaves, the grasshoppers continued feeding through the middle vein to the other side of the leaf. As a result, the distal part of the leaf fell down and was not used. This feeding type produced on the wide *Artemisia* leaves a large amount of leaf litter.

The measured behavioral parameters (relative attractiveness and time spent feeding) did not differ between grasshoppers feeding on differently treated plant material. However, within all groups the variance of both tested parameters was high (Fig. 4.14). Grasshoppers started feeding after several minutes and usually changed the plant at least one time during the recorded first 60 minutes. The mean attractiveness (A) on all food types was determined as 20 to 25. The observed grasshoppers showed a specific feeding behavior, with several minutes of food uptake interrupted by longer periods without feeding. However, no choice behavior and no rejection of food happened, they always started feeding on the first plant they did climb or jump on. The mean time spent feeding laid between 8 and 12 minutes (see Table A4 appendix).

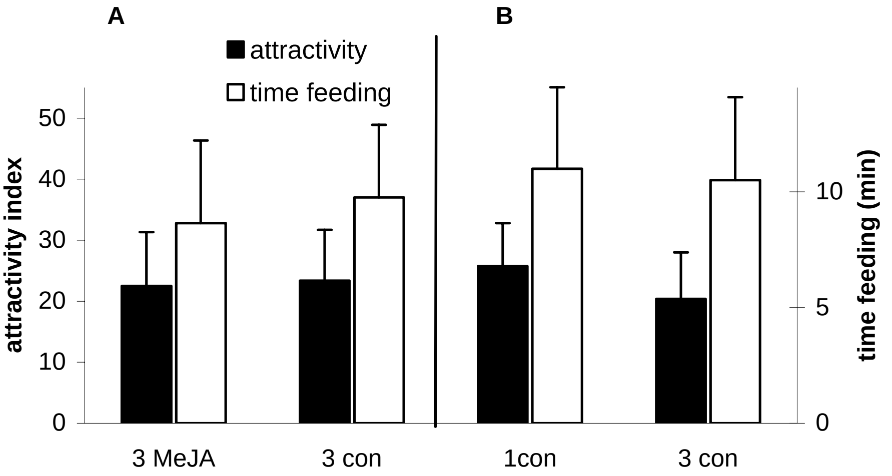

Figure 4.14 Feeding behavior of *Chorthippus mollis* imagines in food choice tests: A) mean (+SE) attractiveness of and cumulative feeding time on induced (3 MeJA) and control (3 con) material of *Artemisia vulgaris* from the unpolluted site 3; B) mean (+SE) attractiveness and cumulative feeding time on material from the polluted site 1 (1 con) and material from the unpolluted site 3 (3 con).

In contrast to this lack of difference in feeding behavior, in terms of the amount of consumed food the grasshoppers clearly distinguished between the different food sources , imagines of *C. mollis* consumed more control material than induced material (*t*-test, t_{14} = 3.221, P = 0.006, Fig. 4.15 A) and more control material from the polluted site 1 than control material from the unpolluted site 3 (*t*-test, t_{14} = 2.806, P = 0.014, Fig. 4.15 B).

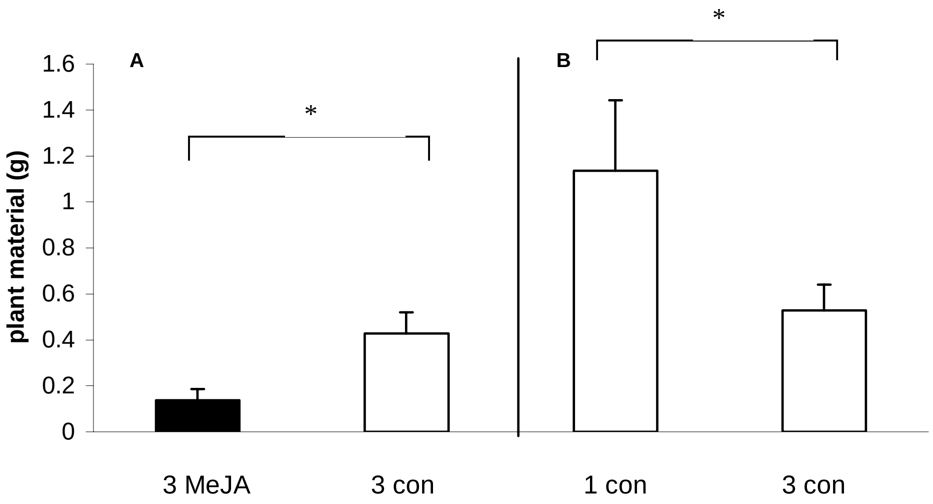

Figure 4.15 Amount of food consumed by *Chorthippus mollis* imagines (each n = 8) in food choice tests. A) mean +SE of consumed food on induced (3 MeJA) and control (3 con) material of *Artemisia vulgaris* from the unpolluted site 3. B) mean +SE of consumed food on material from the polluted site 1 (1 con) and material from the unpolluted site 3 (3 con). Asterisk reflects significant differences at P <0.05.

Although the pattern of consumption for third-instar nymphs was similar the amount of consumed food was smaller and the values showed a higher variance, leading to a lack of significant differences between the treatment groups (Fig. 4.16, one-way ANOVA, $F_{3,31} = 1.476$, $P = 0.243$).

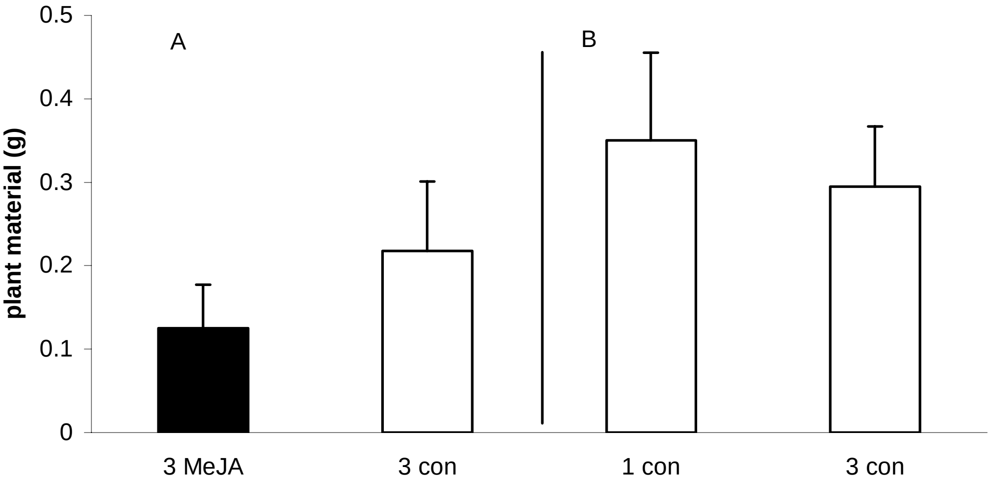

Figure 4.16 Amount of food consumed by third instar nymphs of *C. mollis* in food choice tests: A) mean (+SE) of consumed food on induced (3 MeJA) and control (3 con) material of *A. vulgaris* from the unpolluted site 3; B) mean (+SE) of consumed food on material from the polluted site 1 (1 con) and material from the unpolluted site 3 (3 con).

Discussion

Artemisia suffers increasing stress towards the pollution source

Plants can be stressed by soil degradation during different stages of their life cycle. In the present study increased concentrations of pollutants inhibited seed germination, growth and reproduction of *Artemisia vulgaris*. In field and laboratory trials, *A. vulgaris* seeds showed a strong decline in germination at sites 1 and 2, indicating suboptimal soil conditions. Germination on neutral sand in the lab showed similar results to the germination at field site 3. Therefore, it is clear that the soil at field site 3 offers almost optimal conditions for germination.

Once established *A. vulgaris* plants showed a high tolerance to soil pollution which can be seen in the high survival rates even at the most polluted site 1. However, without watering during the first 3 weeks of extremely dry weather (compare Chapter 2), an extreme decrease of survival of all plants would be expected.

Plants respond to most environmental stress by producing more abscissic acid and less cytokinines, which leads to a decline in growth rate (Chapin 1991). A reduction in shoot growth is described as one of the most sensitive and consistent responses of plants to drought and is incorporated into most definitions of stress, too (Mattson & Haack 1987; Waring & Cobb 1992; Preszler & Price 1995). Therefore, if growth rates are accepted as indicators of plant stress, the plants at site 1 and 2 showed clear stress symptoms. Again soil conditions at site 3 did not reduce growth compared to that of plants grown under optimal conditions in the greenhouse which indicates that plants at site 3 did not suffer intense stress.

Soil pollution decreased Artemisia fitness

The modular construction of plants ensures, that the seed production increases monotonically with shoot dry weight (Samson & Werk 1986); most of the evidence suggests that the relationship between size and fecundity is linear, at least for annual and short-lived perennial plant species (Rees & Crawley 1989). In accord with that statement, the number of *A. vulgaris* seeds showed a significant linear correlation to the size of the plants. Declines in growth rate were obviously reflected in seed numbers.

Flowering phenology in plants can have substantial impact on plant fitness (Pilson 2000). However, time of flowering as well as survival rates of *A. vulgaris* were not influenced by soil quality. In contrast, all other fitness-related parameters measured in *A. vulgaris* were negatively influenced. Combining the effects of soil pollution on the different stages of the life cycle during the experiment, plants grown at site 1 showed a fitness decline of 76 % compared to those grown at site 3.

Plant Stress Hypothesis and Plant Vigor Hypothesis

The Plant Stress Hypothesis (PSH) suggests a positive effect of pollution-caused plant stress on herbivores (White 1984) whereas the Plant Vigor Hypothesis (PVH) predicts better herbivore performance on vigorous tissue that is low in fiber and lignified tissues compared to stressed tissue (Price 1991). Both hypotheses in its most general original form seem not to be true. Today they are seen more than two ends of a spectrum of plant herbivore interactions (Price 1991) with the results depending on characteristics of the investigated system. The patterns were analyzed in a current meta analysis of stress effects on plant-herbivore interactions (Koricheva et al. 1998). The consequences of stress-induced changes in host plants on insects can vary depending on their modes of feeding (Koricheva et al. 1998). The reasons for this are probably related to the feeding sites and tissues utilized by differently feeding insect herbivores. Another source of variation is the differentiation of herbivores into specialist and generalist feeders (Koricheva et al. 1998). Jones & Coleman (1991) suggested that insects which are specialized to feed on a few plant taxa should be limited in their physiological plasticity and, therefore are expected to be more strongly influenced by changes of food quality. In contrast, generalists may show greater behavioral plasticity and may therefore be able to handle abroad range of nitrogen and secondary metabolite concentrations.

Finally the type of stress acting on the system influences the reaction patterns of involved herbivores. For example, differences among guilds of chewers and suckers are less apparent in the case of water stress than in that of pollution (Koricheva et al. 1998).

All investigated herbivores on Artemisia are negatively affected by host-plant stress

In this study, herbivores from three different feeding guilds (gall formers, suckers and chewers), representing three different degrees of specialization, all reacted in a similar way and were negatively affected by soil pollution, however, the extent of the reaction differed.

Reaction pattern of Cryptosiphum artemisiae

The performance of the gall forming aphid *Cryptosiphum artemisiae* was closely correlated with the performance of its host plant. The number of infested mugwort plants and the number of galls/plant increased with decreasing soil pollution. However gall size, which is documented as related to gall-inducer survivorship (Abrahamson et al. 1989; Weis et al. 1992; Sumerford 2000), and the number of aphids/gall, did not change. This result indicates that the negative effect of soil pollution acts more strongly on the process of colonization than on fitness parameters like survival, growth and reproduction. A similar pattern was detected by Koricheva et al. (1998) who found a negative effect of plant stress on colonization and survival of gall formers, but not on fecundity. Among the studied herbivores *C. artemisiae* as a monophagous gall forming insect has the most intimate connection to its host plant and is immediately linked to its physiology. This is consistent with the common idea that those herbivore species most directly involved with the processes of plant growth will match the Plant Vigor Hypothesis more closely (Larsson 1989; Price 1991). Furthermore, Koricheva et al. (1996) describe variable responses of pollution stress even within the feeding guild of gall-makers depending on the structure of galls and therefore the degree of protection from the environment. Complex galls with thick walls and chambers filed with a nutrient-rich parenchymatous tissue are likely to provide better protection from the environment and enough food for their inhabitants even on stressed plants. In contrast, the inhabitants of simple galls such as the leaf rolls of *C. artemisiae* are more exposed to the environment and changing metabolite levels offered by the host plant.

Reaction pattern of Macrosiphoniella artemisiae

The reproduction rate of *M. artemisiae* also exhibited a strong response to plant stress and decreased with increasing soil pollution. At the unpolluted site 3 the enclosed aphids produced significantly more offspring than at both polluted sites. This result stands in contrast to one common idea of PSH, that sucking insects such as aphids typically respond positively to stress-induced changes in host plant quality. However, almost all studies on the PSH were confirmed on woody plants and in some studies the reproductive potential of sucking insects was increased by air pollution but reduced by water stress (Koricheva et al. 1998), which seems to be an important stress factor in Steudnitz. Furthermore the monophagous feeding of *M. artemisia* also leads to a close link between the herbivore and its host plant.

A well studied phenomenon is that aphids phenotype can be influenced by abiotic and biotic stress. Bad host plant quality due to drought stress or pollution as well as intra- or interspecific interactions are capable of inducing the production of winged offspring that is capable of long distance migration (Pons & Tatchell 1995; Dixon 1998; Weisser et al. 1999; Muller et al. 2001). This effect had been shown for several aphid species and numerous stressing agents. In the present study, however, soil pollution or induction did not result in wing induction. In contrast, findings like those of McVean & Dixon (2001), who detected up to 40% of alatae in *Acyrthosiphon pisum* (pea aphid) after one week of drought stress, no alatae were found in the *Macrosiphoniella* field experiment and only 2 of the examined 5000 *Cryptosiphum* aphids were winged. However, it has not yet been investigated how frequently wing induction occurs in the species studied. Both herbivore species investigated along the pollution gradient in the field, reacted in a linear way to increasing soil pollution. Herbivore performance on medium polluted soil laid between those on the heavily polluted and the relatively unpolluted soil. This result, however, does not prove a general linear response of the model herbivores on plant stress. Although at site 3 no stress symptoms were evident in the plants, some soil parameters show atypical high concentrations that possibly do have negative effects on plants (see Chapter 2). Therefore a potential unimodal reaction of the investigated herbivores could be existent although it has not been detected. However, regarding the lack of difference of germination, growth and reproduction of *A. vulgaris* at site 3 compared to controls on neutral soil, this possibility seems unlikely.

Reaction pattern of Chorthippus mollis

In terms of survival, growth and feeding behavior the grasshopper *C. mollis* did not react significantly to pollution-related changes in the quality of its food. In general, the behavioral process of feeding can be divided in 3 different stages: palpation, test bite and feeding. When the palps come in contact with the plant and the food is accepted, the grasshopper dips his head and takes a first bite (Lewis 1984; Chapman

1988; Chapman et al. 1991). *C. mollis*, however, did not distinguish between the offered food sources. The feeding behavior observed during the first hour indicated that the animals chose their food purely by chance and did not reject any food. A reason for that could be that due to the starvation for 24 hours before the experiment the grasshoppers were less choosy and accepted the food first offered. Later during the experiment they made a more subtle distinction between the food sources, which then led to differences in consumption. Almost all grasshoppers are, up to a certain degree, generalist feeders on different plant parts from roots to flowers. The Gomphocerinae are specialized on the leaves of grasses. However, *C. mollis* can be seen as generalist feeder with a broad spectrum of host plants. About 20 different grasses and herbs have been described as food source for this species (Kaufmann 1965; Gottschalk 1993). This high degree of generalism also could explain the low effect of food source on grasshopper fitness.

In 24 hour choice tests, however, *C. mollis* consumed significantly more material from the polluted site 1 compared to material from the unpolluted site 3.

A problem in interpreting this results is that the morphology of *Artemisia* plants grown at the 2 sites was different. Branches harvested at the polluted site 1 usually had thicker leaves and shorter internodes than plants from the unpolluted site 3. The difference in consumed material could therefore possibly be due to a difference in the loss of water during the experiment rather than a real difference in consumption.

Another potential source of bias in the choice experiments could be habituation, which sometimes was observed in laboratory trials. The food spectrum of grasshoppers is not fixed; grasshoppers are able to change their food preferences and in experiments finally accept food that was rejected at the begin (Szentesi & Bernays 1984). As all grasshoppers that had been used for choice tests were reared on control material from site 3, this also could have influenced the choice during the experiments. Reproduction was slightly but not significantly lower when the grasshoppers were fed with plant material from the polluted site 1. That observation is consistent with the fecundity of chewing insects often being reduced by plant stress, but growth, survival and colonization not being affected (Koricheva et al. 1998).

MeJA-elicitation decreased fitness and induced resistance in Artemisia

Simulated herbivory by MeJA-induction caused similar fitness costs in *Artemisia* plants as soil pollution. The MeJA-treatment resulted in a significant reduction of growth and reproductive performance of induced plants compared to uninduced neighbors. Similar results were found for other plant species in the absence of herbivores. When an induced and an uninduced *Nicotiana attenuata* plant compete for limited resources, the uninduced plant will produce significantly more seed capsules and will have a higher lifetime fitness than its induced neighbor (Baldwin & Hamilton 2000; van Dam & Baldwin 1998; Baldwin 1998). These fitness costs of MeJA induction that occur even under conditions of natural herbivory indicates that in the study area during the experiment herbivory had only a weak impact on plant fitness. However, the reduction of herbivore pressure by insecticide spraying caused a slight increase of growth of uninduced plants at site 3. These effect of herbivory seems to be caused by the high densities of *M. artemisiae* forming large colonies on the unsprayed plants. Aphids are known to inflict considerable fitness costs in many crop plants (Dixon 1998; Moran & Thompson 2001). The better performance of insecticide treated plants compared to control plants growing nearby may have been enhanced by behavior of this very mobile aphid species (see Chapter 3) that falls of a plant if disturbed and climb it again later: it can therefore switch to nearby growing plants. This behavior can be influenced by induced changes in host plant quality that makes herbivores more mobile, than they normally would be on an uninduced plant (van Dam et al. 2000).

Similarly, all other treatment pairs differed in view of MeJA-induction but not due to other effects. Neither enclosures nor the different levels of herbivory caused any changes in plant performance. Analogous to the influence of soil pollution, the decline of growth rates in induced plants was mirrored in seed production. Additionally the time of flowering was delayed in MeJA-induced plants which also could have negative effects on plant reproduction. It appears that during the study, induction lowered the fitness of *A. vulgaris* by up to 35%.

In contrast to these negative consequences of induction on plant growth the experiments involving herbivory gave evidence for some resistance effects that potentially can have positive influences on plant fitness. Reproductive rate of the aphid *M. artemisiae* on induced plants at the less polluted sites 2 and 3 was significantly reduced compared to that on control plants. The lifetime reproduction as only measured fitness factor for *Chorthippus mollis* showed a clear response on induction and mirrored the results of the aphid experiments. Although the number of all produced eggs per female decreased only slightly, the number of viable eggs was significantly lower within the females bred on induced plant material.

There was no effect of induction on the feeding behavior of the grasshoppers during the first hour but the uninduced plant material from the unpolluted site was preferred to the induced material of the same site. A possible explanation for this difference could be induced change of nutrients, antifeedant or toxic compounds in the plant. However the results of the choice experiments must be interpreted carefully. One possibility is that the grasshoppers really were repelled from the induced material. An alternative explanation could be that the nutritional value of the control material was lower or the concentration of digestion inhibitors higher in control plants, so that the grasshoppers had to feed more to gain enough energy.

On polluted soil the effect of induction and the induced resistance in *A. vulgaris* was similar but less pronounced. The overall effect and the differences between induced and control plants decrease on polluted soil. Induction did not lead to a decrease in growth and seed production of *A. vulgaris* at the most polluted site 1 and no induced resistance to *M. artemisiae* could be detected at sites 1 and 2. Looking at the aphid resistance in a different way, all aphids were affected negatively by soil pollution but on the more susceptible uninduced plants the effect of soil pollution was stronger than on induced plants. These results are similar to that of Björkmann (2000) who found, that aphids on resistant trees gained from drought stress, whereas those on susceptible trees were affected negatively.

Combining the effects of soil degradation and induction on plant fitness and defense, some interesting patterns appear. MeJA-induction had similar effects on fitness and defense of *A. vulgaris* as soil pollution. Both kinds of stress, biotic and abiotic, reduced plant growth and insect performance. However, there was no addition of consequences but rather a weakening of the effects elicited by MeJA-induction on polluted soil. This suggests an interaction between the reactions elicited by MeJA-induction and those incited by soil pollution. It seems probable that the synthesis or signaling of some compounds of induced resistance is disturbed on polluted soil.

Analysis and multivariate patterns of secondary plant compounds

Introduction

Plants defend against herbivores with secondary metabolites

During Co-evolution, herbivores have selected for traits that allow plants to escape, defend or tolerate the fitness consequences of their attack (Crawley 1989; Rausher 1992; Kahl et al. 2000). Defense traits can function constitutively or induced by physical structures, toxins, antifeedants or anti-nutritive compounds. Alternatively, secondary metabolites can be classified according to their chemical structure, into nitrogen-containing compounds, terpenoids and phenolic compounds (Crawley 1989).

Phenolic compounds are aromatic structures bearing one or more hydroxyl groups. Most are polyphenols, having several hydroxyl substituents, one or more of which may be secondarily substituted by methyl or glycoxy groups. Phenolics share a common biosynthetic origin from phenyl-alanine, one of the three amino acids formed from sedoheptulose via the shikimate-pathway which is an integral part of cell and tissue development (Crawley 1989; Waterman & Mole 1989; Matsuki 1996). Phenolics are the only defensive compounds ubiquitous in plants. In herbivores, they can elicit a number of effects. Most phenolic compounds act as feeding deterrents or digestibility reducers (Rosenthal & Berenbaum 1991). They cause oxidative damage and are involved in plant resistance to a large number of herbivores (Appel 1993; Björkmann 2000; Bi et al. 1997; Dreyer & Jones 1981). However, phenolic compounds (e. g. caffeic acid) are also known to stimulate feeding and/or growth in insects (Bernays & Woodhead 1982; Watermann & Mole 1989; Shaver et al. 1998). Asteracea plants and among these especially the members of the genus *Artemisia* comprise a large number of phenolic compounds (Brown et al. 1975; Wollenweber et al. 1989) that are often discussed with respect to antibacterial (Rabe & van Staden 1997), antimalarial (Hernandez et al. 1990; Kohler et al. 1997; Blum et al. 1998; Sahai & Vishwakarma 1998) or antimycotic (Macchioni et al. 1999) implications.

Biotic stress influences pattern of secondary compounds

In addition to the constitutive function of phenolics and the large variation in concentrations that occur within and between individuals, these compounds also play a role in induced defenses (Watermann & Mole 1989). Most inducible responses result from changes in gene expression influencing the biochemical regulation of secondary metabolite synthesis, turnover or transport (Karban & Baldwin 1997). A large number of studies found phenolic compounds among other secondary metabolites to be induced as a result of previous insect herbivory or mechanical damage (Schultz & Baldwin 1982; Rossiter et al. 1988).

Some effects of herbivory can be mimicked by the application of jasmonic acid. This compound has been shown to regulate of secondary metabolites in a number of systems (e. g. Baldwin 1996, 1999, 2001; Thaler et al. 1996; Beale & Ward 1998; Thaler 1999). When applied to plants, both the free acid and the methyl esters are active. Jasmonates can also inhibit growth and promotes senescence, but these effects generally occur more slowly and only at higher jasmonate levels (Gross & Parthier 1994; Creelman & Mullet 1995, 1997).

Abiotic stress influences the pattern of secondary compounds

Apart from biotic induction of phenolics, abiotic factors also can influence the patterns of these compounds (Bolsinger et al. 1992). Plants of the same species vary in phenolic synthesis depending on their environment. Individuals in habitats characterized by low resource levels produce proportionately more phenolics (Bryant et al. 1987; Reichardt et al. 1991). In addition to nutrients deficiencies, other abiotic stress factors also are involved. For instance, glucosinolate (mustard oil glycoside) levels have been shown to be three or four times higher in drought-stressed plants compared to unstressed plants (Louda et al. 1987). Matsuki (1996) found that drought stress and salinity cause an increase in phenolic concentrations. However, phenolic levels can also be lower in stressed plants, when the secondary metabolism is disturbed (Feller 1995; Hakulinen et al. 1995; Lindroth et al. 2000). Apart from changes in the nutritional quality in plants suffering from abiotic stress, the altered production of phenolics, as well as changes in the nutritional quality of plants (see Chapter 4) may also play a role in plant resistance to herbivores. Overall, these changes could lead to a better herbivore performance on plants suffering from abiotic stress in accordance with the plant stress hypothesis (PSH, White 1984) or to a weaker herbivore performance, such as predicted by the plant vigor hypothesis (PVH, Price 1991). An increase in phenolic concentrations in plants suffering from abiotic stress would support the PVH, whereas a decrease would support the PSH.

In natural populations a multitude of stress factors simultaneously affect plants. However, little is known on the effects of multiple stresses. How do different environmental variables interact in changing plant nutritional quality? For example, what is the net effect of simultaneous changes in heavy metals, phosphate and nitrogen levels? So far only a few multivariate studies, particularly related to the complex changes in secondary chemistry caused by anthropogenic stress have been carried out (Zhang & Lechowicz 1995; Tausz et al. 2001). Also, most studies of environmental effects on plant resistance have focused on constitutive defenses. Much less is known about the effects of plant physiological status on short term inducible responses.

The investigated pollution gradient, together with the expected small scale patchiness of soil quality, forms a environmental pattern. Combining this pattern with the distribution of phenolic compounds and induction treatments offers a possibility for the investigation of correlations between single soil parameters and plant defense compound levels with multivariate statistical techniques.

Main questions were:
How does the pattern of phenolic compounds in *A. vulgaris* react on pollution stress and simulated herbivory?
Which compounds possibly play an important role?
Which soil parameters explain differences in secondary metabolism best?

Material and Methods

High Performance Liquid Chromatography

The investigation of a large number of compounds requires a suitable method of purification. Chromatography encompasses a diverse group of methods that are utilized for the separation of closely related components of mixtures. In High Performance Liquid Chromatography (HPLC), the stationary phase is contained within a narrow tube through which the mobile phase is forced under pressure. The components of the mixture to be analyzed distribute themselves between the mobile and the stationary phase in varying proportions. Compounds that interact strongly with the stationary phase migrate very slowly with the mobile phase; in contrast, compounds that are weakly retained by the packing material migrate rapidly with the mobile phase. As a consequence of the differences in mobility between the individual components of a mixture, the sample components are separated into discrete zones that emerge from the column at specific retention times. These bands may be identified qualitatively and analyzed quantitatively using an appropriate detection mechanism coupled with a data recording system.

For assessment of the distribution of small molecular weight phenolic compounds in *A. vulgaris* plants grown at the Steudnitz field sites a HPLC analysis was conducted on extracts of the youngest fully-expanded leaves of each plant. These leaves had been harvested with a razorblade just before the onset of flowering, frozen at –20 °C and then freeze-dried. A 25 mg aliquot of each sample was extracted with 1 ml of methanol-water (1:2) containing 400 µg/ml quercetin as internal standard in a Fast Prep FP120 extractor (Savant Instruments Inc., Q-BIOgene, Heidelberg, Germany) for 2 cycles of 45 seconds at maximum speed. The extract was centrifuged and the supernatant was analyzed on a Varian HPLC equipped with a 9300 autosampler, a 9012 Q ternary pump and a 9050 UV-vis detector. The detector was tuned to 220 nm, a 100 x 4.6 mm, the inertil column (Phenomenex, Aschaffenburg, Germany) had a particle size of 3 µm and the solvent gradient was as follows (Solvent A = 0.25% H_3PO_4 in water, Solvent B = acetonitrile): 0 min.: 100% A, 0% B, 15 min.: 85% A, 15% B, 65 min.: 45% A, 55% B at a flow rate of 0.5 ml/min. Data recording and processing was done with Varian Saturn chromatographic workstation software version 4.5 and Microsoft Excel 97.

A couple of authentic phenolic compounds that were described as common in species of the genus *Artemisia* (Wollenweber et al. 1989; Valant-Vetschera & Wollenweber 1995; Stefanovic et al. 1973, 1982; Yoshikawa et al. 1996) were tested as external standards (see Table 5.1) and used for the identification of single peaks by comparing their retention times and UV-vis spectra. Peak areas of all samples were calculated and standardized for further statistical tests and multivariate analyses. Corresponding peaks in all samples were selected with the program Datatrans (MPI for Chemical Ecology, Jena) and subsequently used for multivariate analyses, when present in at least 2% of the samples. The amount of identified compounds was quantified with both external and internal standards and expressed as µg /g freeze-dried mass.

Multivariate statistics

Multivariate statistical techniques provide a convincing way for the analyzes of complex ecological data. These procedures not only do allow the handling of large datasets by summarizing the redundancy but also the exploration of data sets for patterns and relationships from which hypothesis can be generated and tested. Multivariate methods have become a common technique in the analysis of ecological data. However, most studies combine environmental data with plant or animal samples. Correlations between ecological data and patterns in plant chemistry rarely are analyzed with this method. That is remarkable because HPLC analyses provide large datasets on the distribution patterns of secondary metabolite peaks in differently treated plants, containing a huge amount of information and therefore being predestined for analysis with multivariate statistics.

Ordination comprises a group of techniques with the main purpose of organizing sampling entities along a meaningful continuum (McGarigal et al. 2000). The approach involves condensing the information contained in the original variables into a smaller set of dimensions, keeping the loss of information at a minimum.

Ordination allows the description of relationships between subjects (Samples, environmental parameters, chromatogram peaks representing compounds) and the reduction of multidimensional connections to the most important dimensions. Samples, peaks and environmental parameters are arranged so that similarities between subjects are reflected in their distances in the ordination plot (Jongman et al. 1987). For choosing the appropriate model for the description of the peak distribution, a Detrendend Correspondance Analysis (DCA) was performed. The given dimensionless value for the length of gradient of the first ordination axis (lg) indicates weather the peaks are better fitted by a unimodal (lg >4) or a linear (lg <3) distribution model. In the present study, lengths of gradient invariably were <1.8, therefore analytical procedures based on linear models such as the Principle Component Analysis (PCA) and the Redundancy Analysis (RDA) were chosen.

Principle Component Analysis

To compare peak distribution within all analyzed plants, a PCA was performed. PCA is a method of indirect gradient analysis and allows the evaluation of patterns in the dataset by plotting the samples in the multidimensional space tightened by the peaks. PCA is a sensitive method for the detection of multivariate patterns in the dataset. It constructs a theoretical variable that minimizes the total residual sum of squares after fitting straight lines to the dataset. The graphical representation as a biplot shows the distribution of samples and peaks. Peaks that are plotted near the origin have no clear distribution pattern in the dataset. In contrast, peaks that are plotted on the periphery are suitable for the comparative discussion of different samples (Jongman et al. 1987).

Redundancy Analysis

Additional insights are gained by incorporating soil analyses results (Chapter 2) and performing a direct gradient analysis. This procedure allows investigation of the relationships between different stress factors and the chemical response in the plant. For a linear response model, RDA is the appropriate method. A number of treatments and measured environmental parameters were included in the calculation. In case of high correlations, environmental variables were combined to a synthetic variable (see Table A1 appendix). The simultaneous analysis of peak data and sample parameters allowed to reveal correlations between those. In the RDA plot the parameters are depicted as vectors, the length indicating the relative importance. An important result of the analysis are the eigenvalues (EV) of the ordination axes, indicating which proportion of the data set variance is explained by the corresponding axis. The axes with high eigenvalues do the best job capturing the sample variance structure, while axes with the lowest eigenvalue do the worst. RDA axes were tested for significance by a Monte Carlo Permutation (999 permutations under the reduced model). For the further identification of environmental variables that are good discriminators a forward selection was performed. This procedure allows to select the most useful subset of environmental variables. The quality of the selected variables also was tested by a Monte Carlo test, checking the significance of the relationship between the peaks and the whole set of environmental parameters. All multivariate analysis were performed with the programm package CANOCO 4.5 (ter Braak & Smilauer 2002).

Test statistics

Concentrations of secondary compounds were log-transformed in order to achieve normal distribution. Normal distribution was tested with the Kolmogorov-Smirnov-test, homogeneity of variances with the Levene-test. A repeated measure linear model (repeated-measure ANOVA) was used to test differences between concentrations for significance. Pairing and MeJA-treatment were used as within-subjects factor, site identity entered the analysis as between-subject factor. Huynh-Feldt correction was used in order to avoid inflation of the F-statistics for within subject factors and their interactions due to a potential failure to meet the assumption of sphericity (von Ende 2001). Because of different group sizes and partly missing homogeneity of variances, Games-Howell *post hoc* tests were used to compare between-site differences. Additional paired t-test or Mann Whitney U tests in case of non-normal distribution were performed to compare within-site differences. SPSS 11.0.1 and Microcal Origin 6.0 software were used for statistical analysis.

Results

HPLC analysis

Altogether, leave samples of 230 of the 360 transplanted *Artemisia* plants were collected and analyzed, of which 76 were harvested at site 1, 35 harvested at site 2 and 119 at site 3.

HPLC analysis indicated that all samples contained mainly the same substances, but with quantitative differences. A typical chromatogram contained about 100 peaks. By comparing the peak pattern of all samples with the program Datatrans (MPI for Chemical Ecology, Jena, Germany) 46 of them were chosen that were present in most samples (Fig. 5.1).

Four compounds were successfully identified. Among the tested external standards four had similar retention times as peak 8, 25, 34 and 36 (Table 5.1) and could be verified by comparison of the UV-spectra.

Table 5.1 List of phenolic compounds that were tested as external standards and had been previously recorded in plants of the genus *Artemisia* (Brown et al. 1975; Wollenweber et al. 1989; Wollenweber & Rustaiyan 1991; Valant-Vetschera & Wollenweber 1995; Stevanovic et al. 1982; Yoshikawa et al 1996; Bhakuni et al. 2001) including specification of retention times in HPLC analysis.

Compound tested as external standard	Exact name	known from *Artemisia* spec.	Retention time in HPLC
Caffeic acid	3,4-dihydroxycinnamic acid	+	
Chlorogenic acid	1,3,4,5-tetrahydroxycyclohexane carboxylic acid 3-(3,4-dihydro xycinnamate)	+	19.8
Cinnamic acid	3-phenylacrylic acid	+	
o-Coumaric acid	o-4-hydroxycinnamic acid		31.8
p-Coumaric acid	p-4-hydroxycinnamic acid	+	
m-Coumaric acid	m-4-hydroxycinnamic acid		
Ferulic acid	4-hydroxy-3-methoxycinnamic acid	+	
Gentisic acid	2,5-dihydroxybenzoic acid	+	
Kaempferol-3-glucoside	3-glucopyranoyloxy-4,5,7-trihydroxyflavone	+	29.9
Luteolin	3,4,5,7-tetrahydroxyflavon	+	
Quercetin	3,3,4,5,7-pentahydroxyflavone	+	
Rutin	Quercetin-3-glucoside	+	26.18
Salicylic acid	2-hydroxybenzoic acid	+	
Scopoletin	7-hydroxy-6-methoxycoumarin	+	
Tamarixetin	3,5,7-Trihydroxy-4-methoxyflavonol		

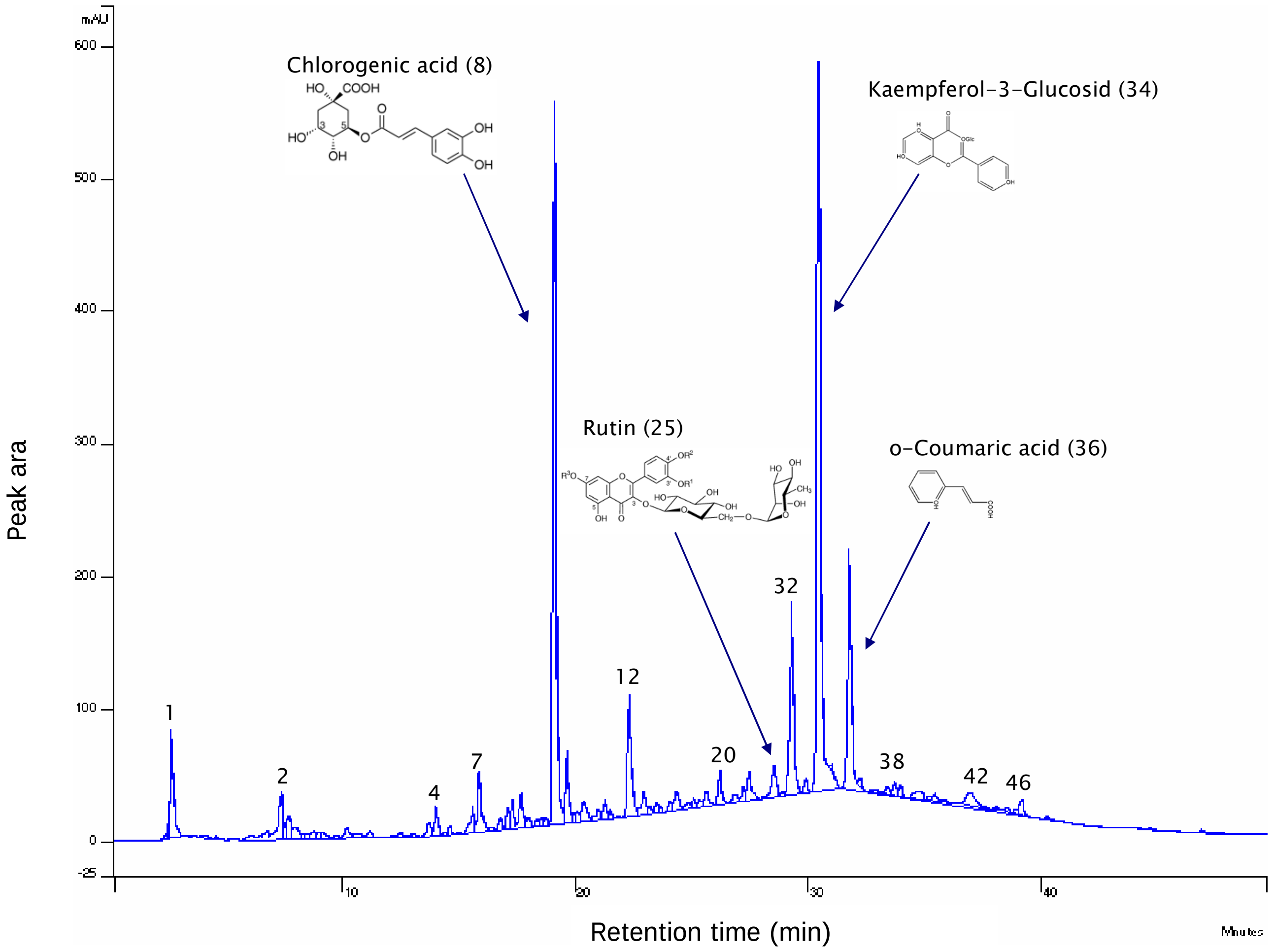

Figure 5.1 Example of a HPLC chromatogram of *Artemisia vulgaris* leaf extracts. Retention time is plotted on the x- and peak area on the y-axis. Unidentified compounds are labeled with numbers. Identified compounds are labeled with names, structural formulas and numbers.

Principle Component Analysis

In a PCA of peak patterns, the first 5 ordination axes explain 50% of the total variance. Axis 1 has by far the highest eigenvalue, whereas axis 2, 3 and 4 explain a relatively small part of the variance (see Table 5.2).

The distribution of investigated plant compounds varies between the sampling sites. Plants grown on polluted soil have a clearly different pattern of phenolic compounds, as the clusters plotted in the PCA indicate (Fig. 5.2). Apart from 2 outlying samples of site 3 and 8 outlying samples of site 1, all samples of site 1 are separated along axis 1 and form a distinct cluster. Interestingly, the outliers are samples from plants with growth rates that differ from the mean of the plants at the corresponding site. Outliers from site 1 had slightly larger and outliers from site 3 slightly smaller growth rates, than the site average (see Table A5 appendix). In the multidimensional cloud of sample points samples from site 1 are plotted closer together than those from site 2 and 3. This indicates a difference in the variance of chemical profiles between plants grown on differently polluted soil. Variance is very low in plants from site 1 and slightly lower in plants from site 2, when compared to those from site 3.

Table 5.2 Characteristics of the first 4 axes of a PCA based on phenolic peak patterns of *Artemisia* samples collected along a pollution gradient.

Axes	1	2	3	4
Eigenvalues	0.268	0.092	0.069	0.063
Cumulative percentage variance of species data	26.8	36.0	42.9	49.2

Some peaks explain a larger proportion of the variance in the pattern than others. Especially peaks represented by long vectors and correlating with axes of high eigenvalues are important for the interpretation of similarity patterns. However, a high number of peaks were plotted near the origin of the coordinates. Besides other reasons, this could be due to low variance in the distribution of these compounds.

Not all compounds could be identified, but among those identified, peak 8 (chlorogenic acid) and peak 34 (kaempferol-3-glucoside) correlate best with the first axis. These two peaks can be seen as proxy for a set of unidentified compounds reacting in a similar way (peaks 2, 5, 7, 32, 40). The vectors of most peaks point in the direction of samples from site 2 and 3. However, some peaks seem to be positively correlated with pollution stress. An example is peak 36, which is negatively correlated with the second axis and absent in samples from the polluted site 1. A strong negative relationship with samples from the polluted site is also evident for peak 37, a strong positive relationship for peaks 21, 26, 28, 31, thus having a lower concentration in plants harvested at the polluted site 1 (see Table A6 appendix).

Most samples from 2 plants of a plant treatment pair are plotted close together, indicating that plants growing close to each other have a high similarity of their compound patterns (compare Fig. A6 appendix). When plotted on axis 1 and 3 the segregation of sample clusters is less clear (Fig. 5.3). Axis 3 is mainly determined by the vector of compound 27, with which it is highly negative correlated. An ordination incorporating axis 4 did not reveal any additional information (Fig. A7 appendix). Induced samples were not separated from control samples along the first 4 axes (Fig. A8 appendix).

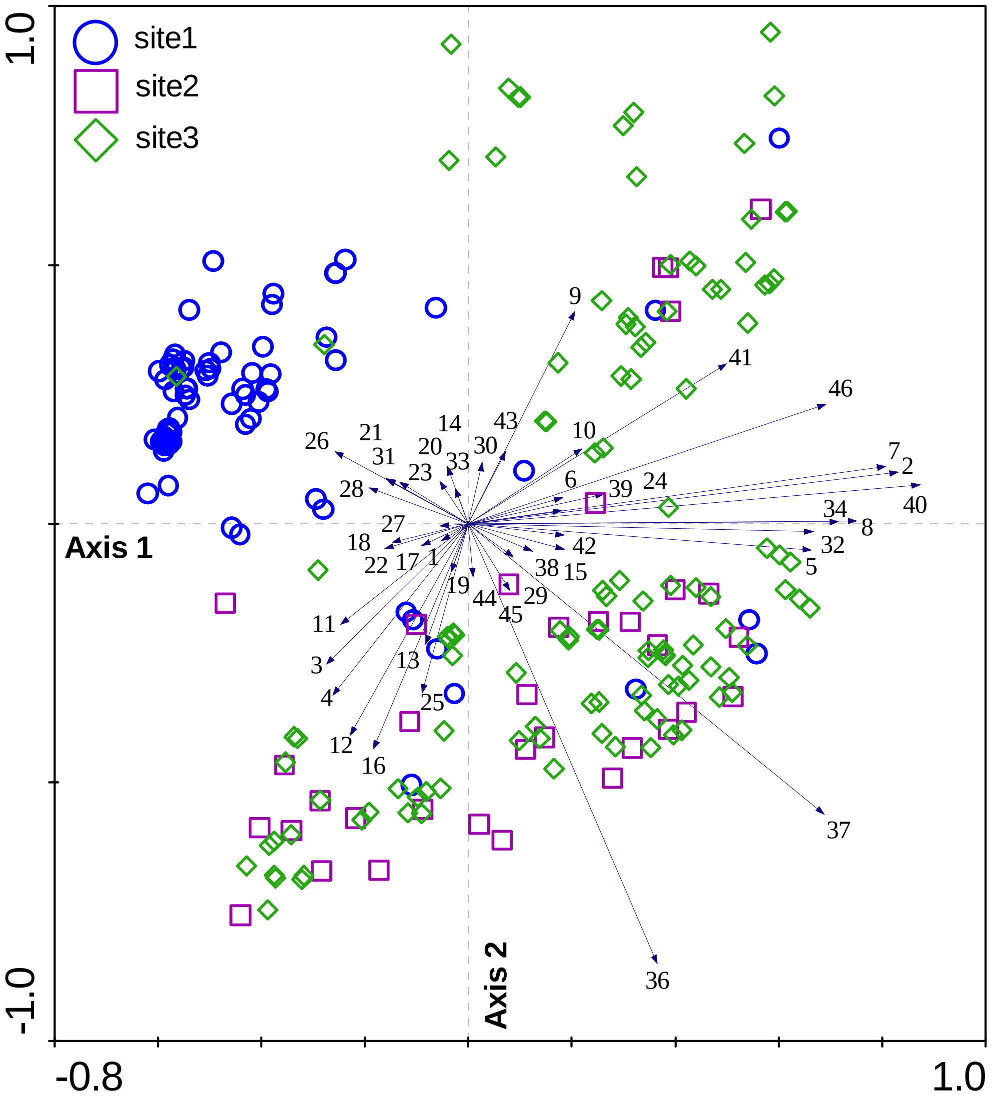

Figure 5.2 Distribution of phenolic compound concentrations (plotted as vectors) in samples of *Artemisia vulgaris* (plotted as symbols) harvested at 3 sites along a gradient of decreasing soil pollution (site 1 - 3) in the ordination plot of a PCA. Axis 1 (EV = 0.268) and axis 2 (EV = 0.092) are presented.

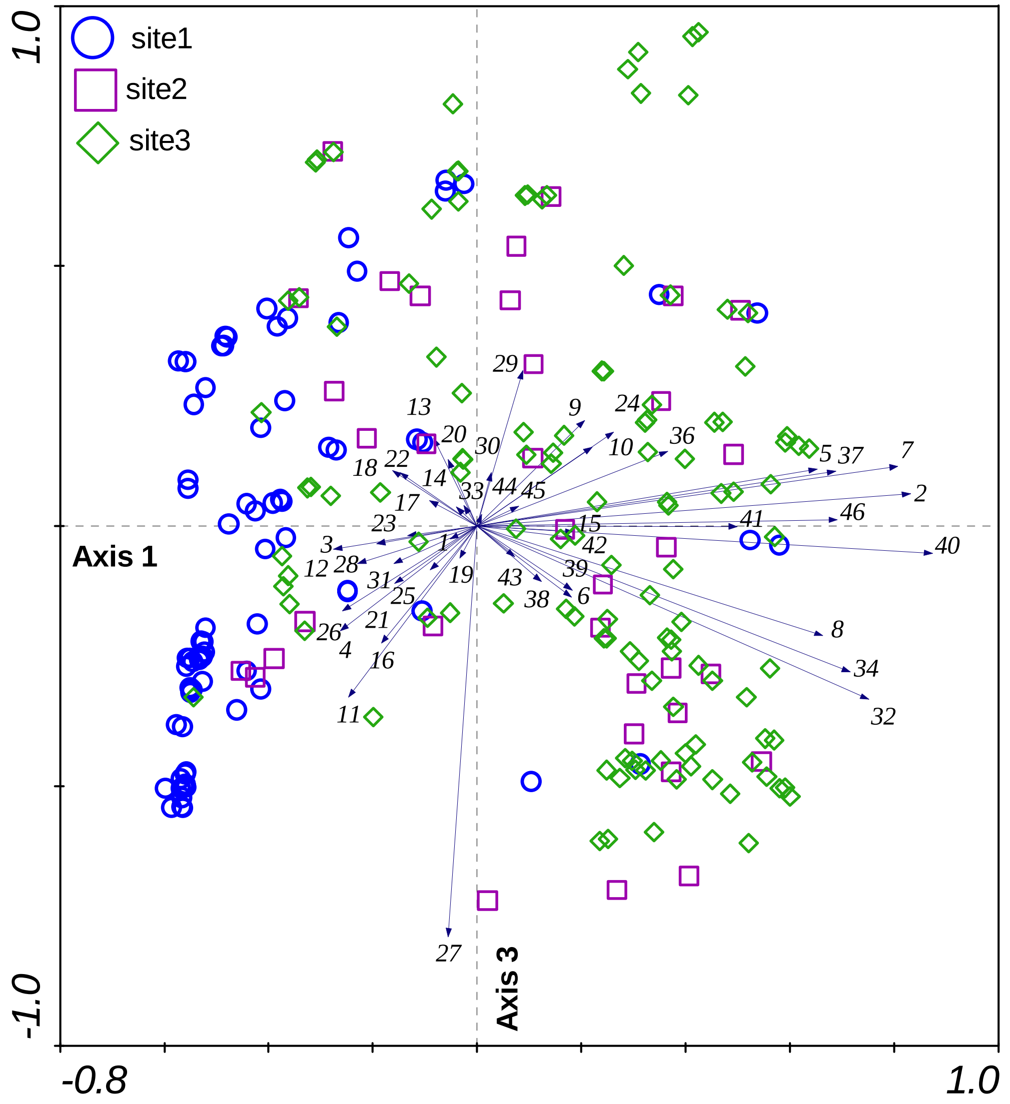

Figure 5.3 Distribution of phenolic compound concentrations (plotted as vectors) in samples of *Artemisia vulgaris* (plotted as symbols) harvested at 3 sites along a gradient of decreasing soil pollution (site 1 - 3) in the ordination plot of a PCA. Axis 1 (EV = 0.268) and axis 3 (EV = 0.069) are presented.

Redundancy Analysis

To extract further information from the dataset, a RDA including additional environmental and treatment parameters was performed. Soil parameters correlating highly significant (*P* >0.01) were combined to new synthetic parameters. Analyzed values for Zn, Pb, Cu and Cd were combined to the synthetic parameter “Heavymetal”. Similarly, a “Ca-mix” parameter was obtained by combining Ca total and $CaCO_3$ (see Chapter 2 and correlation matrices in appendix Table A1). Together with original soil parameters and MeJA-induction as treatment parameter, these synthetic parameters were incorporated in the multivariate analysis.

The first RDA axis explains by far the highest part of the variance, whereas axis 2, axis 3 and axis 4 contain only little additional information (Table 5.3).

A comparison of RDA eigenvalues with PCA eigenvalues allows an assessment of the ability of the chosen environmental parameters for explaining the observed patterns. The RDA of *A. vulgaris* samples with incorporated induction treatment and soil analysis patterns resulted in eigenvalues that were well below those of the PCA (Table 5.3).

Table 5.3 Characteristics of the first 4 ordination axes of a RDA based on phenolic peak patterns of *Artemisia* samples collected along a gradient of increasing soil pollution with incorporated soil and treatment parameters.

Axes	1	2	3	4
Eigenvalues	0.143	0.018	0.010	0.006
Species-environment correlations	0.751	0.441	0.433	0.424
Cumulative percentage variance of species data	14.3	16.1	17.0	17.6
Cumulative percentage variance of species-environment relation	72.8	81.7	86.6	89.6

However, a test for significance of the RDA-axes showed that both the first canonical axis (Eigenvalue = 0.143, F = 35.823, $P = 0.002$), as well as all canonical axes (Trace = 0.197, F = 3.496, $P = 0.002$), are significant and therefore suitable for the interpretation of the dataset. Given the importance of the first axis, those parameters correlating best with this axis explain a large part of the variance and therefore are likely to be mainly responsible for the pattern. In the ordination plot (Fig. 5.4) this is the case for Calcium (Ca-Mix) and heavy metals (heavymet), correlating positively and for total Potassium (K) and the cation exchange capacity (CEC) correlating negatively with axis 1. Axis 2 is primarily determined by the MeJA-induction (MeJA) vector.

The distribution of samples was similar to that in the PCA. Samples from site 1 formed a distinct cluster, whereas samples from site 2 and 3 were arranged together (Fig. 5.4).

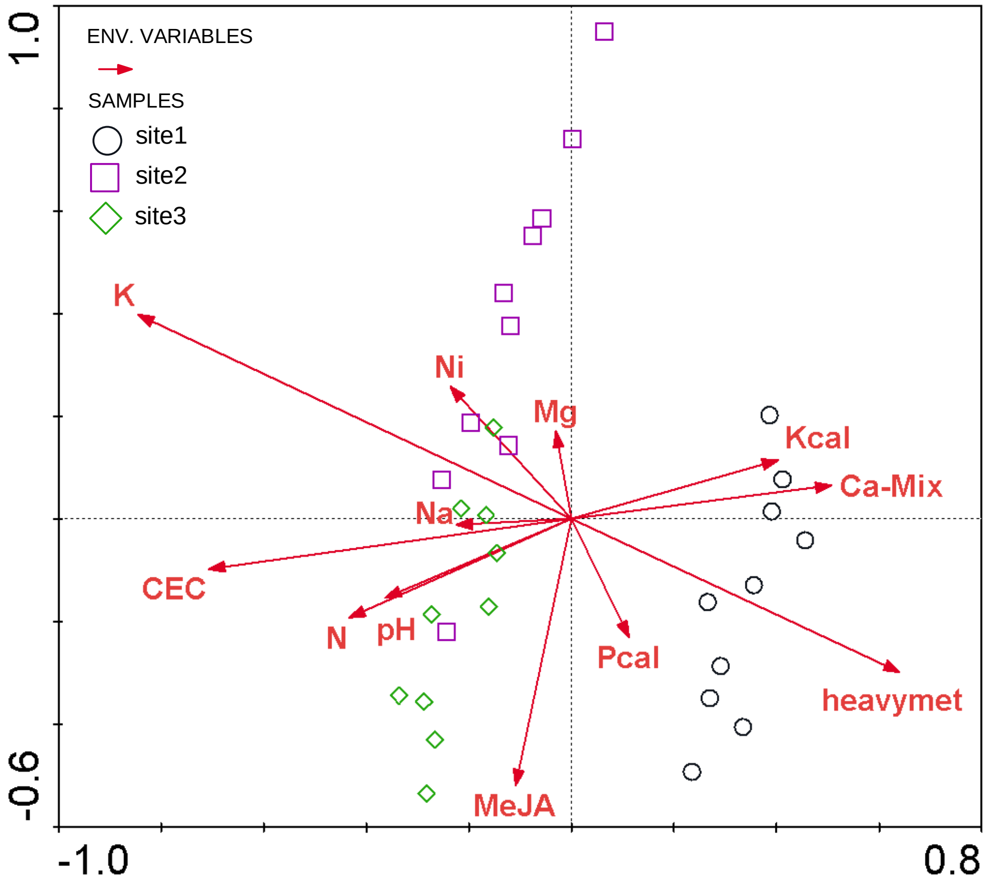

Figure 5.4 Distribution of samples of *Artemisia vulgaris* (plotted as symbols) based on phenolic compound concentrations and environmental and treatment parameters (plotted as vectors) in the ordination biplot of a RDA. Samples were harvested at 3 sites along a gradient of decreasing soil pollution (site 1 - 3). Axis 1 (EV = 0.143) and axis 2 (EV = 0.018) are presented.

The cluster formed by samples harvested at the polluted site 1 is best explained by the plant available phosphate (Pcal) and potassium (Kcal) as well as by the synthetic parameters for Calcium (Ca-Mix) and heavy metals (heavymet) that characterize site 1 samples. Additionally, samples from the polluted site 1 are characterized by a negative association with potassium (K), the cation exchange capacity, Sodium (Na), Nickel (Ni), Nitrogen (N) and pH. Samples from sites 2 and 3 are not clearly separated from each other, but also differ with respect to the correlation with some parameters. Nitrogen, Sodium, pH and MeJA-induction link positively with samples from site 3. Soil contents in Magnesium (Mg) and Nickel explain the plotted positions of site 2 samples. (Fig. 5.4, Correlation coefficients Table A1 appendix).

The associations between different soil parameters plotted in the RDA mirror the results of the tests for correlations (Chapter 2). The synthetic parameter “heavymet” is strongly negative correlated with total potassium content (K); the synthetic parameter Ca-mix is negatively correlated with CEC and phosphate (Pcal) is negatively correlated with Ni and Mg. A strong positive correlation exists between pH and nitrogen and between CEC and sodium.

The biplot of peaks and environmental parameters (Fig. 5.5) allows an additional analysis of correlations between single phenolic compounds and treatment or soil factors. Along axis 1, most peaks are to a higher or lower degree negatively correlated with the soil contents in potassium, phosphate, calcium and heavy metals.

Some single phenolic compounds strongly correlate with soil parameters: For instance the compound represented by peak 2 positively correlates with the cation exchange capacity and negatively with calcium concentrations and plant available potassium. Peak 46 correlates with nitrogen and pH, peak 29 with nickel, peak 34 with sodium and peak 30 with phosphate.

Among the identified compounds some also showed clear correlations with single influencing factors. The compound o-coumaric acid (peak 36) is negatively correlated with the factor heavymet and positively with potassium. Kaempferol-3-glucoside (peak 34) and chlorogenic acid (peak 8) show a negative correlation with Ca-Mix and a positive one with Na and CEC.

No compounds proved a strong correlation with MeJA treatment, however a group of compounds is weakly positively associated with induction (peaks 6, 9, 20, 25, 30, 41).

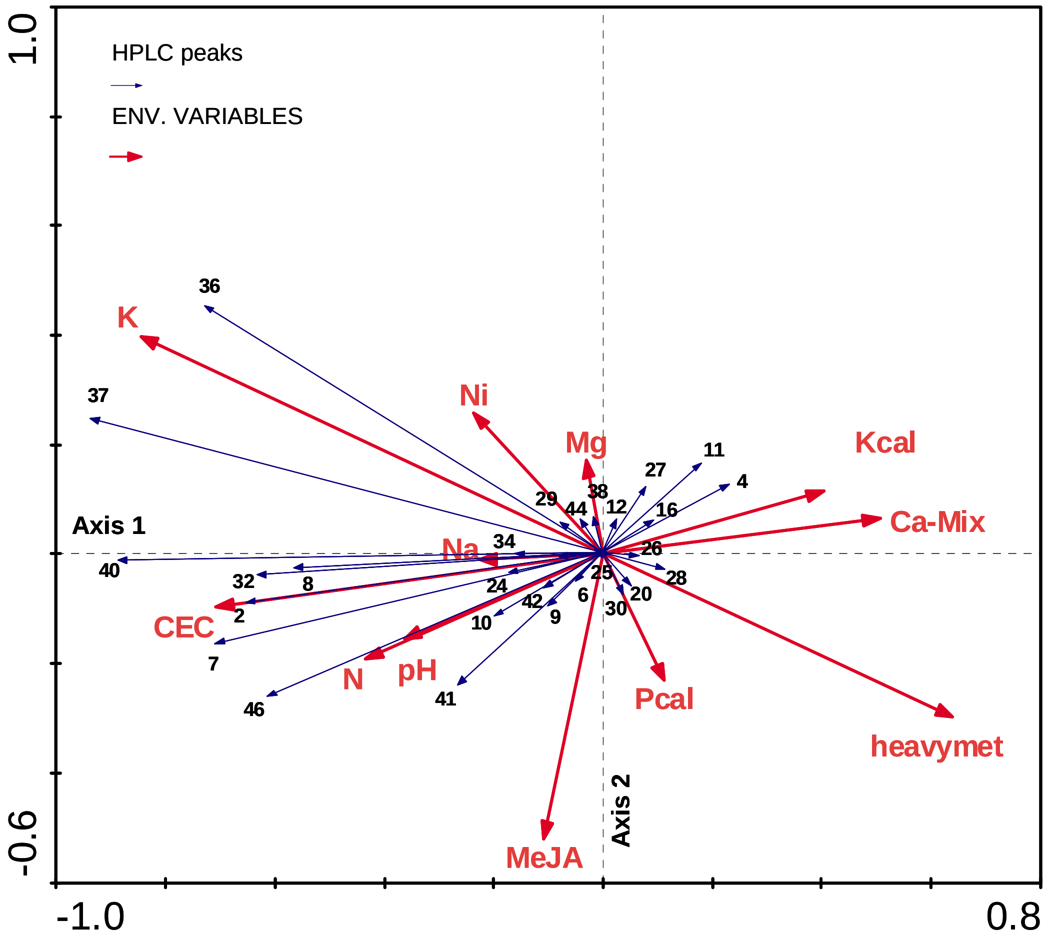

Figure 5.5 Distribution of phenolic compound concentrations of *Artemisia vulgaris* (plotted as vectors) and environmental and treatment parameters (plotted as vectors) in the ordination biplot of a RDA. Samples were harvested at 3 sites along a gradient of decreasing soil pollution (site 1 - 3). Axis 1 (EV = 0.143) and axis 2 (EV = 0.018) are presented.

To reveal the relative importance of the incorporated treatment and environmental parameters, a forward selection followed by a Monte-Carlo Permutation test was performed. The effect of potassium, CEC, heavy metals and MeJA-induction were found to be most important, having a significant relationship with the whole set of peaks (Table 5.4).

For the identified compounds the effects of MeJA-treatment and soil pollution were quantified and statistically tested (Fig. 5.6 - Fig. 5.9)

Table 5.4 Results of a forward selection with Monte-Carlo Permutation-test of 999 permutations of the RDA.

Parameter	Selected	F-value	P-value
Potassium (K)	1.	13.48	<0.001
Cation Exchange Capacity (CEC)	2.	4.42	<0.001
Heavy metal (heavymet)	3.	2.93	<0.001
MeJA-Induction (MeJA)	4.	2.21	0.002

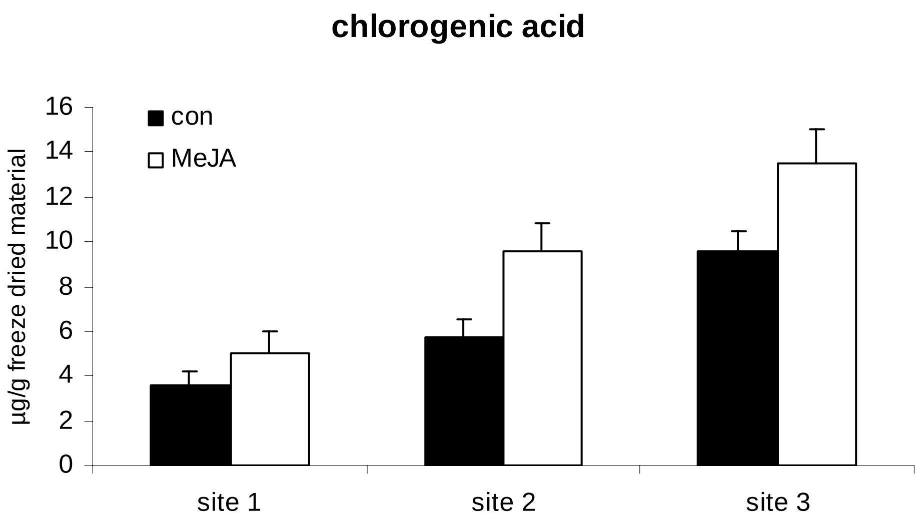

Figure 5.6 Mean (+SE) concentrations of chlorogenic acid in *Artemisia vulgaris* leave extracts from MeJA-induced (MeJA) and control (con) plants harvested at 3 sites of decreasing soil pollution (site 1 - site 3).

Mean concentrations of chlorogenic acid reach from 3.58 µg at site 1, to 13.54 µg at site 3 (Fig. 5.6). There was a significant effect of site and MeJA-induction on the concentration of chlorogenic acid (Repeated-measure ANOVA, site: F_2 = 19.795, *P* <0.001, induction: F_2= 13.176, *P* = 0.02, site x induction: F_2= 1.141, *P* = 0.327). With increasing soil pollution chlorogenic acid concentrations decreased. Concentrations between all sites differed significantly (Games-Howell *post-hoc*, all *P*s <0.044). Induced plants at all sites had a higher content of chlorogenic acid. However paired tests found only at site 3 significant differences between induced and control plants (paired *t*-test, t_{23} = -2.779, *P* = 0.011) but not at sites 1 (Wicoxon Signed Rank test, W = 136, T+ = 218, T- = 82, *P* = 0.054) and 2 (paired *t*-test t_{11} = 2.042, *P* = 0.066).

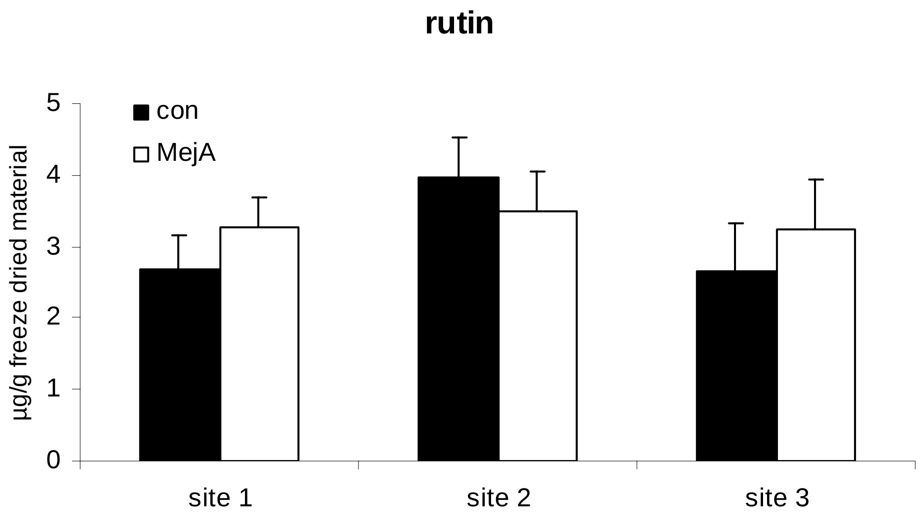

Figure 5.7 Mean (+SE) concentrations of rutin in *A. vulgaris* leave extracts from MeJA-induced (MeJA) and control (con) plants harvested at 3 sites of decreasing soil pollution (site 1 - site 3).

Mean concentrations of rutin ranged between 2.66 and 3.97 µg (Fig. 5.7). No clear effect of site or induction could be shown (Repeated-measure ANOVA, site: F_2 = 0.685, P = 0.508, induction: F_2= 0.213, P = 0.647, site x induction: F_2= 0.26, P = 0.772). In paired t-tests this compound also did not significantly respond to the treatments (all P-values >0. 368).

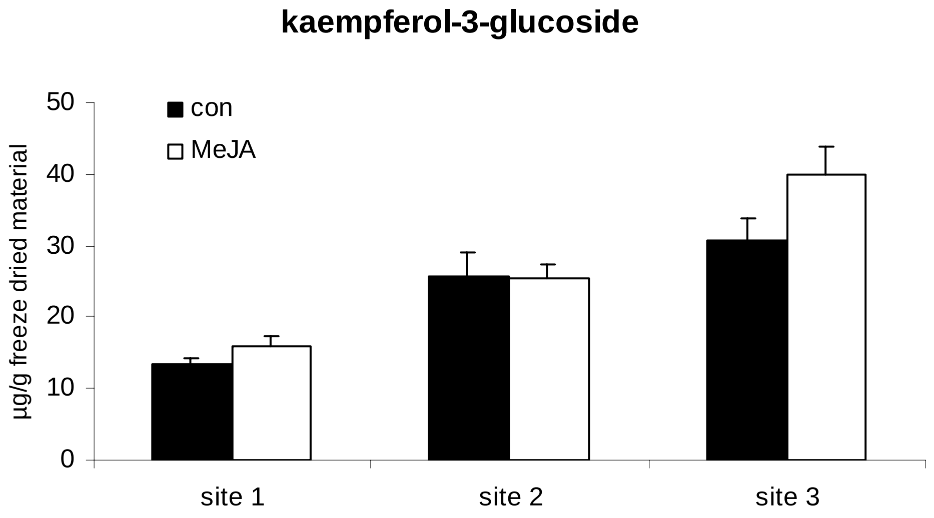

Figure 5.8 Mean (+SE) concentrations of kaempferol-3-glucoside in *Artemisia vulgaris* leave extracts from MeJA-induced (MeJA) and control (con) plants harvested at 3 sites of decreasing soil pollution (site 1 - site 3).

Mean concentrations of kaempferol-3-glucoside ranged from 13.36 µg at site to 39.97 µg at site 3 (Fig. 5.8). A repeated-measure ANOVA showed effects of site and of induction (Repeated-measure ANOVA, site: $F_2 = 27.578$, $P < 0.001$, induction: $F_2 = 4.891$, $P = 0.031$, site x induction: $F_2 = 1.662$, $P = 0.199$). Concentrations differed signigicantly between all sites (Games-Howell *post-hoc* test, all *P*-values <0.001). Paired tests found only at site 3 a trend of induced increase in kaempferol-3-glucoside (paired *t*-test, $t_{23} = -1.936$, $P = 0.065$) but not at site 2 (paired *t*-test, $t_{11} = -0.06$, $P = 0.861$) and site 1 (paired *t*-test, $t_{23} = --1.843$, $P = 0.078$).

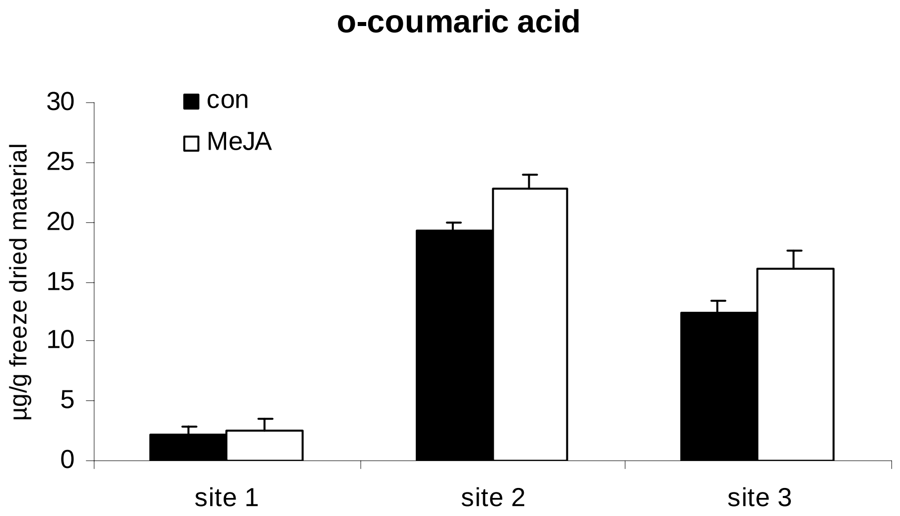

Figure 5.9 Mean (+SE) concentrations of o-coumaric acid in *Artemisia vulgaris* leave extracts from MeJA-induced (MeJA) and control (con) plants harvested at 3 sites of decreasing soil pollution (site 1 - site 3).

Concentrations in o-coumaric acid were much lower at the polluted site 1 (mean 2.17 – 2.5 µg) compared to site 2 and 3 (means from 12.42 – 22.72 µg). Highest values were reached at the medium polluted site 3 (Fig. 5.9). This effect of site was significant as well as an effect of induction (Repeated-measure ANOVA (site: $F_2 = 11.015$, $P < 0.001$, induction: $F_2 = 22.211$, $P < 0.001$, site x induction: $F_2 = 11.161$, $P < 0.001$). Games-Howell posthoc test found significant higher concentrations at site 2 compared to site 1 and 3 (all *P*-values <0.001). The mean values of o-coumaric acid at sites 2 and 3 were higher in induced than in control plants. However, no significant MeJA-induced increase in concentrations could be confirmed by paired t-tests (all *P*-values >0.23).

Discussion

Secondary metabolites

Secondary plant compounds often inhibit growth and development of insects and can even cause mortality (Todd et al. 1971; Isman & Duffey 1982; Manuwoto & Scriber 1986; Bryant et al. 1987; Lindroth et al. 2000; Kelly & Curry 1991; Matsuki & MacLean 1994; Hemming & Lindroth 1995; Ayres et al. 1997). As feeding inhibitors and deterrents, they also frequently influence insect behaviour (Kraft & Denno 1982; Matsuda & Senbo 1986; Bernays et al. 1991; Kelly & Curry 1991; Mori et al. 1992; Gross & Hilker 1994, 1995; Van Dam et al. 2000). Overall, many insect herbivores may continuously face optimization of capturing essential nutrient while avoiding harmful secondary compounds (Rhoades & Cates 1985; Cates 1980; Meyer & Montgomery 1987; Van Dam et al. 2000).

A main group of secondary metabolites, the phenolic compounds, can have a multitude of possible functions in the plant e. g. the protection against environmental stress (Felton et al. 1992; Close & McArthur 2002) or against bacteria and fungi (Harborne et al. 1976; Waterman & Mole 1989). However, a primary effect seems to be that of anti-herbivore defense (Horwath & Stamp 1993).

A further feature of phenolics and other secondary metabolites is that increases in concentration may be induced dynamically in response to mechanical damage, herbivore feeding or the application of jasmonic acid, a compound that can elicit defense reactions (Karban & Baldwin 1997; Baldwin 1999, 2001).

Several hypotheses deal with environmental influences on the defense capabilities of plants (Lombardero et al. 2000). Models explaining environmental patterns in plant secondary metabolism include carbon nutrient balance (Bryant et al. 1983), growth differentiation balance (Lorio 1986; Herms & Mattson 1992), optimal allocation (Tuomi et al. 1990) and the plant stress hypothesis (White 1984). The plant stress hypothesis in its general form predicts a better performance of herbivores on host plants suffering from abiotic stress caused by an increase of nutritional quality and a decrease of defensive capabilities in such plants. In contrast the plant vigor hypothesis predicts a lower herbivore fitness on stressed slow growing plants. In addition to this abiotic stress, possibly leading to an altered pattern of defensive compounds, biotic stressing agents such as herbivores are also able to induce changes of plant secondary metabolites.

Phenolic patterns in Artemisia respond to soil pollution and MeJA-elicitation

In the present study, the investigated species *Artemisia vulgaris* revealed a diverse pattern of phenolic compounds, which was not static. The distribution and the variance of phenolics changed with altered soil quality and after simulated herbivory.

Four different compounds could be identified, of which 3 had been found in previous studies on *Artemisia* species (Brown et al. 1975; Wollenweber et al. 1989; Rodriguez et al. 1972, Valant-Vetschera & Wollenweber 1995; Stevanovic et al. 1982; Yoshikawa et al. 1996; Bhakuni et al. 2001). The presence of kaempferol-3-glycoside, chlorogenic acid and rutin and of o-coumaric acid could be proven with the techniques applied in this study, the latter, however, was not yet described for *Artemisia spec.*

Principle Component Analysis

PCA analysis revealed a high variance phenolic compounds patterns, being responsible for the relatively low eigenvalues of the ordination axes. The first 4 axes explained only 50 % of the whole variance, which, however, is acceptable for datasets of chemical compounds, usually having a high variance. Both multivariate analyses, PCA and RDA clearly separated the most polluted study site 1 from the less polluted study sites 2 and 3 along the first ordination axis, which explained the largest part of the variance in the dataset. This pattern indicates major changes in the production of phenolic compounds at the highly polluted site 1. Sites 2 and 3 were only weakly separated along the second ordination axis. The chemical profile of plants growing at these sites is therefore similar.

Additionally, the variance of compounds at the polluted site 1 was lower than at the two less polluted sites. This loss of diversity in the patterns of phenolics occurring in stressed plants could become important with respect to the moving target theory (Adler & Karban 1994) predicting an increased herbivore resistance in plants with highly variable patterns of secondary metabolites. Even a relatively small qualitative variation in plants usually has a great effect on the behavior and physiology of insect herbivores (Larsson and Björkman 1993; Bryant et al. 1987; Meyer & Montgomery 1987; Bingaman & Hart 1993; Matsuki & MacLean 1994; Hemming & Lindroth 1995; Van Dam et al. 1995; Orians et al. 1997). This phenomenon could also be one of the reasons for a better herbivore performance on stressed plants and would therefore support the plant stress hypothesis.

The concentration of 3 of the 4 identified compound and most of the unidentified compounds decreased with increasing soil pollution in concentration. Nevertheless, some unidentified compounds were positively correlated with soil pollution (e. g. peak 26). Depending on the possible effect of a particular compound on herbivores this could lend support to both theories (PSH and PVH). The main pattern, however, together with the fact that phenolics in most cases have negative effects on herbivores, would support the predictions of the PVH.

Obvious changes in secondary metabolite patterns occurred in plants grown on polluted soil. However, the analyzed phenolic compounds reacted differently on the influence of pollution stress. According to the PCA plot, concentration of three of the four identified and most of the unidentified compounds was negatively correlated with soil pollution. For instance chlorogenic acid (peak 8) and kaempferol-3-glycoside (peak 34) correlated strongly with first ordination axis and are less important in samples from site 1.

Samples from 2 plants of a plant treatment pair were usually plotted closely together and therefore had a similar pattern of phenolic compounds. This indicates that the paired experimental design was the correct approach to cope with the existing smale scale patchiness of soil quality. Apart from the soil, microclimatic effects also could have been responsible for this similarity.

The different growth rates of outlying samples compared to samples lying within the clusters re-emphasize that growth is an important influence explaining a part of the variance in the metabolite pattern.

Redundancy Analysis

The eigenvalues of the ordination axes in RDA were much smaller compared to PCA leading to the conclusion, that the included soil parameters and treatments do not explain a high portion of variance in the dataset. Other, not included environmental parameters also seem to have a strong impact on *Artemisia* secondary metabolite patterns.

Factors influencing the distribution of phenolics

In contrast to the general decrease of phenolics in pollution stressed plants, for most of the identified compounds an increase could be seen as result of the induced defense reactions. However, the general pattern of phenolics was less influenced by induction than by soil quality. In both, PCA and RDA, there was no clear separation between plants resulting from MeJA-induction, although a RDA with forward selection proved a significant impact of MeJA induction on secondary metabolite patterns. Induction treatment therefore rather leads to a quantitative change in compound concentrations, whereas soil quality caused qualitative changes as well. This comparison between the importance of environmental caused variation and the variation caused by simulated herbivory shows that in *A. vulgaris* the soil quality had a much higher impact than induction. Similarly, in *Betula pubescens,* the factor with the greatest influence on total phenolics was the environment (nutrients and light), whereas genotype and damage played a minor role (Ruohomäki et al. 1996). In contrast, studies on the mean concentrations of Xanthotoxin in the foliage of *Pastinaca sativa* found the most dramatic changes to be a function of damage, followed by ontogenetic changes, whereas light and nutrients had only small effects (Zangerl & Berenbaum 1990, Li et al. 2000, Harrison et al. 2001). In *Plantago lanceolata* (Fajer et al. 1992) and *Betula pendula* (Keinanen et al. 1999) the genotype seems to be the most important factor determining secondary metabolites, environment and damage explained only a small part of the variance.

Interpreting the RDA biplots, phenolic patterns can be more precisely linked to single soil parameters. According to these analyses the distribution of compounds at site 1 is mainly determined by relatively high levels of calcium, phosphate and heavy metals and low values of CEC, total potassium, magnesium, sodium, nickel, pH and nitrogen. The peak pattern at site 2 is primarily explained by the concentrations magnesium and nickel. Site 3 peak patterns are determined by CEC, nitrogen, sodium, pH and MeJA.
The importance of the induction treatment for the distribution of phenolic compounds at site 3 reveals that the induction effect on phenolic compounds at this site is higher than at the two more polluted sites.

The environmental parameters whose vectors were longest and strongly correlated to the first axis explained a large part of the variance. These were heavy metals, total potassium and the cation exchange capacity. The forward selection procedure confirmed this finding. Apart from MeJA, being correlated to the second axis, the parameters K, CEC and the synthetic parameter heavymet (calculated from Cd, Pb and Zn) were explaining a significant part of the variance in the peak pattern.

The RDA results allow ranking of soil parameters according to their influence on secondary chemistry in *A. vulgaris*. The high concentrations of heavy metals and potassium are the main cause for changes in phenolic metabolites. It can be assumed that these environmental factors also affect other aspects of the plants physiology and therefore represent the main stress agents for *A. vulgaris*.

Response of particular phenolic compounds

Some compounds strongly correlate with single soil parameters, suggesting a linkage between single environmental influences and the production of a particular secondary compound. For instance, o-coumaric acid represented by peak 36 is negatively correlated with the factor heavymet and positively with sodium. This indicates a suppression of this compound in plants deficient in K or growing on soil with increased heavy metal levels.

The reaction of identified compounds on soil quality and induction treatment uncovered different patterns. Each of the 4 identified compounds can be seen as a proxy for a group of compounds reacting in a similar way.

The compound rutin can be considered as representative for a group of other phenolic compounds that were plotted near the origin. The concentrations of these compounds in *A. vulgaris* was not affected by MeJA induction or differences in soil quality. This result contradicts findings of Wilkens et al. (1996), who showed a relationship between soil nutrient content and the concentrations of rutin and chlorogenic acid in tomato. They found phenolic contents to be highest at intermediate nutrient levels. Depending on the definition of “intermediate nutrient level”, their results could be transferred to the other 3 identified compounds. Nutrient supply is difficult to asses in Steudnitz, because of the combination of high phosphate concentrations and low nitrogen levels near the emission source. Interactions between these two main nutrients, however, could explain the pattern.

Levels of o-coumaric acid that in previous studies has been shown to cause oxidative stress leading to reduced growth rates in lepidopteran larvae (Summers & Felton 1994), are highest at the medium polluted site 2. Chlorogenic acid and kaempferol-3-glucoside are present in highest concentrations at the unpolluted site 3. Chlorogenic acid, an ester of caffeic and quinic acid is one of the major products of phenylpropanoid metabolism in vascular plants (Mølgaard & Ravn 1988). It is often used as a model phenolic in the study of plant anti-herbivore defence, due to its ubiquitous occurrence among terrestrial plants and well-documented toxicity to insect herbivores (Cole 1985; Friedman 1997a, 1997b; Hoover et al. 1998). In the insect it promotes lipid peroxidation, oxidation of proteins and the release of free iron in the midgut, resulting in oxidative damage (Felton et al. 1992). In contrast to the present study chlorogenic acid levels are known to increase in a variety of plant tissues in response to a wide range of environmental stresses. However, the soil at site 3 still has high phosphate concentrations and it is possible, that under a lack of nutrients the concentrations would further decrease.Together with kaempferol-3-glucoside, chlorogenic acid could therefore be responsible for potential beneficial effects of soil pollution on herbivore performance, which had been found in studies supporting the PSH. However, Swiatek et al. (1998) found a chlorogenic acid content of 14.6 µg/g dried plant material in not explicitly stressed naturally grown *Artemisia vulgaris*, which is comparable to the upper range in the present study. In the present study, an amplified toxic effect on herbivores caused by exceptional high concentration should therefore not be expected.

Induction also affected the concentration of identified compounds in several ways. Chlorogenic acid concentrations increased significantly in MeJA-induced plants, however this effect was only significant at the unpolluted site 3. Kaempferol-3-glucoside and o-coumaric acid also tended to increase in induced plants. The general effect of an increase in phenolic compound as reaction to herbivory or induction with methyl jasmonate can therefore be confirmed by the findings in *A. vulgaris*.

However, regarding the different reaction patterns of particular phenolic compounds to abiotic stress and induction, the common technique of testing total phenolic content (e. g. Kainulainen et al. 1993; Hakulinen et al. 1995; Inderjit & Foy 1999; Willis et al. 1999) seems not to be suitable for the investigation of plant defense.

The reaction of the 4 identified compounds partly indicated interactions between soil pollution and MeJA-induction. Miles et al. (1982) found that phenolic content increased in control plants after attack, but not in water-stressed plants and at temperatures above 30 °C. This pattern of decreasing induction effects with increasing abiotic stress is consistent with the findings for a group of compounds in the present study represented by chlorogenic acid.

Summarizing the results of chemical and multivariate analyses of secondary compounds in *A. vulgaris*, it is obvious that the pattern of leaf chemistry does react to pollution and induction in a complex way. Heavy metals, potassium and sodium concentrations in the soil, together with induction treatment, explain best the distribution of phenolics. With respect to the theories of plant stress and herbivore performance, the analyzed compounds would support the PVH rather than the PSH.

Furthermore, the results show that multivariate methods offer a suitable tool for the investigation of correlations between secondary metabolite patterns and environmental or treatment influences.

General discussion

Soil pollution in Steudnitz

An increasing number of studies have investigated indirect effects of anthropogenic pollution on plant-insect relationships (Bolsinger et al. 1992; Koricheva et al. 1996; Masters & McNeill 1996; Redak et al. 1997; Zvereva et al. 1997a; Lappalainen et al. 1999; Zvereva & Kozlov 2000). Although soil is the most important mediator between a plant and its environment and therefore has an impact of exceptional importance on plant physiology, industrial emissions contaminating soil have attracted attention in only few studies (e. g. Perner et al. 1996.). Soil analyses revealed that soil in Steudnitz is heavily degraded and probably many factors are responsible for plant stress. Soil quality varies along a gradient between sites, and within sites it is locally patchy. Phosphate, high salinity and heavy metal concentrations partly attain levels of toxicity and, together with a low soil nitrogen content, seem to be the most important factors stressing plants in Steudnitz. The degraded soil composition not only causes occasional water stress but also nutrient stress due to leaching effects. These conditions are likely to influence the plants’ metabolism, and in turn influence the plants’ vigour and growth (Fogal et al. 1999, 2002). For these reasons the Steudnitz field site offers ideal conditions for the study of plant-herbivore interactions under the influence of abiotic stress.

A. vulgaris is a suitable model plant

Artemisia vulgaris has many characteristics that make it suitable for experiments to investigate the influence of soil pollution on plant defense against herbivores. *A. vulgaris* is one of the species in Steudnitz that tolerates degraded soil conditions (Heinrich 1984) and, in my experiments, showed high survivorship even on the most polluted site. Its phenology is convenient for fitness measurement and it has a wide range of secondary metabolites that are potentially defense-related. Although it is a food source for many herbivores, few studies have focused on *Artemisia* species in the context of plant-herbivore interactions (e. g. Strauss 1987).

Soil pollution affects plant fitness

As plant fitness is a difficult quantity to measure in the field, most studies investigating plant fitness are limited in their assessment. I covered a wide spectrum of the *Artemisia* life cycle by measuring germination, survival, growth, seed production and flowering phenology in *Artemisia* plants and found that growth rate can be used as a proxy for overall fitness. A general phenomenon in stressed plants is slower cell growth; this in turn leads to smaller structures e. g. buds, leaves and shoots (Kozlowski 2000; Kozlowski & Pallardy 2002). This reaction may be due to a plant's response to the majority of environmental stressors by producing more abscissic acid and fewer cytokinins, which in turn reduces growth rate (Chapin 1991) and presumably increases plant survival under stressful conditions. Furthermore, Foggo & Speight (1993) suggested that plant stress should be defined as a decrease in plant growth and reproduction.

Artemisia growth and fitness were markedly lower at the polluted sites in Steudnitz. Therefore I can clearly rule out the possibility that *A. vulgaris* did not suffer from stress. My field and laboratory experiments demonstrated that increasing levels of soil pollution at sites 1 and 2 were associated with decreased germination rates, growth and reproduction of *A. vulgaris*. The conditions at the relatively unpolluted site 3 provided close to optimal conditions for germination and growth, comparable to germination in neutral sand and growth under optimal glass house conditions. To control for undetected genotypic variance in my experiments I used a uniform seed source. Germination patterns failed to detect any maternal effect of MeJA-induction on the next generation or any adaptation to soil conditions. These results therefore are likely to be representative for the *Artemisia* population in Steudnitz.

The interaction of biotic and abiotic stress

Apart from abiotic stress, plants must fend off a myriad of aggressive biotic agents during their lifetime, and both factors often interact in their effect (Kahl et al. 2000). The impact of abiotic stress influenced many theories on plant defense. The main focus, however, was on the interaction between nutrient availability in the soil and plant defense. For example the Carbon Nutrient Balance Theory (Bryant 1983) predicts an increase in phenolics under nitrogen deficiency and, more generally, the Resource Availability Hypothesis (Coley et al. 1985) expects that both the quantity and identity of defenses produced by plants will depend on the availability of resources.

The Plant Stress Hypothesis generated by White (1984) focused on the phenomenon that herbivores often reach higher fitness when their host plants suffer from abiotic stress and explained it by a higher content of nitrogen being available in stressed plants. Rhoades (1979) complemented this argument by noting the likelihood of stressed plants being less able to synthesize defensive chemicals. However the opposing Plant Vigor Hypothesis (Price 1991) predicts a lower herbivore performance on stressed plants. Some authors (Larsson 1989; Koricheva et al. 1998) tried to integrate the results of many studies on plant-herbivore interactions and found that, in their original form, neither hypothesis seemed to be generally true. Plant stress has no overall effect on insect performance; the two hypotheses rather represent two opposite ends of a wide spectrum of insect responses to plant stress with a multiplicity of parameters influencing the final outcome. The results of my experiments, that tested the influence of plant-mediated pollution effects on feeding pattern of herbivores varying according to feeding guild and level of specialization, confirm some of the predictions, but not all. A reason for the mismatches could be that almost all studies of the PSH have been carried out on woody plants, which may react differently from ruderal plants and that many authors suggest that herbivores will respond non-linearly to plant stress (English-Loeb 1989; Willis et al. 1993; Koricheva et al. 1998).

Feeding guild and host plant specialization do not explain resistance patterns

In previous studies, the feeding guild of the herbivorous insect was identified as being the most important determinant of the effects of abiotic stress on second trophic level feeders: sucking insects typically respond in accord with the PSH, whereas chewers respond in accord with the predictions of the PVH (Larsson 1989; Koricheva et al. 1998). Therefore I expected to find increased aphid performance on the stressed plants of site 1 in comparison to the unstressed plants of site 3, and the opposite pattern of performance for the grasshopper *Chorthippus mollis.* The grasshopper was suitable for choice and performance tests as in preliminary trials it could be reared on *Artemisia vulgaris*. It reacted in the expected way with an decreased reproduction on polluted sites. However, the measured fitness parameters of the aphid species *Macrosiphoniella artemisiae* and *Cryptosiphum artemisiae* similarly showed a decline at the most polluted site. This is not a surprising finding for the gall forming aphid *Cryptosiphum*, as gall formers are most intimately associated with the processes involved in plant growth and therefore are expected to conform to the Plant Vigor Hypothesis more closely (Price 1991). Additionally, Jones & Coleman (1991) suggested that insects that specialize on only a few plant taxa should be limited in their physiological plasticity, and therefore should be able to respond to altered plant characteristics only over a narrow range. In contrast, generalists may show greater behavioral plasticity and may therefore be able to handle a broad range of nitrogen and secondary metabolite concentrations. However this pattern could not be confirmed by my experiments: the generalist grasshopper *C. mollis* reacted to plant stress with a similar decrease in reproduction as exhibited by the aphids. Furthermore, *C. artemisiae* was negatively influenced only in colonization rate, but not in performance.

Herbivore traits and pollution type explain resistance pattern

Neither feeding guild nor host-plant specialization explain the outcome of herbivore performance in Steudnitz, however some patterns were evident when I considered herbivore traits and the factors causing pollution stress in Steudnitz. Koricheva et al. (1998) found that herbivore traits can be diversely affected by host plant stress. Survival and colonization of gall-forming insects are negatively affected by plant stress, as is the reproductive rate of chewing insects, whereas other traits are not influenced or even show a positive reaction. If and how a herbivore is affected can also depend on the type of stress a plant is exposed to. Some pollution types enhanced fecundity in suckers but not chewers, as shown by an increased population growth, whereas water stress tended to decrease the fecundity of both herbivore types (Koricheva et al. 1998). The few manipulative studies on the relationship between drought stress and sucking insects that were conducted outside the laboratory showed, without exception, that host plant drought-stress decreases aphid abundance (Michels & Undersander 1986; Oswald & Brewer 1997). It is likely that, during drought periods, water stress in Steudnitz can become an important factor, especially on the sandy soil near the former factory, and this could explain the apparent correlation between reduced reproduction in *M. artemisiae* and pollution levels. Existing literature, however, shows the effects of drought stress on aphid abundance, survival and fecundity to vary. Most studies demonstrating negative effects of drought stress (Miles 1982; Pons & Tatchell 1995) have been carried out in the laboratory. Some investigations (e. g. Prezler & Price 1995) found no influence of drought stress, or reported positive responses of aphids to drought stressed in plants (Wearing 1972; Miles et al. 1982; Dorschner et al. 1986; Mattson & Haack 1987; McVean & Dixon 2001). For example, Archer et al. (1995) found no effect of nutrient stress but significant positive effects of water stress on the Russian wheat aphid (*Diuraphis noxia*). Furthermore, herbivores are often particularly sensitive to heavy metal pollution (Koricheva et al. 1998), and this could also be responsible for decreased herbivore performance, in addition to drought stress, particularly in the grasshoppers.

Feeding behavior of C. mollis was affected diversely

In contrast to the reproduction of *Chorthippus mollis*, that was negatively affected, food choice tests with plant material from the different polluted sites can be interpreted diversely. The grasshoppers did not show any differences in feeding behavior that could be interpreted by a deterrent or stimulating function of the plant material, but they clearly favored material from the polluted site and consumed significantly more from this food source. Similarly Endress & Post (1985) described a feeding preference of bean beetles for stressed plant material. An explanation for these findings could be a lower level of feeding deterrent substances in plant tissue grown on polluted soil or, if the grasshoppers chose the plant material randomly and didn’t switch between the food types, greater feeding levels could have reflected a lower nutritional value of this food source.

Herbivores responded linearly to host plant stress

For many model systems a non-linear reaction of the model system can be expected (Larsson 1989). For example Masters & McNeill (1996) found a pattern of unimodal response curves for aphid resistance in various plant genotypes suffering from air pollution. Under medium stress impact aphid performance was highest, whereas on non-stressed plants or those suffering from extreme stress, aphid performance was lower. Despite the occurrence of such non-linear patterns, only a few previous studies used several levels of stress, which would have allowed detection of any non-linear relations. The gradient of soil pollution in Steudnitz, however, provides an ideal opportunity for investigating potential non-linearities. The measured parameters for fitness and resistance of *A. vulgaris* were linearly distributed along the pollution gradient. At the moderately polluted site 2 all investigated herbivore species showed a response level intermediate to those observed for the heavily polluted site 1 and the relatively unpolluted site 3. This reaction pattern supports the PVH. However, it could be that the investigated gradient of soil quality covered only one side of the unimodal response curve and that the soil at site 3 still is heavily polluted. If this were the case, by further expanding the spatial gradient one could observe whether plant resistance would eventually decrease. Nevertheless, the fact that *A. vulgaris* fitness among plants at the relatively unpolluted field site 3 was similar to that for control plants grown in greenhouse leads me to assume that the apparent linear reaction for plant resistance is in fact real.

MeJA elicitation affects plant fitness in a similar way to soil pollution

Jasmonic acid (JA) occurs in many plants including the genus *Artemisia* (Preston & Baldwin 1999; Preston et al. 2002). It is considered to be an important component of the octadecanoid acid wound signaling pathway (Creelman & Mullet 1997a, 1997b). The use of JA or its methyl ester (MeJA) as an elicitor of plant wound responses has been proposed as a method to study more effectively the biochemical and evolutionary framework of induced responses by uncoupling wound responses from actual wounding and stimulating plant resistance in the field (Baldwin 1996; Thaler et al. 1996). In this context, jasmonate has been shown to be a regulator of secondary metabolites in a number of systems (e. g. Thaler 1999; Baldwin 2001). Moreover, jasmonate elicitation is known to slow the growth and decrease the competitive ability of plants (Beale & Ward 1998; Baldwin 2001; Heil & Baldwin 2002). Although negative consequences for fitness are not always found and, where they are found, relative effects may vary among the range of fitness components (Agrawal 1998; Thaler 1999; Cipollini & Sipe 2001), such effects have been reported for several species. Treatments with MeJA reduced seed production in *Nicotiana attenuata* (Baldwin 1998) and treatment with jasmonic acid reduced pollen production and delayed flowering in *Raphanus raphanistrum* (Agrawal et al. 1999). In my study, MeJA-induction had an effect on *A. vulgaris* fitness similar to that for soil degradation: growth and reproduction of MeJA-elicited plants was reduced. However, the effects were not the same in every respect. In contrast to soil quality, MeJA failed to affect plant germination performance, and only MeJA-induction significantly delayed flowering of *A. vulgaris*. It follows that the cost of producing defensive compounds could have contributed to any lower fitness levels recorded from induced *Artemisia* plants during my field experiments.

MeJA is involved in plant resistance

A fitness cost of induction may become a fitness benefit for the induced plant when herbivores are present (van Dam et al. 2000.). However, only few studies found clear evidence for such fitness benefits in induced plants (e. g. Baldwin 1998). Most studies measured plant resistance only in terms of herbivore performance, and most concentrated on chewing insects (lepidopterous larvae or leaf beetles). More recently, aphids have been reported to elicit the jasmonate cascade and to respond to jasmonate mutants of *Arabidopsis* (Moran & Thompson 2001). Aphid feeding on tomato stimulates lipoxygenase mRNA production, which codes for enzymes that catalyze the production of 13-hydropoxide fatty acids (Fidantsef et al. 1999). These fatty acids are the initial precursors of jasmonate biosynthesis and the results suggest that plant responses to phloem feeding also involve jasmonate synthesis and signaling. However, field experiments that have examined the effects of JA application have not found strong evidence for increased resistance to sucking insects (Thaler et al. 2001).

I could prove a MeJA-induced resistance against different herbivore species on *A. vulgaris* plants grown in Steudnitz. The grasshopper *Chorthippus mollis*, had a significant lower reproductive success when reared on MeJA-treated plant material than when reared on control material. Similarly, in enclosure experiments at the

Steudnitz field site, the aphid species *M. artemisia* showed a significant decrease in reproduction on induced plants compared to control plants.

Naturally occurring herbivores in Steudnitz have a low impact on plant fitness

Surprisingly, the field experiments investigating *Artemisia* fitness under different combinations of MeJA- and insecticide treatments did not indicate higher costs for induced plants in a herbivore free environment. The lack of fitness impact, however, was not due to an insufficient toxicity of the insecticide or to an absence of naturally-occurring herbivores. The insecticide clearly was effective against herbivores: large colonies of *M. artemisiae* could only be found on plants that had not been treated with the insecticide. Furthermore, previous studies revealed that since the end of the pollutant emission in Steudnitz an increasing diversity of herbivores could be found (Perner et al. 1996). However, herbivore pressure in Steudnitz was probably not strong enough to reveal clear benefits of induction.

Naturally occurring herbivores in Steudnitz are influenced by soil pollution

It is possible for soil pollution to have a direct influence upon herbivore performance if direct contact with pollutants invokes immediately harmful effects. For instance, Köhler (1984) found a direct negative effect of Steudnitz industrial dust on grasshoppers laying their eggs in contaminated soil. However, in my experiments the investigated herbivores did not come in direct contact with the pollutants; therefore no direct effects are expectable within the model system. Moreover, previous work at this site found no direct effect of toxic soil compounds accumulated by plants on the 2nd trophic level (Seifert et al. 1999). I therefore conclude that the observed effects on herbivores were likely to have been mediated by host plant quality.

Distribution of phenolic compounds mediates between stress and resistance

The genus *Artemisiae* of the Asteraceae comprises a large number of species that are still used as medicinal plants due to their high content of secondary plant compounds (Wollenweber & Mann 1989). One group of secondary compounds, the phenolics, are considered to be important defensive compounds against herbivores, either by interfering with the herbivores digestive enzymes or by lowering the nutritive value of the plant tissue by precipitating ingested proteins (Bernays & Chapman 2000). The phenolic content of plants is known to change in response to biotic and abiotic stress

(Matsuki 1996; Karban & Baldwin 1997). Given that the pattern of low-molecular weight phenolic compounds plays a significant role in defining plant defense capabilities, the variation of phenolic levels in leaf extracts offers a possible mechanism mediating between biotic and abiotic stress acting on *A. vulgaris* and the pattern of plant resistance that were observed.

Distribution of phenolic compounds react to biotic and abiotic stress

High Performance Liquid Chromatography (HPLC) revealed that the pattern of *A. vulgaris* leaf secondary chemical variation reacted to changes in soil quality. Most phenolic compounds were present at lower concentrations in plants growing at the heavily polluted site 1, compared to those in plants growing at the less polluted sites. This would therefore support the assumption of the PVH, that secondary metabolism is disturbed in stressed plants. In RDA, potassium and sodium concentrations together with heavy metals were identified as the most important soil factors. These results contradict the findings of Watermann & Mole (1989) who found that both drought stress and salinity are comparable stress factors, often leading to increases in phenolic concentrations.

In contrast to the general stress response that is postulated for plant growth, the response expressed by the patterns of phenolic compounds in *A. vulgaris* showed a wide variation. Some phenolics increased in concentration with increasing soil pollution (phenolic 28) while most others decreased (chlorogenic acid, kaempferol-3-glucoside) or were not affected (rutin). Similarly, Kainulainen et al. (1995) found no effect of SO_2 emission on total phenolic content in spruce and pine but higher concentrations of specific phenolic compounds such as monoterpenes, palustric acid abinene and beta-pinene. Furthermore, my multivariate analyses of *A. vulgaris* leaf chemistry revealed that both various soil parameters and MeJA elicitation affect diversely the levels of various phenolic compounds, and seemingly interact in their effects . In this context, it also is important to differentiate between the possible effects of different phenolics. Bernays & Woodhead (1982) found that ferulic acid reduced growth and reproduction of herbivores, whereas gallic and caffeic acid increased them. The usual method of assessing the defensive capabilities of a plant purely from its total phenolic content therefore would seem to be inappropriate.

Several studies have previously discussed the influence of biotic and abiotic stress on phenolic compounds. Miles et al. (1982) found an interaction between drought stress and phenolic induction. Plants responded to the attack of chewing insects by increasing their phenolic content, but as soil water became strongly limiting this response was no longer elicited. My studies confirmed a similar pattern: MeJA-induction led to increased levels of most phenolic compounds, but for several identified compounds (e. g. kaempferol-3-glucosid) significantly higher levels could only be verified in plants growing at the relatively unpolluted site.

Phenolics influence performance and food choice of herbivores

Naturally occurring plant compounds can act beneficially for insects as feeding stimuli (Dreyer & Jones 1981), or detrimentally by functioning as feeding deterrents and/or effecting a decrease in growth or reproduction (Matsuki & MacLean 1994). For example the phenolic compounds quercetin, luteolin and rutin have been shown to be toxic towards the aphids *Schizaphis graminum* and *Myzus persicae* (Dreyer & Jones 1981). However, the pattern of insect responses is multifaceted. A particular compound can be a deterrent for one species but act as a stimulant for another species, as in the case of Phlorizin (Montgomery & Arn 1974; Klingauf 1976; Schoonhoven & Derksenkoppers 1976). The relative concentration of a compound may also determine the nature of its effects, some acting as feeding stimuli at low concentrations but become deterrents at higher concentrations (Bernays et al. 1991; 2000, Bernays & Chapman 2000). Similarly, in my experiments, the reaction of the investigated herbivores can be explained by an influence of abiotic and biotic stress on the synthesis of defense related secondary compounds. However, the simple approach of explaining herbivore performance merely by phenolic toxicity is not sufficient.

Phenolics and resistance

Focusing on the distribution of phenolics, no particular compound exactly mirrors the pattern of herbivore resistance. Chlorogenic acid and Kaempferol-3-Glucoside together with several unidentified compounds increase in response to MeJA-elicitation and would therefore allow explanation of the patterns of induced herbivore resistance against *M. artemisiae* and *C. mollis*. But because poor soil quality leads to lower concentrations of these compounds, any observed decrease of herbivore performance under conditions of high soil pollution fails to be explained in terms of concentrations of these compounds. In contrast, the pattern of other phenolic compounds, such as the unidentified peak 20 that correlates positively with both MeJA-induction and soil pollution, probably could account for the reaction pattern of herbivore resistance (although not for food choice behavior) of *C. mollis*. In choice tests, for *C. mollis* the pollution stressed plant material seemed to taste better compared to the control and worse compared to induced material. So induction in this case had an opposite effect on the amount of consumed tissue to that of soil pollution. Rutin was described as a phagostimulant for the grasshopper *Schistocera americana* (Bernays et al. 1991). However, I found concentrations of this compound to remain constant, regardless of pollution levels or induction, and therefore rutin levels cannot explain the results of the grasshopper choice tests. Grasshopper food choice rather mirrored the distributions of chlorogenic acid and kaempferol-3 glucoside. Similarly, correlations of peak 4, positively with soil pollution and negatively with induction, suggests a possible stimulant effect explaining the reactions of grasshoppers in food choice tests. However, in some cases it is not physiologically necessary for a insect to avoid secondary metabolites, or plants in which they occur, even if it is deterred by them (Bernays & Graham 1988; Bernays & Chapman 2000). This uncoupling of toxic and deterrent effects would explain why the effects of soil quality and MeJA-induction on reproduction differ from those on feeding behavior.

Furthermore, earlier studies suggest that effects of individual secondary substances on insect herbivores may depend on the background variation of other plant chemicals (Renwick & Radke 1987; Soetens & Pasteels 1994). For example, the leaf beetle *A. alni* was a deterred by chlorogenic acid only on the leaves of its secondary host, *S. phylicifolia*, but not on the leaves of the primary host, *A. incana* (Ikonen et al. 2001).

It also must be taken into account, that the investigated patterns of leaf chemistry are only a small part of the total chemical defense system of a plant and that phenolic compounds are only one group of substances with a potential defense function. In addition, other factors (e. g. water deficiency, nutrient supply) could override the influence of phenolic compounds on herbivores. In particular, the effect of low nitrogen concentration in soil at the polluted sites could have influenced plant resistance. Furthermore, I sampled comparable leafs of *A. vulgaris* at a single point in time. Defense chemistry, however, can vary within different tissues of a plant (Bowers 1993) and during different stages of a plant's lifecycle (Pilson 1992; Baldwin 2001).

The interrelating effects of biotic and abiotic stress

Both abiotic stress (soil pollution) and biotic stress (MeJA-elicitation) decreased fitness and increased herbivore resistance of *A. vulgaris* plants. However, depending on cause and effect, either the intensity of induction effect waned with increasing pollution or the effect of soil pollution was lower in induced plants. Similarly, one of the few studies combining plant-stress response and induced plant defense (Miles et al. 1982) found that total phenolic content increased after herbivore attack in control plants but not in plants suffering from water stress. A comparable interaction between soil quality and the effect of herbivory also was evident in work on *Solidago altissima* by Meyer & Root (1993). They found that total seed production was reduced by herbivory but there was a strong interaction between insect impact and soil fertility so that, for all three insects investigated, total seed production was reduced only when soil fertility was high. Similarly in my study, the fitness of *A. vulgaris* was most intensively reduced by MeJA-application at the relatively unpolluted site 3.

MeJA is involved in plant tolerance to pollution

The fitness cost of MeJA-elicitation in plants should not solely be caused by a higher resistance to herbivores, but also by a higher tolerance to pollution. The jasmonate cascade is known to mediate a variety of responses in addition to induced resistance, including the signaling of drought and pollution stress (Sembdner & Parthier 1993; Gross & Parthier 1994; Creelman & Mullet 1995, 1997a, 1997b). Recent work with *Arabidopsis* micro-arrays found substantial overlap between the expression of transcripts after herbivore and pathogen attack and after various abiotic stresses

(Reymond et al. 2000; Chen et al. 2002). Interestingly, laboratory studies with rice found that exposure to copper increased jasmonate levels (Rakwal 1996; Xiang & Oliver 1998) and in *Arabidopsis* plants MeJA-elicitation increased tolerance to copper and cadmium exposure (Maksymiec & Krupa 2002). In my study, the same pattern was obvious for the fitness of induced *A. vulgaris* plants that were less affected by soil pollution than control plants. My results therefore provide the first field based evidence for a role of the jasmonate cascade in heavy metal tolerance and suggest that an understanding of the jasmonate cascade may provide a mechanistic understanding of the PVH.

Consequences for investigations of the graduate research group

Soil quality clearly affects plants at Steudnitz and several studies have already shown that spatial vegetation patterns in Steudnitz can be explained by soil quality (e. g. Scholze 1997). My findings indicate effects of soil degradation on fitness and defense on the level of the individual and present the jasmonate cascade as a possible mechanism mediating plant responses to biotic and abiotic stress. Apart from direct toxic effects, herbivores in Steudnitz are affected indirectly by soil pollution. Although I found no strong impact of natural herbivory on plants in Steudnitz, herbivory could become increasingly important as soil quality continues to improve. Along with continuing soil quality in future years, it could be expected that the influence of herbivory would become increasingly important for the succession of Steudnitz plant communities.

Summary

Point-source emissions provide ecologists with well-defined pollution gradients with which to study the effects of plant stress on induced resistance against herbivores. I investigated a gradient of severely degraded soil from the dust emissions of a former fertilizer factory and characterized three sites along this gradient by performance of soil analyses to study the interaction of abiotic stress and herbivore-resistance in a single genotype of *Artemisia vulgaris*, which naturally colonizes this disturbed habitat. To provide a phytocentric characterization of the effect of pollution stress, I measured *A. vulgaris* fitness and inducible herbivore resistance by eliciting plants at each site with methyl jasmonate (MeJA) and/or exposing them to insecticide, and measured the performance of 3 native herbivores, the aphids *Cryptosiphum artemisiae* and *Macrosiphoniella artemisiae* (in field trials) and the grasshopper *Chorthippus mollis* (in the laboratory). Soil pollution showed smale scale patchiness but predominantly decreased with distance from the former factory while *A. vulgaris* fitness (measured in both field and laboratory assays) increased, demonstrating pollution-mediated plant stress. MeJA elicitation decreased *A. vulgaris* growth and seed production and the reproductive performance of the 3 herbivores, but different insect traits were affected. MeJA-induced herbivore resistance waned in plants grown on polluted soils and was correlated with changes in concentrations of leaf secondary metabolites. Simultaneous multivariate statistical analyses of phenolics and soil parameters revealed a reaction of the pattern of secondary metabolites to abiotic and biotic stress. Induction treatment, the concentrations of potassium and heavy metals and the cation exchange capacity influenced the distribution of phenolic compounds.

Different phenolic compounds reacted differently to abiotic and biotic stress. Among the identified phenolic compounds rutin was unaffected whereas o-coumaric acid concentrations decreased in plants grown on heavily polluted soil. Chlorogenic acid and kaempferol-3-glucoside increased dramatically in MeJA-induced plants grown on moderately polluted soil but not in those on heavily polluted soil.

I conclude that herbivore-induced resistance is constrained in slow-growing plants on polluted soils and that the jasmonate cascade likely mediates plant responses to both types of environmental stresses. This central role for the jasmonate cascade provides a mechanistic understanding of hypotheses dealing with the impact of environmental stress on plant-herbivore-interactions.

Zusammenfassung

Diese Arbeit untersucht die Interaktion zwischen Schadstoff-Stress und Herbivoren-Resistenz von *Artemisia vulgaris* an drei Standorten entlang eines aus den Staubemissionen einer ehemaligen Düngemittelfabrik resultierenden Belastungsgradienten. Mittels Bodenanalysen wurde die Belastungssituation charakterisiert. Um die Auswirkungen des Schadstoff-Stresses zu untersuchen wurden Messungen von Fitness und induzierter Abwehr in einem Genotyp von *A. vulgaris* durchgeführt, indem die Pflanzen mit Methyljasmonat (MeJA) induziert und/oder mit einem Insektizid behandelt wurden. Die Herbivorenresistenz mass ich an dem Verhalten und der Reproduktion von drei verschiedene Herbivorenarten verschiedener Fraßtypen: zwei Aphiden-Arten im Freiland sowie einer Orthopteren-Art im Laborversuch. Die Bodenbelastung zeigt kleinräumige Verteilungsmuster, nimmt aber mit zunehmender Entfernung von der ehemaligen Belastungsquelle ab, während die Fitness von *A. vulgaris* (in Freiland- und Laborexperimenten) zunimmt, was einen schadstoffbedingten Pflanzenstress belegt. Die MeJA-Induktion verringert Wachstum und Samenproduktion von *A.vulgaris*, sowie die Reproduktion der untersuchten Herbivoren, bei denen jedoch unterschiedliche Parameter beeinflusst werden. Die MeJA-induzierte Herbivoren-Resistenz nimmt mit zunehmender Bodenbelastung ab und korreliert Änderungen im Muster der phenolischen Sekundärmetabolite. Simultane multivariate Analysen von phenolischen Substanzen und Bodenparametern belegen eine Reaktion der Sekundärmetabolite in Abhängigkeit von abiotischem und biotischem Stress. Eine signifikante Korrelation von MeJA-Induktion, Kalium- und Schwermetallkonzentrationen im Boden, sowie dessen Kationenaustauschkapazität mit dem Muster der phenolischen Verbindungen ist nachweisbar. Die phenolische Substanzen reagieren unterschiedlich auf abiotischen und biotischen Stress. Unter den identifizierten Substanzen reagiert Rutin nicht auf die Behandlungen, während o-Coumarinsäure Konzentrationen in Pflanzen auf stark belasteten Böden abnehmen. Chlorogensäure und Kämpferol-3-glucosid nehmen in induzierten Pflanzen auf mäßig belasteten Böden stark zu, aber nicht in Pflanzen an den stark belasteten Standorten. Eine Schlussfolgerung ist, dass die von Herbivoren induzierte Resistenz in gestressten, langsam wachsenden Pflanzen gehemmt wird und dass möglicherweise der Jasmonsäureweg ein Bindeglied zwischen der Reaktion von *A. vulgaris* auf beide Arten von Umweltstress darstellt. Eine zentrale Rolle des Jasmonsäureweges in der Antwort auf biotisch und abiotisch bedingten Pflanzenstress würde ein mechanistisches Verständnis der Theorien zum Einfluss von Umweltstress auf Pflanzen-Herbivoren-Interaktionen ermöglichen.

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Appendix

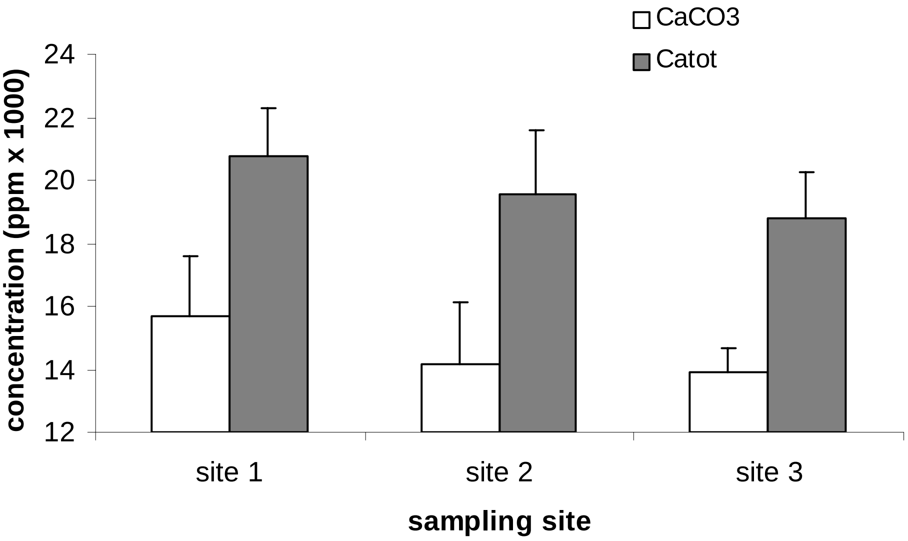

Figure A1 Mean (+SE) concentrations of the total content in calcium (Catot) and calcium carbonate ($CaC0_3$) in the topsoil along three sites of decreasing soil pollution (1 - 3).

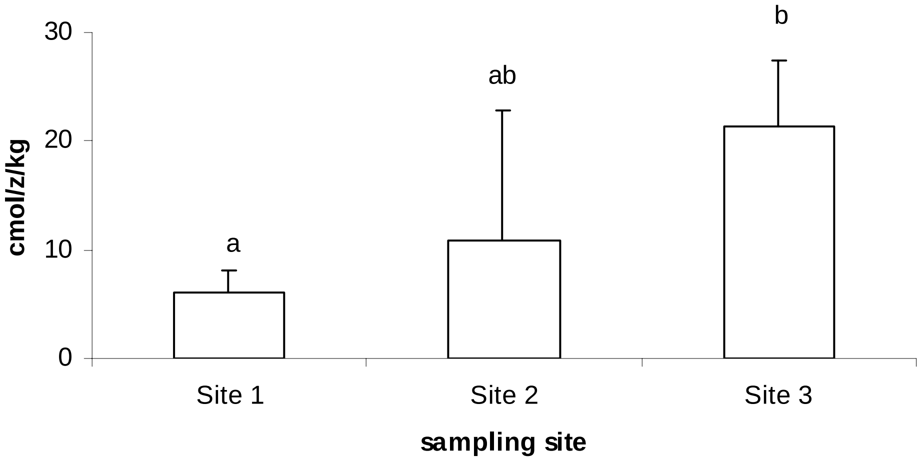

Figure A2 Mean +SE concentrations of the cation exchange capacity (CEC) in the topsoil along three sites of decreasing soil pollution (1-3). Different letters reflect significant differences for each group at *P* <0.05.

Table A1 A) correlative matrix of values for soil nitrogen, soil pH, CEC and element contents measured along a gradient of polluted soil. Presented are correlation coefficients (Coeff.) and *P*-values (P) of the 2-tailed test. B) synthetic parameters formed by a linear combination of soil factors showing a highly significant positive correlation (*P* <0.01).

	A	**pH**	**$CaCO_3$**	**N**	**Pcal**	**Kcal**	**CEC**	**Zn**	**Pb**	**Cu**	**Ni**	**Cd**	**Na**	**K**	**Mg**	**Ca**
pH	Coeff.	1.000	.256	.131	.374	.243	-.038	-.283	-.084	-.213	-.297	-.066	.336	-.240	.250	.277
	P	.	.357	.642	.187	.382	.894	.307	.766	.446	.282	.815	.220	.390	.368	.317
$CaCO_3$	Coeff.	.256	1.000	-.055	-.446	.329	-.146	-.389	-.071	-.557	-.089	.125	-.007	.000	.743	.918
	P	.357	.	.845	.110	.232	.603	.152	.800	.031	.752	.657	.980	1.000	.022	.000
N	Coeff.	.131	-.055	1.000	-.097	-.066	.543	-.298	-.297	-.263	.459	-.386	-.029	.218	.281	-.020
	P	.642	.845	.	.742	.815	.036	.280	.283	.344	.085	.155	.919	.435	.311	.945
Pcal	Coeff.	.374	-.446	-.097	1.000	.081	-.226	.499	.508	.719	-.543	.415	.574	-.600	-.648	-.240
	P	.187	.110	.742	.	.782	.436	.069	.064	.014	.045	.140	.032	.023	.012	.409
Kcal	Coeff.	.243	.329	-.066	.081	1.000	-.514	.225	.539	.121	-.157	.404	.261	-.314	.039	.425
	P	.382	.232	.815	.782	.	.050	.420	.038	.666	.576	.136	.348	.254	.889	.114
CEC	Coeff.	-.038	-.146	.543	-.226	-.514	1.000	-.314	-.721	-.125	.646	-.825	-.329	.564	.189	-.271
	P	.894	.603	.036	.436	.050	.	.254	.002	.657	.029	.020	.232	.028	.499	.328
Zn	Coeff.	-.283	-.389	-.298	.499	.225	-.314	1.000	.636	.782	-.111	.500	.193	-.539	-.611	-.357
	P	.307	.152	.280	.069	.420	.254	.	.009	.001	.694	.058	.491	.038	.016	.191
Pb	Coeff.	-.084	-.071	-.297	.508	.539	-.721	.636	1.000	.425	-.454	.804	.214	-.768	-.461	.054
	P	.766	.800	.283	.064	.038	.002	.009	.	.114	.089	.000	.443	.021	.084	.850
Cu	Coeff.	-.213	-.557	-.263	.719	.121	-.125	.782	.425	1.000	-.218	.314	.396	-.389	-.739	-.411
	P	.446	.031	.344	.014	.666	.657	.001	.114	.	.435	.254	.143	.152	.012	.128
Ni	Coeff.	-.297	-.089	.459	-.543	-.157	.646	-.111	-.454	-.218	1.000	-.671	-.593	.436	.346	-.271
	P	.282	.752	.085	.045	.576	.029	.694	.089	.435	.	.006	.020	.104	.206	.328
Cd	Coeff.	-.066	.125	-.386	.415	.404	-.825	.500	.804	.314	-.671	1.000	.454	-.618	-.318	.325
	P	.815	.657	.155	.140	.136	.020	.058	.000	.254	.006	.	.089	.014	.248	.237
Na	Coeff.	.336	-.007	-.029	.574	.261	-.329	.193	.214	.396	-.593	.454	1.000	-.154	-.339	.218
	P	.220	.980	.919	.032	.348	.232	.491	.443	.143	.020	.089	.	.585	.216	.435
K	Coeff.	-.240	.000	.218	-.600	-.314	.564	-.539	-.768	-.389	.436	-.618	-.154	1.000	.282	-.036
	P	.390	1.000	.435	.023	.254	.028	.038	.021	.152	.104	.014	.585	.	.308	.899
Mg	Coeff.	.250	.743	.281	-.648	.039	.189	-.611	-.461	-.739	.346	-.318	-.339	.282	1.000	.636
	P	.368	.022	.311	.012	.889	.499	.016	.084	.012	.206	.248	.216	.308	.	.011
Ca	Coeff-	.277	.918	-.020	-.240	.425	-.271	-.357	.054	-.411	-.271	.325	.218	-.036	.636	1.000
	P	.317	.000	.945	.409	.114	.328	.191	.850	.128	.328	.237	.435	.899	.011	.

B Camix = CaCO3 x Ca

Heavymet = Zn x Pb x Cu x Ni x Cd

Table A2 Mean ±SE values (each n = 3) for soil parameters (element contents: ppm, N: %, CEC: cmol/z/kg) measured at 5 plots for each of the three field sites lying along a gradient of polluted soil and demonstrating the intrasite variation.

Site	**Plot**	**pH**	**CaCO3**	**N**	**Pcal**
Site 1	**1**	8,13 ± 0,03	7,43 ± 1,68	0,17 ± 0,10	18724,66 ± 654,87
	2	8,80 ± 0,07	8,37 ± 1,29	0,11 ± 0,07	17846,11 ± 819,04
	3	8,07 ± 0,03	25,27 ± 1,15	0,26 ± 0,08	4775,84 ± 1816,85
	4	8,60 ± 0,07	21,17 ± 1,94	0,12 ± 0,01	7379,70 ± 595,91
	5	8,30 ± 0,10	15,90 ± 0,59	0,14 ± 0,02	12516,20 ± 1290,66
Site 2	**1**	8,30 ± 0,06	24,87 ± 0,49	0,16 ± 0,01	4826,57 ± 715,42
	2	7,80 ± 0,00	8,40 ± 0,82	0,11 ± 0,00	13704,00 ± 574,59
	3	8,07 ± 0,18	6,03 ± 1,41	0,26 ± 0,20	4358,63 ± 2492,48
	4	7,87 ± 0,03	19,70 ± 0,25	0,17 ± 0,01	5158,12 ± 109,33
	5	8,37 ± 0,03	10,33 ± 0,41	0,21 ± 0,04	6871,29 ± 1035,55
Site 3	**1**	8,27 ± 0,07	12,03 ± 0,53	0,22 ± 0,08	4322,72 ± 1624,54
	2	8,47 ± 0,06	14,00 ± 0,20	0,48 ± 0,02	12466,09 ± 736,32
	3	8,87 ± 0,18	14,70 ± 1,04	0,30 ± 0,01	13057,86 ± 731,68
	4	8,83 ± 0,06	17,27 ± 3,00	0,17 ± 0,03	6343,45 ± 1717,84
	5	8,40 ± 0,06	11,83 ± 2,51	0,51 ± 0,00	11607,61 ± 3126,89

Site	**Plot**	**Kcal**	**CEC**	**Zntot**	**Pbtot**
Site 1	**1**	990,70 ± 25,64	7,77 ± 0,27	94,05 ± 3,19	261,64 ± 2,49
	2	722,19 ± 169,27	6,77 ± 1,02	137,10 ± 2,16	270,84 ± 1,92
	3	822,00 ± 84,55	7,08 ± 1,36	96,23 ± 7,01	258,06 ± 2,28
	4	887,34 ± 124,98	4,57 ± 0,72	190,34 ± 1,87	170,57 ± 0,65
	5	726,80 ± 126,35	4,21 ± 0,38	86,23 ± 13,24	150,98 ± 1,93
Site 2	**1**	337,09 ± 43,21	8,72 ± 0,35	53,92 ± 2,63	19,44 ± 4,96
	2	506,11 ± 7,43	21,33 ± 0,33	107,17 ± 2,07	24,76 ± 0,15
	3	579,05 ± 174,52	10,85 ± 4,11	74,57 ± 33,95	22,86 ± 0,85
	4	438,70 ± 197,63	10,01 ± 0,25	75,08 ± 0,41	32,28 ± 5,01
	5	714,08 ± 149,41	7,21 ± 0,41	80,25 ± 3,71	29,94 ± 2,95
Site 3	**1**	579,22 ± 56,54	11,56 ± 0,14	92,20 ± 52,27	22,12 ± 117,00
	2	510,55 ± 326,28	26,40 ± 0,49	72,23 ± 12,92	28,47 ± 143,49
	3	755,84 ± 163,51	16,31 ± 1,45	82,74 ± 19,43	24,96 ± 66,67
	4	446,37 ± 42,70	16,45 ± 0,46	57,51 ± 6,79	21,83 ± 73,00
	5	589,28 ± 103,77	36,46 ± 1,54	105,75 ± 7,42	27,79 ± 15,42

Site	Plot	Cutot	Nitot	Cdtot	Natot
Site 1	**1**	33,14 ± 3,23	12,34 ± 1,14	10,80 ± 0,85	7,18 ± 0,77
	2	31,10 ± 0,60	12,04 ± 1,07	9,70 ± 0,60	7,12 ± 0,79
	3	21,45 ± 0,57	19,17 ± 1,41	6,24 ± 0,96	5,94 ± 0,17
	4	21,88 ± 0,98	10,89 ± 0,09	8,85 ± 0,25	6,36 ± 0,65
	5	26,06 ± 2,22	14,82 ± 2,14	7,41 ± 0,45	9,13 ± 1,21
Site 2	**1**	16,14 ± 0,43	13,28 ± 0,58	4,49 ± 1,06	8,23 ± 0,31
	2	56,72 ± 5,33	21,53 ± 1,65	2,62 ± 0,22	5,03 ± 0,49
	3	17,90 ± 4,84	27,43 ± 12,01	2,74 ± 0,83	3,87 ± 0,51
	4	21,77 ± 0,32	14,67 ± 1,28	5,52 ± 0,56	6,57 ± 0,04
	5	27,84 ± 0,49	8,48 ± 0,39	4,49 ± 2,24	22,85 ± 0,53
Site 3	**1**	26,55 ± 1,30	18,92 ± 0,53	4,07 ± 2,02	6,62 ± 0,75
	2	21,00 ± 0,73	17,50 ± 1,12	3,04 ± 1,23	5,44 ± 0,44
	3	26,28 ± 0,78	17,34 ± 1,04	3,86 ± 3,21	8,33 ± 1,73
	4	15,61 ± 1,44	17,01 ± 0,41	1,95 ± 1,78	4,52 ± 0,36
	5	23,91 ± 2,25	15,92 ± 0,77	4,38 ± 2,16	13,72 ± 1,02

Site	Plot	Ktot	Mgtot	Catot
Site 1	**1**	2,25 ± 0,58	4,66 ± 0,95	134,12 ± 6,68
	2	2,54 ± 0,44	4,82 ± 0,49	141,10 ± 2,89
	3	3,22 ± 0,50	12,23 ± 0,35	194,17 ± 8,54
	4	3,44 ± 0,38	10,23 ± 0,63	211,13 ± 12,76
	5	3,59 ± 0,46	7,86 ± 0,26	181,73 ± 2,45
Site 2	**1**	12,36 ± 0,45	12,36 ± 0,49	218,11 ± 2,28
	2	8,47 ± 0,05	7,29 ± 0,68	98,25 ± 10,08
	3	12,54 ± 3,28	9,64 ± 2,43	142,45 ± 106,11
	4	9,85 ± 0,41	9,37 ± 0,41	189,55 ± 2,01
	5	6,22 ± 1,52	5,39 ± 0,29	170,42 ± 5,38
Site 3	**1**	8,24 ± 0,26	9,63 ± 0,07	170,93 ± 3,13
	2	7,89 ± 0,13	9,82 ± 0,25	175,50 ± 4,76
	3	7,96 ± 0,34	10,99 ± 2,99	177,30 ± 21,24
	4	7,01 ± 0,20	10,41 ± 1,46	162,60 ± 26,59
	5	8,95 ± 0,52	7,03 ± 1,14	147,12 ± 5,54

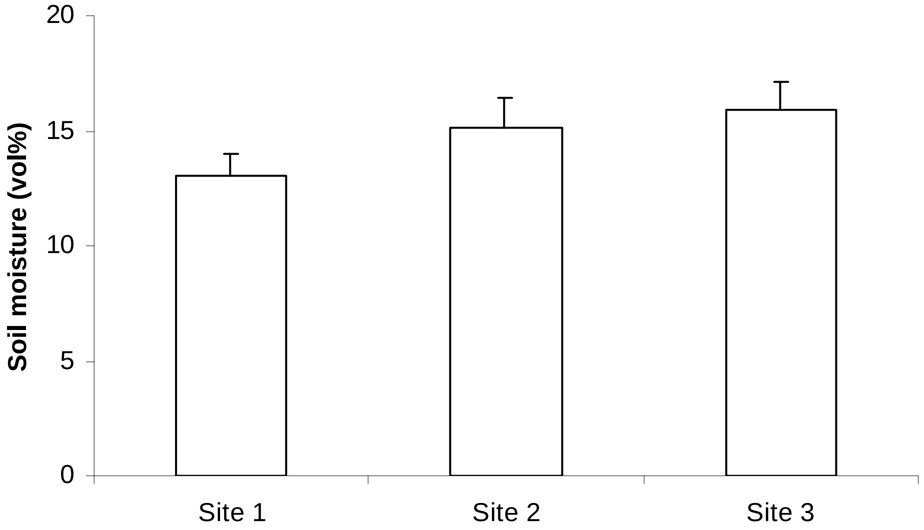

Figure A3 Mean +SE relative soil moisture (each n = 50) measured in the topsoil along three sites of decreasing soil pollution (1-3).

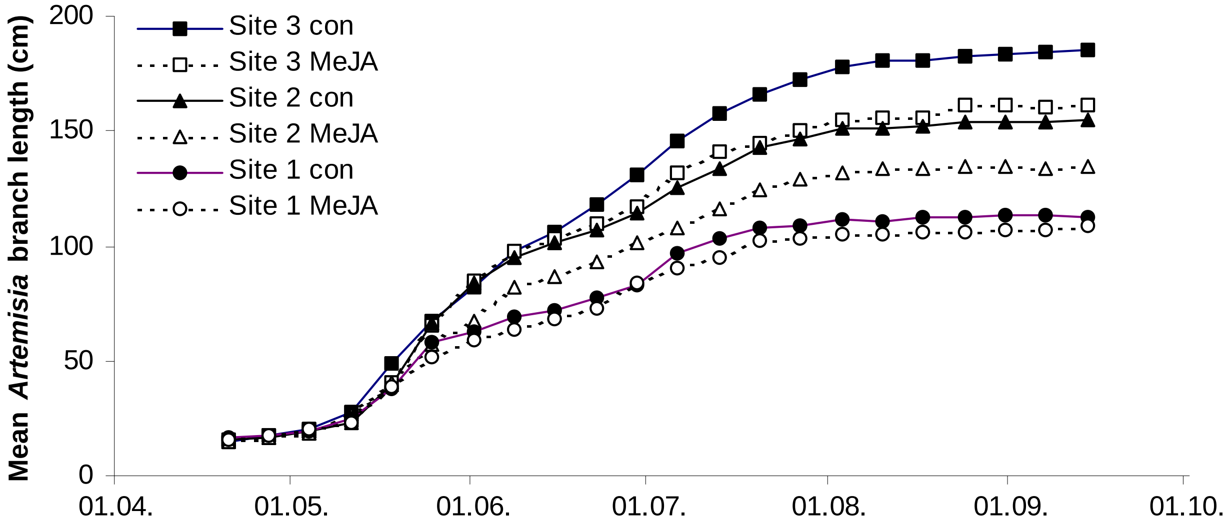

Figure A4 Mean growth of induced (MeJA) and non-induced (con) *Artemisia vulgaris* plants (n = 328) along three sites of decreasing soil pollution (1-3) during vegetation period 2000.

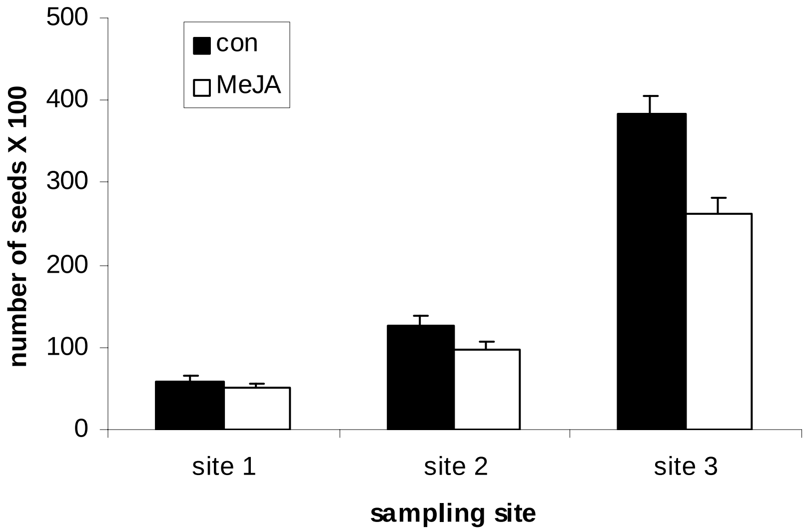

Figure A5 Mean +SE number of seeds produced by *Artemisia vulgaris* control plants (con) and plants treated with methyl jasmonate (MeJA) at the three field sites of decreasing soil pollution (1-3).

Table A3 relation between branch length and the number of seeds produced by *Artemisia vulgaris* plants tested for all single treatments tested with linear regressions (see Chapter 4).

Treatment	R	R Square	Adjusted R Square	Std. Error of the Estimate	*P*-value
1,00	,927	,860	,859	69,5500	<0.01
2,00	,805	,648	,642	45,0317	<0.01
3,00	,919	,845	,841	73,6041	<0.01
4,00	,757	,574	,564	54,8362	<0.01
5,00	,910	,829	,822	91,7769	<0.01
6,00	,845	,714	,701	57,2183	<0.01
7,00	,917	,841	,834	66,6010	<0.01

a Predictors: LENGTH
b Dependent Variable: SEEDS

Table A4 Feeding behavior of 8 *Chorthippus mollis* imagines in 2 food choice tests monitored for 60 minutes (see Chapter 4). A) attractiveness of and feeding time on induced (3 MeJA) and control (3 con) material of *Artemisia vulgaris* from the unpolluted site 3. B) attractiveness and feeding time on material from the polluted site 1 (1 con) and material from the unpolluted site 3 (3 con).

Food choice A	**Site 3 MeJA**		**Site 3 con**	
Individuals	**time feeding (min)**	**attractivity**	**time feeding (min)**	**attractivity**
1	10	52	11	29
2	6	13	22	44
3	0	0	17	46
4	33	56	0	0
5	12	48	0	0
6	0	0	22	11
7	0	0	7	57
8	9	11	0	0
mean	8,75	22,50	9,88	23,38
SE	3,86	8,84	3,39	8,33

Food choice B	**Site 1 con**		**Site 3 con**	
Individuals	**time feeding (min)**	**attractivity**	**time feeding (min)**	**attractivity**
1	8	26	11	13
2	0	0	0	0
3	12	49	30	33
4	14	52	10	11
5	32	36	0	5
6	0	0	12	44
7	4	15	22	57
8	19	28	0	0
mean	11,13	25,75	10,63	20,38
SE	3,81	7,04	3,88	7,63

Table A5 Growth rates of 328 *A. vulgaris* plants belonging to plant pairs (a – h) treated with methyl jasmonate (MeJA), insecticide or partly covered by aphid enclosures (aphid) (for explanation of treatment numbers see Chapter 4).

plant#	pair	growth rate	MeJA	insecticide	aphid	treatment	plant#	pair#	growth rate	MeJA	insecticide	aphid	treatment
1	a	4.23	1	0	0	treatment1	5	a	5.16	1	1	0	treatment 4
28	b	4.56	1	0	0	treatment1	15	b	3.92	1	1	0	treatment 4
32	c	4.92	1	0	0	treatment1	46	c	2.83	1	1	0	treatment 4
35	d	3.82	1	0	0	treatment1	57	d	4.41	1	1	0	treatment 4
78	e	3.24	1	0	0	treatment1	68	e	4.27	1	1	0	treatment 4
85	f	3.99	1	0	0	treatment1	98	f	3.66	1	1	0	treatment 4
106	g	3.54	1	0	0	treatment1	114	g	3.28	1	1	0	treatment 4
116	h	3.86	1	0	0	treatment1	119	h	3.87	1	1	0	treatment 4
7	a	4.33	0	0	0	treatment1	11	a	5.99	1	0	0	treatment 4
26	c	4.58	0	0	0	treatment1	21	b	4.03	1	0	0	treatment 4
29	d	4.88	0	0	0	treatment1	40	c	2.74	1	0	0	treatment 4
34	b	3.96	0	0	0	treatment1	51	d	3.84	1	0	0	treatment 4
84	e	5.34	0	0	0	treatment1	62	e	4.46	1	0	0	treatment 4
91	f	4.03	0	0	0	treatment1	104	f	3.81	1	0	0	treatment 4
100	g	4.67	0	0	0	treatment1	113	h	3.72	1	0	0	treatment 4
110	h	3.75	0	0	0	treatment1	120	g	4.31	1	0	0	treatment 4
14	a	4.22	0	1	0	treatment2	131	a	7.22	1	1	0	treatment 4
36	b	4.02	0	1	0	treatment2	141	b	3.38	1	1	0	treatment 4
52	c	5.57	0	1	0	treatment2	160	c	4.77	1	1	0	treatment 4
56	d	3.88	0	1	0	treatment2	171	d	5.21	1	1	0	treatment 4
64	e	3.12	0	1	0	treatment2	182	e	6.04	1	1	0	treatment 4
71	f	3.89	0	1	0	treatment2	224	f	6.38	1	1	0	treatment 4
97	g	3.59	0	1	0	treatment2	233	g	4.51	1	1	0	treatment 4
117	h	3.91	0	1	0	treatment2	240	h	4.82	1	1	0	treatment 4
20	a	4.45	0	0	0	treatment2	251	a	7.74	1	0	0	treatment 4
30	b	4.55	0	0	0	treatment2	261	b	2.94	1	0	0	treatment 4
50	d	4.76	0	0	0	treatment2	280	c	5.33	1	0	0	treatment 4
58	c	3.68	0	0	0	treatment2	291	d	6.36	1	0	0	treatment 4
65	f	5.12	0	0	0	treatment2	302	e	6.65	1	0	0	treatment 4
70	e	4.13	0	0	0	treatment2	344	f	6.18	1	0	0	treatment 4
103	g	4.55	0	0	0	treatment2	353	h	5.93	1	0	0	treatment 4
111	h	3.78	0	0	0	treatment2	360	g	5.99	1	0	0	treatment 4
121	a	5.82	1	0	0	treatment1	251	a	8.53	1	1	0	treatment 4
148	b	8.33	1	0	0	treatment1	261	b	9.88	1	1	0	treatment 4
152	c	4.73	1	0	0	treatment1	280	c	10.72	1	1	0	treatment 4
155	d	5.03	1	0	0	treatment1	291	d	8.86	1	1	0	treatment 4
198	e	4.67	1	0	0	treatment1	302	e	10.76	1	1	0	treatment 4
205	f	6.32	1	0	0	treatment1	344	f	9.79	1	1	0	treatment 4
226	g	5.92	1	0	0	treatment1	353	g	11.39	1	1	0	treatment 4
236	h	5.16	1	0	0	treatment1	360	h	9.22	1	1	0	treatment 4
127	a	7.23	0	0	0	treatment1	371	a	10.94	1	0	0	treatment 4
146	c	7.82	0	0	0	treatment1	381	b	9.44	1	0	0	treatment 4
149	d	7.92	0	0	0	treatment1	400	c	11.07	1	0	0	treatment 4
154	b	8.94	0	0	0	treatment1	411	d	9.37	1	0	0	treatment 4
204	e	5.23	0	0	0	treatment1	422	e	7.62	1	0	0	treatment 4
211	f	6.99	0	0	0	treatment1	464	f	8.44	1	0	0	treatment 4
220	g	8.18	0	0	0	treatment1	473	h	9.87	1	0	0	treatment 4
230	h	6.55	0	0	0	treatment1	480	g	9.2	1	0	0	treatment 4

Appendix

plant#	pair	growth rate	MeJA	insecticide	aphid	treatment	plant#	pair#	growth rate	MeJA	insecticide	aphid	treatment
134	a	6.82	0	1	0	treatment2	3	a	2.93	0	1	0	treatment 5
156	b	6.87	0	1	0	treatment2	8	b	4.47	0	1	0	treatment 5
172	c	8.24	0	1	0	treatment2	24	c	4.55	0	1	0	treatment 5
176	d	6.91	0	1	0	treatment2	54	d	5.21	0	1	0	treatment 5
184	e	5.13	0	1	0	treatment2	79	e	4.89	0	1	0	treatment 5
191	f	6.66	0	1	0	treatment2	83	f	3.63	0	1	0	treatment 5
217	g	8.09	0	1	0	treatment2	101	g	5.18	0	1	0	treatment 5
237	h	6.55	0	1	0	treatment2	109	h	4.41	0	1	0	treatment 5
140	a	7.92	0	0	0	treatment2	2	b	2.99	1	0	0	treatment 5
150	b	7.73	0	0	0	treatment2	9	a	2.02	1	0	0	treatment 5
170	d	7.62	0	0	0	treatment2	18	c	5.31	1	0	0	treatment 5
178	c	9.89	0	0	0	treatment2	60	d	5.88	1	0	0	treatment 5
185	f	4.76	0	0	0	treatment2	73	e	5.04	1	0	0	treatment 5
190	e	6.81	0	0	0	treatment2	77	f	4.95	1	0	0	treatment 5
223	g	9.38	0	0	0	treatment2	107	g	4.09	1	0	0	treatment 5
231	h	6.73	0	0	0	treatment2	115	h	4.34	1	0	0	treatment 5
241	a	9.43	1	0	0	treatment1	123	a	9.32	0	1	0	treatment 5
268	b	11.82	1	0	0	treatment1	128	b	10.03	0	1	0	treatment 5
272	c	8.27	1	0	0	treatment1	144	c	8.43	0	1	0	treatment 5
275	d	7.37	1	0	0	treatment1	174	d	8.29	0	1	0	treatment 5
318	e	10.04	1	0	0	treatment1	199	e	7.14	0	1	0	treatment 5
325	f	9.92	1	0	0	treatment1	203	f	7.26	0	1	0	treatment 5
346	g	8.35	1	0	0	treatment1	221	g	6.64	0	1	0	treatment 5
356	h	9.32	1	0	0	treatment1	229	h	8.17	0	1	0	treatment 5
247	a	15.23	0	0	0	treatment1	122	b	9.13	1	0	0	treatment 5
266	c	14.56	0	0	0	treatment1	129	a	4.42	1	0	0	treatment 5
269	d	13.88	0	0	0	treatment1	138	c	4.91	1	0	0	treatment 5
274	b	17.96	0	0	0	treatment1	180	d	5.06	1	0	0	treatment 5
324	e	16.77	0	0	0	treatment1	193	e	5.29	1	0	0	treatment 5
331	f	16.05	0	0	0	treatment1	197	f	6.47	1	0	0	treatment 5
340	g	15.83	0	0	0	treatment1	227	g	5.13	1	0	0	treatment 5
350	h	13.21	0	0	0	treatment1	235	h	5.77	1	0	0	treatment 5
254	a	18.45	0	1	0	treatment2	243	a	19.21	0	1	0	treatment 5
276	b	18.82	0	1	0	treatment2	248	b	14.46	0	1	0	treatment 5
292	c	17.25	0	1	0	treatment2	264	c	16.4	0	1	0	treatment 5
296	d	14.27	0	1	0	treatment2	294	d	15.88	0	1	0	treatment 5
304	e	17.46	0	1	0	treatment2	319	e	14.06	0	1	0	treatment 5
311	f	18.54	0	1	0	treatment2	323	f	18.98	0	1	0	treatment 5
337	g	16.48	0	1	0	treatment2	341	g	16.01	0	1	0	treatment 5
357	h	13.92	0	1	0	treatment2	349	h	16.43	0	1	0	treatment 5
260	a	17.38	0	0	0	treatment2	242	b	15.72	1	0	0	treatment 5
270	b	16.25	0	0	0	treatment2	249	a	5.05	1	0	0	treatment 5
290	d	15.39	0	0	0	treatment2	258	c	8.85	1	0	0	treatment 5
298	c	14.88	0	0	0	treatment2	300	d	9.51	1	0	0	treatment 5
305	f	14.94	0	0	0	treatment2	313	e	9.21	1	0	0	treatment 5
310	e	18.06	0	0	0	treatment2	317	f	11.95	1	0	0	treatment 5
343	g	15.76	0	0	0	treatment2	347	g	9.89	1	0	0	treatment 5
351	h	14.89	0	0	0	treatment2	355	h	10.03	1	0	0	treatment 5
13	a	4.79	0	0	1	treatment7	6	a	5.55	1	0	1	treatment 6
16	b	4.87	0	0	1	treatment7	25	b	4.21	1	0	1	treatment 6
43	c	4.14	0	0	1	treatment7	27	c	3.88	1	0	1	treatment 6
59	d	4.62	0	0	1	treatment7	42	d	4.34	1	0	1	treatment 6
76	e	4.85	0	0	1	treatment7	61	e	3.57	1	0	1	treatment 6
86	f	3.39	0	0	1	treatment7	75	f	3.89	1	0	1	treatment 6

plant#	pair	growth rate	MeJA	insecticide	aphid	treatment	plant#	pair#	growth rate	MeJA	insecticide	aphid	treatment
105	g	5.11	0	0	1	treatment7	89	g	4.11	1	0	1	treatment 6
108	h	4.63	0	0	1	treatment7	112	h	4.87	1	0	1	treatment 6
19	a	4.47	0	0	0	treatment7	12	a	4.88	0	0	1	treatment 6
22	b	5.04	0	0	0	treatment7	31	b	5.02	0	0	1	treatment 6
37	c	3.92	0	0	0	treatment7	33	c	4.63	0	0	1	treatment 6
53	d	4.55	0	0	0	treatment7	48	d	3.98	0	0	1	treatment 6
82	e	4.18	0	0	0	treatment7	67	e	3.62	0	0	1	treatment 6
92	f	3.69	0	0	0	treatment7	81	f	4.44	0	0	1	treatment 6
99	g	3.96	0	0	0	treatment7	95	g	3.79	0	0	1	treatment 6
102	h	4.02	0	0	0	treatment7	118	h	5.86	0	0	1	treatment 6
133	a	8.33	0	0	1	treatment7	126	a	5.77	1	0	1	treatment 6
136	b	6.65	0	0	1	treatment7	145	b	5.81	1	0	1	treatment 6
163	c	5.94	0	0	1	treatment7	147	c	6.72	1	0	1	treatment 6
179	d	7.72	0	0	1	treatment7	162	d	4.88	1	0	1	treatment 6
196	e	6.02	0	0	1	treatment7	181	e	6.77	1	0	1	treatment 6
206	f	7.54	0	0	1	treatment7	195	f	5.35	1	0	1	treatment 6
225	g	7.88	0	0	1	treatment7	209	g	4.71	1	0	1	treatment 6
228	h	8.03	0	0	1	treatment7	232	h	6.29	1	0	1	treatment 6
139	a	8.29	0	0	0	treatment7	132	a	6.71	0	0	1	treatment 6
142	b	6.75	0	0	0	treatment7	151	b	8.82	0	0	1	treatment 6
157	c	6.34	0	0	0	treatment7	153	c	8.42	0	0	1	treatment 6
173	d	7.88	0	0	0	treatment7	168	d	8.13	0	0	1	treatment 6
202	e	5.96	0	0	0	treatment7	187	e	8.09	0	0	1	treatment 6
212	f	7.72	0	0	0	treatment7	201	f	6.83	0	0	1	treatment 6
219	g	8.09	0	0	0	treatment7	215	g	7.91	0	0	1	treatment 6
222	h	9.15	0	0	0	treatment7	238	h	9.68	0	0	1	treatment 6
253	a	13.67	0	0	1	treatment7	246	a	10.07	1	0	1	treatment 6
256	b	16.39	0	0	1	treatment7	265	b	7.72	1	0	1	treatment 6
283	c	11.634	0	0	1	treatment7	267	c	9.84	1	0	1	treatment 6
299	d	14.86	0	0	1	treatment7	282	d	8.18	1	0	1	treatment 6
316	e	17.28	0	0	1	treatment7	301	e	8.99	1	0	1	treatment 6
326	f	19.19	0	0	1	treatment7	315	f	9.51	1	0	1	treatment 6
345	g	16.34	0	0	1	treatment7	329	g	9.82	1	0	1	treatment 6
348	h	16.11	0	0	1	treatment7	352	h	11.01	1	0	1	treatment 6
259	a	15.23	0	0	0	treatment7	252	a	13.41	0	0	1	treatment 6
262	b	16.25	0	0	0	treatment7	271	b	15.77	0	0	1	treatment 6
277	c	15.39	0	0	0	treatment7	273	c	11.58	0	0	1	treatment 6
293	d	14.88	0	0	0	treatment7	288	d	17.22	0	0	1	treatment 6
322	e	14.94	0	0	0	treatment7	307	e	17.88	0	0	1	treatment 6
332	f	18.06	0	0	0	treatment7	321	f	19.21	0	0	1	treatment 6
339	g	15.33	0	0	0	treatment7	335	g	16.03	0	0	1	treatment 6
342	h	14.89	0	0	0	treatment7	358	h	17.81	0	0	1	treatment 6
17	a	5.21	1	1	0	treatment 3							
45	b	4.33	1	1	0	treatment 3							
47	c	4.18	1	1	0	treatment 3							
49	d	3.87	1	1	0	treatment 3							
66	e	2.94	1	1	0	treatment 3							
69	f	4.63	1	1	0	treatment 3							
74	g	4.11	1	1	0	treatment 3							
96	h	3.45	1	1	0	treatment 3							
23	a	5.02	0	0	0	treatment 3							
39	b	4.42	0	0	0	treatment 3							
41	c	3.75	0	0	0	treatment 3							
55	d	3.98	0	0	0	treatment 3							

plant#	pair	growth rate	MeJA	insecticide	aphid	treatment
63	f	3.62	0	0	0	treatment 3
72	e	4.06	0	0	0	treatment 3
80	g	3.92	0	0	0	treatment 3
90	h	4.88	0	0	0	treatment 3
137	a	4.88	1	1	0	treatment 3
165	b	5.86	1	1	0	treatment 3
167	c	6.73	1	1	0	treatment 3
169	d	4.99	1	1	0	treatment 3
186	e	5.71	1	1	0	treatment 3
189	f	5.76	1	1	0	treatment 3
194	g	6.94	1	1	0	treatment 3
216	h	6.92	1	1	0	treatment 3
143	a	7.92	0	0	0	treatment 3
159	b	6.81	0	0	0	treatment 3
161	c	8.33	0	0	0	treatment 3
175	d	6.78	0	0	0	treatment 3
183	f	8.95	0	0	0	treatment 3
192	e	7.98	0	0	0	treatment 3
200	g	9.49	0	0	0	treatment 3
210	h	7.76	0	0	0	treatment 3
257	a	11.18	1	1	0	treatment 3
285	b	7.78	1	1	0	treatment 3
287	c	10.06	1	1	0	treatment 3
289	d	8.03	1	1	0	treatment 3
306	e	9.53	1	1	0	treatment 3
309	f	9.77	1	1	0	treatment 3
314	g	8.91	1	1	0	treatment 3
336	h	9.29	1	1	0	treatment 3
263	a	19.26	0	0	0	treatment 3
279	b	11.81	0	0	0	treatment 3
281	c	17.79	0	0	0	treatment 3
295	d	16.05	0	0	0	treatment 3
303	f	17.33	0	0	0	treatment 3
312	e	17.26	0	0	0	treatment 3
320	g	13.38	0	0	0	treatment 3
330	h	16.33	0	0	0	treatment 3

Table A6 Matrix of HPLC values (area counts/1000) for 46 peaks of phenolic compounds (columns) based on leaf extracts of 230 *A. vulgaris* plants (lines).

plant #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	32.79	0.00	0.00	55.50	15.92	0.00	0.00	15.49	0.00	0.00	0.00	11.21	0.00	0.00	0.00	9.87
2	31.42	8.42	0.00	0.00	98.78	0.00	18.28	91.52	15.36	18.00	0.00	14.79	0.00	0.00	0.00	15.00
3	66.61	0.00	0.00	21.72	9.06	0.00	0.00	9.32	0.00	0.00	10.98	48.20	0.00	0.00	0.00	42.92
5	62.49	0.00	0.00	47.13	9.89	0.00	0.00	10.19	0.00	76.06	0.00	84.77	0.00	0.00	0.00	76.37
6	33.69	0.00	0.00	26.32	0.17	0.00	0.00	8.19	0.00	0.00	0.00	14.77	0.00	0.00	0.00	14.69
7	41.50	0.00	0.00	70.26	0.16	0.00	0.00	7.78	0.00	0.00	0.00	14.19	0.00	0.00	0.00	13.84
8	40.05	0.00	0.00	21.02	16.31	0.00	0.00	15.79	0.00	0.00	0.00	99.77	0.00	167.77	0.00	100.54
9	54.99	0.00	0.00	12.50	12.35	0.00	11.95	11.88	0.00	0.00	13.82	42.29	0.00	0.00	0.00	40.02
11	0.14	4.62	0.00	43.22	25.99	0.00	26.44	27.40	0.00	0.00	0.00	33.49	0.00	0.00	0.00	33.46
13	26.23	6.47	0.00	77.53	79.75	0.00	43.71	70.80	0.00	0.00	0.00	11.65	0.00	0.00	0.00	10.40
17	71.66	0.00	98.07	24.91	12.48	0.00	20.58	12.44	43.86	13.55	0.00	12.45	0.00	0.00	0.00	11.23
18	88.04	0.00	0.00	29.42	10.79	0.00	0.00	10.72	0.00	84.56	57.84	54.97	0.00	0.00	0.00	51.45
23	24.64	0.00	0.00	92.77	11.91	0.00	26.69	11.78	0.00	0.00	0.00	11.28	0.00	0.00	0.00	10.17
24	38.24	0.00	0.00	48.57	0.15	0.00	0.00	0.10	0.00	0.00	0.00	43.38	0.00	0.00	0.00	41.08
25	30.85	4.78	0.00	8.13	28.35	0.00	28.04	30.03	11.59	0.00	0.00	8.58	0.00	0.00	0.00	0.00
26	17.15	0.00	20.58	23.39	11.93	0.00	0.00	11.79	0.00	0.00	0.00	74.47	0.00	39.98	0.00	67.02
27	53.61	0.00	0.00	40.43	17.46	0.00	0.00	16.23	0.00	134.25	0.00	149.61	0.00	0.00	0.00	140.93
28	9.76	0.00	43.40	22.29	19.43	0.00	0.00	19.99	0.00	0.00	0.00	54.28	0.00	0.00	0.00	49.15
29	36.11	0.00	0.00	61.13	13.97	0.00	44.57	13.92	0.00	0.00	0.00	14.89	0.00	0.00	0.00	15.32
32	10.59	0.00	0.00	46.40	12.47	0.00	10.21	12.19	0.00	0.00	0.00	21.14	14.26	0.00	0.00	21.35
34	31.75	0.00	0.00	7.96	15.64	0.00	0.00	15.11	0.00	0.00	27.91	74.82	0.00	0.00	0.00	67.52
35	48.96	0.00	0.00	82.89	15.60	0.00	60.43	15.03	0.00	0.00	0.00	20.19	0.00	0.00	0.00	19.45
36	69.36	0.00	10.28	17.59	11.85	0.00	17.10	11.77	0.00	0.00	0.00	49.71	0.00	32.93	0.00	43.26
39	32.90	0.00	0.00	25.74	0.15	0.00	0.00	0.13	0.00	0.00	0.00	23.13	0.00	0.00	0.00	23.52
41	14.43	0.00	68.59	16.95	17.65	0.00	0.00	16.76	0.00	0.00	80.45	54.56	0.00	0.00	0.00	49.45
42	53.47	6.34	0.00	65.71	74.76	0.00	28.38	65.44	0.00	0.00	13.72	22.77	0.00	0.00	56.43	23.27
43	18.71	4.98	0.00	11.26	32.54	0.00	52.25	33.75	0.00	0.00	63.31	33.98	0.00	0.00	0.00	33.47
44	69.12	13.31	0.00	110.50	133.97	0.00	152.25	104.69	0.00	0.00	0.00	125.71	0.00	16.86	0.00	119.17
45	36.88	0.00	0.00	19.36	14.49	0.00	0.00	14.22	0.00	0.00	0.00	44.65	0.00	75.08	0.00	41.51
46	81.78	0.00	86.57	28.40	18.88	0.00	0.00	18.67	0.00	0.00	0.00	65.46	97.93	0.00	0.00	60.64
47	48.18	0.00	0.00	12.08	12.68	0.00	0.00	13.30	0.00	0.00	42.36	113.56	0.00	0.00	0.00	112.86
48	57.37	0.00	0.00	23.83	13.64	0.00	0.00	13.70	0.00	0.00	0.00	8.54	0.00	0.00	0.00	0.00
49	25.99	0.00	0.00	20.33	15.85	0.00	0.00	15.16	0.00	0.00	0.00	18.27	0.00	0.00	0.00	17.35
50	72.46	0.00	0.00	38.68	9.45	0.00	0.00	9.85	0.00	0.00	82.61	44.55	0.00	0.00	0.00	41.49
52	49.22	0.00	0.00	41.52	9.11	0.00	0.00	9.35	0.00	12.06	0.00	92.15	0.00	0.00	0.00	90.85
53	42.22	0.00	0.00	35.62	16.74	0.00	0.00	15.97	0.00	21.28	0.00	162.64	0.00	0.00	0.00	166.98
54	53.38	0.00	0.00	22.18	15.87	0.00	0.00	15.32	0.00	0.00	0.00	7.94	0.00	0.00	0.00	0.00
55	26.90	6.63	0.00	79.49	83.06	0.00	44.81	75.67	0.00	0.00	0.00	11.94	0.00	0.00	0.00	10.72
56	26.76	0.00	0.00	36.12	8.16	0.00	0.00	8.81	0.00	0.00	50.48	39.99	0.00	0.00	0.00	38.31
57	67.57	0.00	0.00	28.07	0.15	0.00	0.00	0.13	0.00	0.00	0.00	10.06	0.00	0.00	0.00	0.00
58	22.13	0.00	0.00	29.87	14.04	0.00	0.00	14.20	0.00	0.00	41.75	33.07	0.00	0.00	0.00	32.32
59	88.18	0.00	0.00	29.42	17.68	0.00	0.00	17.24	0.00	0.00	0.00	38.66	0.00	0.00	0.00	38.07
60	19.95	0.00	0.00	37.68	17.53	0.00	0.00	16.73	0.00	0.00	65.08	56.12	0.00	0.00	0.00	54.55
63	57.14	0.00	0.00	18.63	16.69	0.00	0.00	15.93	0.00	0.00	19.37	85.07	0.00	0.00	0.00	78.01
66	22.39	5.80	0.00	0.00	56.62	0.00	0.11	46.47	0.00	13.99	0.00	40.91	0.00	0.00	0.00	38.31
69	94.47	0.00	10.15	31.35	11.47	0.00	0.00	11.43	0.00	0.00	0.00	74.50	0.00	0.00	0.00	67.19
72	17.69	4.58	0.00	0.00	25.85	0.00	0.08	27.39	0.00	11.05	0.00	32.32	0.00	0.00	0.00	30.37
73	13.79	6.38	0.00	59.27	75.55	0.00	30.67	66.82	87.42	0.00	0.00	32.31	0.00	0.00	0.00	30.24
74	32.66	0.00	0.00	36.86	16.19	0.00	0.00	15.68	0.00	0.00	0.00	153.27	0.00	0.00	0.00	160.36
77	38.07	0.00	0.00	42.97	0.19	0.00	0.00	8.47	0.00	0.00	0.00	86.84	0.00	0.00	0.00	79.85
78	63.88	5.75	0.00	58.31	54.11	0.00	28.80	44.83	0.00	0.00	0.00	53.42	0.00	0.00	0.00	48.25

plant #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
79	37.82	3.36	0.00	42.69	22.69	0.00	0.00	24.61	0.00	0.00	0.00	86.27	0.00	0.00	0.00	78.18
80	46.69	0.00	0.00	24.50	8.23	0.00	0.00	9.28	0.00	0.00	0.00	56.53	0.00	95.05	0.00	55.34
81	39.72	3.35	0.00	33.50	22.50	0.00	0.00	23.75	0.00	9.73	0.00	74.36	0.00	0.00	0.00	66.89
83	54.79	0.00	35.10	49.17	12.58	0.00	18.48	12.84	0.00	0.00	0.00	14.75	0.00	0.00	0.00	14.55
84	30.21	0.00	0.00	38.37	15.73	0.00	0.00	15.14	0.00	0.00	0.00	34.27	0.00	0.00	0.00	33.57
85	0.14	4.79	0.00	44.77	28.38	0.00	27.39	30.34	0.00	0.00	0.00	31.47	0.00	0.00	0.00	28.39
88	62.16	0.00	0.00	33.18	17.27	0.00	0.00	16.04	0.00	0.00	145.80	78.64	0.00	0.00	0.00	71.80
90	62.79	3.30	0.00	53.80	22.38	0.00	23.40	23.57	76.36	0.00	0.00	14.77	0.00	0.00	0.00	14.79
91	10.23	0.00	0.00	34.98	20.54	0.00	9.86	21.61	0.00	0.00	0.00	22.50	15.18	0.00	0.00	22.76
92	22.96	0.00	0.00	30.99	16.30	0.00	0.00	15.73	0.00	0.00	89.09	70.58	0.00	0.00	0.00	63.26
96	59.23	5.91	0.00	23.54	63.02	0.00	90.64	48.73	0.00	24.74	0.00	76.25	0.00	0.00	0.00	70.28
100	64.76	0.00	0.00	21.11	14.53	0.00	0.00	14.38	0.00	0.00	10.67	46.86	0.00	0.00	0.00	42.61
101	89.69	0.00	0.00	36.91	19.20	0.00	0.00	19.12	0.00	0.00	0.00	55.59	0.00	0.00	0.00	52.58
104	73.20	0.00	0.00	55.51	11.02	0.00	0.00	11.15	0.00	0.00	0.00	73.17	0.00	0.00	0.00	65.56
106	58.29	0.00	0.00	24.21	15.10	0.00	0.00	15.02	0.00	0.00	0.00	8.67	0.00	0.00	0.00	0.00
107	40.18	0.00	0.00	10.08	0.15	0.00	0.00	0.10	0.00	0.00	35.33	94.71	0.00	0.00	0.00	94.86
108	91.34	0.00	0.00	30.47	10.42	0.00	0.00	10.71	0.00	0.00	0.00	36.33	0.00	0.00	0.00	36.40
109	28.91	0.00	0.00	20.79	16.11	0.00	0.00	15.56	0.00	0.00	0.00	11.67	0.00	0.00	0.00	10.46
110	31.23	0.00	0.00	24.39	13.99	0.00	0.00	14.11	0.00	0.00	0.00	13.69	0.00	0.00	0.00	11.90
112	20.67	0.00	0.00	43.93	10.12	0.00	0.00	10.28	0.00	0.00	36.87	31.80	0.00	0.00	0.00	30.14
113	87.72	0.00	33.10	44.99	12.54	0.00	22.69	12.71	0.00	0.00	0.00	70.89	0.00	0.00	0.00	63.47
115	35.45	0.00	0.00	29.24	13.62	0.00	0.00	13.62	0.00	0.00	0.00	26.28	0.00	0.00	0.00	26.32
116	44.71	0.00	0.00	56.77	12.93	0.00	0.00	13.40	0.00	0.00	0.00	50.71	0.00	0.00	0.00	45.10
117	25.88	6.27	0.00	87.10	73.33	0.00	38.68	61.06	0.00	0.00	0.00	13.97	0.00	0.00	0.00	13.57
121	48.08	9.36	0.00	15.56	106.13	0.00	26.29	96.98	0.00	0.00	0.00	21.70	0.00	0.00	0.00	21.76
127	11.42	0.00	0.00	37.79	14.91	0.00	0.00	14.53	0.00	0.00	0.00	64.91	9.87	0.00	0.00	60.20
137	46.62	0.00	68.80	39.37	20.12	0.00	14.15	21.17	0.00	0.00	0.00	102.18	0.00	0.00	0.00	105.52
143	9.77	0.00	12.72	38.49	18.02	0.00	0.00	17.67	0.00	10.97	26.71	72.76	0.00	0.00	0.00	65.12
146	11.60	4.56	0.00	40.40	24.32	0.00	19.11	27.21	0.00	0.00	0.00	29.69	0.00	0.00	0.00	27.17
148	20.45	4.95	0.00	68.81	32.18	0.00	30.56	32.28	0.00	0.00	0.00	11.04	0.00	0.00	0.00	9.02
149	124.78	0.00	0.00	66.60	15.08	0.00	0.00	14.93	0.00	0.00	142.25	76.72	0.00	0.00	0.00	71.04
152	92.91	0.00	0.00	38.23	11.50	0.00	0.00	11.48	0.00	0.00	0.00	52.23	0.00	0.00	0.00	47.56
154	68.91	0.00	0.00	36.18	9.77	0.00	0.00	10.10	0.00	10.57	12.61	51.58	0.00	0.00	0.00	46.77
155	37.75	5.84	0.00	0.00	57.58	0.00	36.52	47.30	0.00	0.00	0.00	11.95	0.00	0.00	0.00	10.83
158	23.74	5.58	0.00	0.00	49.01	0.00	35.86	44.25	0.00	0.00	0.00	75.76	0.00	0.00	0.00	70.25
159	68.54	5.26	0.00	53.98	39.22	0.00	26.64	36.50	0.00	0.00	70413.00	156.77	0.00	0.00	0.00	162.92
161	33.65	6.35	0.00	33.79	74.86	0.00	30.43	66.08	0.00	12.65	0.00	14.33	0.00	0.00	0.00	13.90
164	13.55	6.93	0.00	37.18	92.82	0.00	52.18	80.16	0.00	0.00	0.00	39.84	0.00	0.00	0.00	38.15
165	79.90	6.13	0.00	62.92	69.86	0.00	31.06	53.60	0.00	0.00	39.66	88.82	0.00	0.00	0.00	84.29
167	93.48	6.68	0.00	93.32	83.19	0.00	56.66	76.39	0.00	0.00	0.00	90.22	0.00	0.00	49.76	85.16
169	29.47	6.51	0.00	11.02	81.19	0.00	94.82	72.28	0.00	0.00	0.00	83.75	0.00	0.00	0.00	75.61
177	16.56	0.00	19.87	22.58	20.08	0.00	0.00	20.84	0.00	0.00	0.00	79.26	0.00	42.55	0.00	72.41
194	49.87	0.00	0.00	27.75	9.28	0.00	0.00	9.48	0.00	11.48	0.00	81.45	0.00	0.00	0.00	72.50
198	75.26	5.23	0.00	37.45	38.81	0.00	20.98	36.22	0.00	0.00	0.00	100.90	0.00	0.00	0.00	101.69
200	39.13	6.06	0.00	10.31	66.77	0.00	35.57	51.43	14.70	0.00	0.00	10.88	0.00	0.00	0.00	0.00
204	10.12	0.00	13.18	39.87	10.90	0.00	0.00	10.92	0.00	11.36	27.67	68.37	0.00	0.00	0.00	62.25
205	35.26	6.79	0.00	56.37	87.53	0.00	77.66	77.98	0.00	0.00	0.00	64.12	0.00	13.85	0.00	57.89
207	84.99	0.00	0.00	28.40	17.84	0.00	0.00	17.30	0.00	81.63	55.84	58.50	0.00	0.00	0.00	55.96
211	37.09	5.28	0.00	47.50	40.04	0.00	31.49	38.13	0.00	0.00	0.00	9.16	0.00	0.00	0.00	0.00
212	36.77	5.89	0.00	56.99	57.87	0.00	35.23	48.13	0.00	91.38	0.00	10.11	0.00	0.00	0.00	0.00
219	48.30	6.28	0.00	0.00	73.73	0.00	23.38	64.02	0.00	0.00	0.00	49.88	0.00	0.00	0.00	44.57
220	23.79	0.00	0.00	89.56	2018.00	0.00	25.77	20.42	0.00	0.00	0.00	12.01	0.00	0.00	0.00	11.07
225	34.19	9.10	0.00	20.58	99.89	0.00	95.45	96.69	0.00	0.00	115.67	62.08	0.00	0.00	0.00	57.47
226	51.62	6.12	0.00	63.43	69.38	0.00	27.40	52.48	0.00	0.00	25.65	24.23	0.00	0.00	60.06	25.68
227	45.61	5.93	0.00	80.50	63.74	0.00	26.50	49.65	0.00	0.00	0.00	46.93	0.00	0.00	0.00	42.75

plant #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
230	11.81	0.00	35.23	56.88	11.52	0.00	0.00	11.52	0.00	0.00	26.39	71.18	38.81	0.00	0.00	64.54
236	11.40	0.00	34.01	54.91	19.31	0.00	0.00	19.57	0.00	0.00	25.48	75.76	41.31	0.00	0.00	70.25
238	34.23	5.32	0.00	46.93	40.75	0.00	29.55	38.89	11.93	92.42	0.00	33.43	0.00	0.00	0.00	33.46
240	25.50	6.60	0.00	0.00	81.43	0.00	0.12	72.44	0.00	15.93	0.00	46.60	0.00	0.00	0.00	42.36
241	37.24	5.89	0.00	0.00	58.25	0.00	26.95	48.15	0.00	20.17	0.00	68.07	0.00	0.00	0.00	62.12
242	19.98	0.00	45.98	43.26	18.39	0.00	0.00	18.30	0.00	0.00	0.00	55.18	0.00	0.00	0.00	52.32
243	22.66	6.17	0.00	99.90	70.69	0.00	30.90	53.64	0.00	0.00	0.00	97.53	0.00	0.00	0.00	98.78
244	27.85	5.36	0.00	44.53	42.55	0.00	61.36	39.43	0.00	0.00	0.00	50.66	0.00	10.94	0.00	44.88
245	34.02	6.42	0.00	34.16	75.56	0.00	30.76	67.85	0.00	12.78	0.00	14.49	0.00	0.00	0.00	14.04
246	50.57	5.50	0.00	86.65	47.50	0.00	32.43	42.48	77.36	92.49	0.00	89.44	0.00	0.00	0.00	84.67
247	32.34	5.90	0.00	17.28	63.00	0.00	27.24	48.26	0.00	31.94	0.00	21.94	0.00	0.00	0.00	22.14
248	32.26	0.00	0.00	48.91	17.92	0.00	0.00	17.63	0.00	0.00	0.00	73.68	0.00	0.00	0.00	66.59
249	28.07	6.98	0.00	0.00	94.28	0.00	37.97	81.04	13.72	10.82	0.00	12.63	0.00	0.00	0.00	11.31
250	41.25	5.66	0.00	93.23	53.81	0.00	25.83	44.64	0.00	0.00	0.00	17.32	0.00	0.00	0.00	16.60
251	23.28	5.15	0.00	8.70	37.70	0.00	74.90	35.77	0.00	0.00	0.00	66.17	0.00	0.00	0.00	60.94
252	84.13	6.27	0.00	39.18	73.65	0.00	27.68	62.43	0.00	0.00	75.00	20.31	0.00	0.00	0.00	19.98
253	12.05	0.00	0.00	39.87	9.37	0.00	0.00	9.83	0.00	0.00	0.00	68.47	10.42	0.00	0.00	62.59
254	53.09	0.00	0.00	12.07	20.36	0.00	11.54	21.28	0.00	0.00	25.84	45.01	0.00	0.00	0.00	42.26
255	11.73	3.16	0.00	0.00	22.19	0.00	15.24	23.27	0.00	0.00	0.00	11.40	0.00	0.00	0.00	10.35
256	52.19	5.68	0.00	89.43	53.97	0.00	33.47	44.66	79.84	95.46	0.00	92.31	0.00	0.00	0.00	94.61
257	42.51	6.36	0.00	0.00	75.51	0.00	86.98	66.44	0.00	0.00	0.00	10.66	0.00	0.00	0.00	0.00
258	46.51	7.36	0.00	0.00	96.44	0.00	33.66	85.01	0.00	25.19	0.00	85.02	0.00	0.00	0.00	77.54
259	28.24	5.80	0.00	0.00	57.13	0.00	33.19	47.11	0.00	0.00	0.00	15.27	0.00	0.00	0.00	15.64
260	36.03	4.69	0.00	63.60	26.87	0.00	20.94	29.43	0.00	0.00	0.00	37.07	0.00	0.00	0.00	37.16
261	10.71	5.47	0.00	29.37	45.62	0.00	41.22	41.02	0.00	0.00	0.00	31.48	0.00	0.00	0.00	28.88
262	41.04	0.00	0.00	22.84	14.54	0.00	0.00	14.46	0.00	9.45	0.00	67.03	0.00	0.00	0.00	61.61
263	33.59	5.03	0.00	0.00	33.06	0.00	68.72	34.30	0.00	0.00	0.00	8.42	0.00	0.00	0.00	0.00
264	75.42	11.28	0.00	0.00	109.23	0.00	154.31	98.39	0.00	0.00	0.00	12.11	0.00	0.00	0.00	11.11
265	54.34	5.75	0.00	13.03	54.15	0.00	14.78	45.28	0.00	12.28	37.05	23.43	0.00	0.00	0.00	24.37
266	24.09	6.48	0.00	0.00	79.86	0.00	31.29	71.71	0.00	0.00	0.00	23.40	0.00	0.00	0.00	23.97
267	46.95	6.68	0.00	60.13	87.15	0.00	39.85	77.73	0.00	0.00	0.00	11.59	0.00	0.00	0.00	10.38
268	18.36	3.35	0.00	9.81	22.63	0.00	15.46	24.61	0.00	8.81	0.00	13.17	0.00	0.00	0.19	11.42
269	14.28	6.61	0.00	61.40	82.73	0.00	31.76	72.77	90.55	0.00	0.00	30.35	0.00	0.00	0.00	27.63
270	59.59	11.48	0.00	95.26	121.95	0.00	131.25	99.10	0.00	0.00	0.00	108.37	0.00	14.53	0.00	108.58
271	32.90	5.54	0.00	47.41	48.07	0.00	27.82	43.03	0.00	24.17	0.00	30.62	0.00	0.00	0.00	28.07
272	38.35	6.45	0.00	55.27	78.21	0.00	32.43	70.50	0.00	13.69	0.00	17.35	0.00	0.00	0.00	16.71
273	29.36	4.56	0.00	40.26	25.63	0.00	25.35	27.22	10.23	163.12	0.00	59.01	0.00	0.00	0.00	56.72
274	48.29	0.00	71.27	40.78	12.32	0.00	14.66	11.80	0.00	0.00	0.00	96.01	0.00	0.00	0.00	96.07
275	31.99	5.12	0.00	49.58	37.47	33.46	30.65	126.90	0.00	79.50	0.00	10.72	0.00	0.00	0.00	0.00
276	14.95	0.00	71.05	17.56	10.33	0.00	0.00	10.36	0.00	0.00	83.33	51.27	0.00	0.00	0.00	46.43
277	26.38	6.20	0.00	0.00	72.70	0.00	39.86	55.03	0.00	0.00	0.00	84.21	0.00	0.00	0.00	75.67
278	35.39	4.85	0.00	79.98	30.13	0.00	22.16	31.16	0.00	0.00	0.00	30.57	0.00	0.00	0.00	27.76
279	41.44	5.39	0.00	0.00	44.04	0.00	20.06	40.87	0.00	0.00	0.00	88.04	0.00	0.00	0.00	83.02
280	26.97	5.54	0.00	0.00	48.71	0.00	31.69	43.11	0.00	0.00	0.00	14.58	0.00	0.00	0.00	14.10
281	37.70	6.88	0.00	20.15	90.67	0.00	31.75	79.27	0.00	18.10	0.00	12.43	0.00	0.00	0.00	11.12
282	10.57	4.72	0.00	41.06	27.64	0.00	26.43	29.75	0.00	0.00	0.00	64.69	0.00	0.00	0.00	59.78
283	34.37	6.49	0.00	34.51	80.18	0.00	31.08	72.23	0.00	12.92	0.00	14.64	0.00	0.00	0.00	14.27
284	42.78	0.00	0.00	23.81	16.77	0.00	0.00	16.01	0.00	20.26	0.00	143.77	0.00	0.00	0.00	119.30
285	16.79	5.60	0.00	63.94	49.79	46.10	42.95	135.08	0.00	0.00	0.00	27.78	0.00	0.00	0.00	27.03
286	33.02	6.43	0.00	10.69	75.76	0.00	18.06	67.86	0.00	0.00	0.00	14.90	0.00	0.00	0.00	15.59
287	90.25	6.45	0.00	90.09	77.33	0.00	54.70	69.56	0.00	0.00	0.00	96.02	0.00	0.00	52.96	96.66
288	72.17	5.38	0.00	33.61	43.94	0.00	23.74	39.49	0.00	0.00	132.37	35.85	0.00	0.00	0.00	34.01
289	45.63	13.01	0.00	0.00	130.80	0.00	64.83	99.43	0.00	23.54	0.00	20.62	0.00	0.00	0.00	21.16
290	10.33	0.00	0.00	34.20	16.89	0.00	0.00	16.01	0.00	0.00	0.00	120.85	18.38	0.00	0.00	118.29
291	71.62	6.01	0.00	62.63	64.40	0.00	26.70	50.08	0.00	0.00	0.00	78.21	0.00	0.00	0.00	71.76

plant #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
292	20.70	0.00	47.62	44.81	10.99	0.00	0.00	11.05	0.00	0.00	0.00	51.85	0.00	0.00	0.00	47.54
293	54.16	0.00	0.00	26.98	11.75	0.00	0.00	11.75	0.00	0.00	0.00	53.70	0.00	0.00	0.00	48.49
294	32.54	4.33	0.00	41.71	23.07	0.00	28.20	25.96	0.00	9.36	0.00	18.78	23.80	0.00	0.00	18.05
295	22.17	5.52	0.00	0.00	47.66	0.00	30.00	42.77	10.84	8.54	0.00	9.98	0.00	0.00	0.00	0.00
296	60.48	9.39	0.00	82.93	108.61	0.00	52.22	97.84	21.08	163.30	0.00	59.07	0.00	0.00	0.00	57.05
297	74.19	6.23	0.00	64.88	73.26	0.00	27.66	58.95	0.00	0.00	0.00	73.49	0.00	0.00	0.00	66.03
298	42.93	4.54	0.00	10.29	24.16	0.00	11.68	27.06	0.00	9.70	29.27	18.51	0.00	0.00	0.00	17.75
299	84.71	0.00	89.67	29.41	11.07	0.00	0.00	11.29	0.00	0.00	0.00	61.50	92.01	0.00	0.00	57.22
300	59.69	6.06	0.00	46.06	68.31	0.00	47.00	51.73	0.00	0.00	0.00	75.39	0.00	0.00	0.30	68.70
301	12.77	0.00	57.93	13.91	11.68	0.00	0.00	11.69	0.00	0.00	0.00	82.69	0.00	0.00	0.00	75.45
302	59.12	0.00	0.00	31.04	17.33	0.00	0.00	16.09	0.00	18.66	22.25	91.05	0.00	0.00	0.00	86.96
303	14.72	6.44	0.00	65.56	76.37	0.00	30.53	68.76	14.36	0.00	0.00	18.16	0.00	0.00	0.00	17.13
304	56.08	5.94	0.00	13.45	63.98	0.00	15.25	49.91	0.00	12.67	38.24	24.18	0.00	0.00	0.00	25.19
305	68.24	5.09	0.00	31.78	36.50	0.00	22.45	35.26	0.00	0.00	60.84	16.48	0.00	0.00	0.00	15.97
306	19.03	5.12	0.00	0.00	37.52	0.00	24.72	35.53	0.00	0.00	0.00	18.49	0.00	0.00	0.00	17.49
307	25.55	6.19	0.00	85.97	71.10	0.00	38.18	54.55	0.00	0.00	0.00	13.79	0.00	0.00	0.00	11.95
308	27.73	4.68	0.00	41.45	26.42	0.00	62.70	28.65	0.00	0.00	0.00	10.74	0.00	0.00	0.00	0.00
309	19.28	5.50	0.00	0.00	47.06	0.00	27.39	42.39	0.00	9.95	0.00	8.71	0.00	0.00	0.00	0.00
310	19.72	4.36	0.00	7.37	23.25	0.00	63.43	26.38	0.00	0.00	0.00	56.03	0.00	0.00	0.00	54.01
311	10.11	0.00	44.95	23.09	11.59	0.00	0.00	11.53	0.00	0.00	0.00	51.00	0.00	0.00	0.00	45.35
312	39.05	6.05	0.00	10.29	66.59	0.00	35.50	51.23	14.67	0.00	0.00	10.86	0.00	0.00	0.00	0.00
313	19.44	5.30	0.00	85.70	40.62	0.00	26.51	38.45	0.00	0.00	0.00	172.14	0.00	0.00	0.00	170.61
314	37.27	4.85	0.00	65.77	28.38	0.00	21.65	30.46	0.00	0.00	0.00	49.74	0.00	0.00	0.00	44.15
315	30.36	4.56	0.00	58.60	25.69	0.00	26.11	27.36	0.00	10.62	0.00	64.46	0.00	0.00	0.00	58.49
316	70.67	0.00	0.00	53.59	18.53	0.00	0.00	18.45	0.00	0.00	0.00	77.87	0.00	0.00	0.00	71.48
317	23.47	6.29	0.00	0.00	74.40	0.00	13.65	64.96	11.47	13.44	0.00	11.05	0.00	0.00	0.00	9.40
318	38.43	5.78	0.00	74.18	55.63	0.00	33.05	46.25	0.00	13.44	0.00	81.59	0.00	0.00	0.00	74.43
319	26.09	5.08	0.00	8.44	35.33	0.00	14.26	35.19	0.00	0.00	0.00	11.77	0.00	0.00	0.00	10.57
320	35.48	5.49	0.00	0.00	47.02	0.00	34.33	42.22	0.00	0.00	0.00	11.23	0.00	0.00	0.00	9.91
321	28.03	4.33	0.00	0.00	23.12	0.00	27.12	26.27	0.00	0.00	0.00	8.87	0.00	0.00	0.00	0.00
322	91.20	0.00	9.80	30.27	19.06	0.00	0.00	19.02	0.00	0.00	0.00	79.29	0.00	0.00	0.00	72.42
323	14.80	2.03	0.00	33.44	21.93	0.00	9.26	22.37	0.00	0.00	0.00	6.21	0.00	6.67	0.00	0.00
324	14.21	6.22	0.00	63.29	72.94	0.00	29.47	57.32	13.86	0.00	0.00	19.33	0.00	0.00	0.00	19.21
325	30.56	5.14	0.00	44.05	37.65	0.00	25.85	35.55	0.00	10.91	34.57	13.82	0.00	0.00	0.00	12.61
326	12.14	7.79	0.00	0.00	97.78	0.00	39.69	90.77	0.00	0.00	0.00	95.91	0.00	0.00	25.98	95.78
327	47.50	7.14	0.00	91.69	94.40	0.00	40.85	81.09	0.00	16.61	0.00	100.85	0.00	0.00	0.00	100.96
328	23.69	6.30	0.00	14.26	74.54	0.00	66.13	65.20	0.00	0.00	80.14	43.01	0.00	0.00	0.00	40.77
329	40.47	0.00	0.00	0.65	21.72	0.00	0.00	121.93	0.00	0.00	0.00	30.24	0.00	0.00	0.00	27.55
330	20.84	4.90	0.00	0.00	31.06	0.00	31.49	31.43	0.00	0.00	0.00	66.52	0.00	0.00	0.00	61.52
331	31.54	5.05	0.00	48.89	35.01	0.00	30.22	34.68	0.00	161.28	0.00	17.85	0.00	0.00	0.00	16.87
332	21.61	7.21	0.00	82.29	94.76	59.32	55.27	150.10	0.00	0.00	0.00	35.75	0.00	0.00	0.00	33.87
333	63.33	9.01	0.00	81.11	99.61	0.00	53.76	91.70	0.00	0.00	0.00	15.64	0.00	0.00	0.00	15.70
334	43.89	4.46	0.00	33.87	23.90	0.00	34.56	26.94	0.00	0.00	0.00	55.44	0.00	0.00	0.22	52.39
335	52.29	0.00	0.00	26.04	19.64	0.00	0.00	20.27	0.00	0.00	0.00	57.16	0.00	0.00	0.00	55.50
336	24.28	6.04	0.00	0.00	65.36	0.00	32.85	50.49	11.87	9.36	0.00	10.92	0.00	0.00	0.00	0.00
337	77.95	5.42	0.00	38.79	44.73	0.00	21.73	40.97	0.00	0.00	0.00	94.80	0.00	0.00	0.00	95.54
338	103.86	14.78	0.00	133.02	134.34	0.00	88.17	106.05	0.00	0.00	0.00	25.65	3.35	0.00	0.56	25.96
339	12.33	0.00	55.92	13.43	19.48	0.00	0.00	20.16	0.00	0.00	0.00	88.00	0.00	0.00	0.00	80.34
340	29.42	4.65	0.00	0.00	26.00	0.00	21.29	28.03	0.00	15.93	0.00	53.77	0.00	0.00	0.00	49.00
341	42.50	5.53	0.00	6.65	47.77	0.00	20.57	42.87	0.00	0.00	0.00	43.89	0.00	0.00	0.00	41.39
342	11.72	7.52	0.00	0.00	96.97	0.00	38.32	89.62	0.00	0.00	0.00	102.08	0.00	0.00	27.65	104.65
343	14.06	7.18	0.00	38.56	94.64	0.00	54.11	82.89	0.00	0.00	0.00	41.32	0.00	0.00	0.00	38.72
344	78.56	6.10	42.71	29.43	69.17	0.00	18.68	51.88	0.00	0.00	0.00	54.82	0.00	0.00	0.00	49.54
345	35.10	5.93	0.00	52.47	63.07	0.00	79.37	49.00	0.00	0.00	0.00	13.60	0.00	0.00	0.00	11.83
346	43.07	7.28	0.00	64.38	95.90	0.00	97.39	84.34	0.00	0.00	0.00	16.69	0.00	0.00	0.00	16.30

plant #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
347	13.27	4.42	0.00	50.51	23.33	36.42	33.93	133.52	0.00	0.00	0.00	21.95	0.00	0.00	0.00	22.26
348	41.19	5.48	0.00	52.80	46.12	0.00	35.69	41.63	0.00	11.85	0.00	23.77	30.12	0.00	0.00	24.70
349	25.92	4.90	0.00	0.00	30.94	0.00	69.72	31.31	14.92	0.00	0.00	10.99	0.00	23.16	0.00	0.02
350	28.06	4.46	0.00	8.46	24.12	0.00	30.18	27.05	8.25	0.00	0.00	8.02	0.00	0.00	0.00	0.00
351	80.96	3.79	0.00	64.76	22.73	0.00	13.81	25.58	0.00	0.00	0.00	76.38	15.38	0.00	0.00	70.31
352	76.31	4.91	0.00	30.77	32.05	0.00	18.17	32.11	0.00	0.00	0.00	58.27	0.00	0.00	0.00	55.55
353	39.01	0.00	0.00	0.63	12.66	0.00	0.00	12.85	0.00	0.00	0.00	29.16	0.00	0.00	0.00	27.14
354	32.90	6.22	0.00	0.00	72.89	0.00	88.52	56.01	18.94	0.00	0.00	13.95	0.00	29.40	0.00	13.37
355	18.54	4.97	0.00	0.00	32.51	0.00	10.78	32.86	9.06	10.62	0.00	8.73	0.00	0.00	0.00	0.00
356	35.52	5.65	0.00	10.71	53.70	0.00	38.20	44.47	10.44	0.00	0.00	10.15	0.00	0.00	0.00	0.00
357	33.45	4.45	0.00	42.87	23.89	0.00	28.98	26.45	0.00	9.62	0.00	19.30	24.46	0.00	0.00	18.77
358	32.44	5.16	0.00	9.78	38.65	0.00	34.89	36.07	9.53	0.00	0.00	9.27	0.00	0.00	0.00	0.00
359	84.69	0.00	31.95	43.43	21.38	0.00	21.91	22.05	0.00	0.00	0.00	75.44	0.00	0.00	0.00	69.05
360	14.69	5.77	0.00	51.14	55.24	0.00	24.19	46.06	0.00	0.00	0.00	37.59	0.00	0.00	0.00	37.56

plant #	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	0.00	9.50	0.00	0.00	0.00	0.00	0.00	0.00	56.59	0.00	17.76	22.88	64.81	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.18	0.00	35.98	0.00	75.16	16.14	0.00
3	0.00	0.00	0.00	0.00	20.99	0.00	0.00	0.00	18.39	0.00	48.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.18	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.12	0.00	19.18	22.63	0.00	0.00	0.00
7	0.00	12.03	0.00	0.00	0.00	0.02	0.00	0.00	57.02	0.00	22.49	28.96	82.04	76.95	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	44.75	43.30	26.37	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.12	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.36	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.19	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	66.14	0.00	20.76	0.00	0.00	0.00	0.00
18	11.08	35.99	0.00	0.00	0.00	64.80	0.00	0.00	65.07	0.00	0.00	0.00	0.00	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.14	0.00	12.46	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.19	66.65	0.00	0.00	0.00	33.56	0.00	0.00
26	0.00	21.10	0.00	0.00	0.00	53.25	0.00	0.00	61.09	0.00	0.00	0.00	0.00	0.00	0.00
27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	53.54	0.00	0.00	0.00	0.00	0.00	0.00
28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.67	0.00	19.52	0.00	0.00	0.00	0.00
29	0.00	12.62	0.00	0.00	0.00	41.77	0.00	0.00	58.08	0.00	23.59	30.38	86.06	97.40	0.00
32	0.00	0.00	0.00	12.28	0.00	0.00	61.56	0.00	12.80	0.00	0.00	0.00	0.00	0.00	0.00
34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.11	0.00	61.87	0.00	0.00	0.00	0.00
35	0.00	17.11	0.00	0.00	0.00	42.07	0.00	0.00	60.53	0.00	31.99	0.00	116.69	110.65	0.00
36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.40	0.00	20.20	0.00	0.00	0.00	0.00
39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.60	0.00	15.88	0.00	0.00	0.00	77.34
41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.91	0.00	28.08	0.00	0.00	0.00	0.00
42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.52	0.00	0.00	0.00	0.00	0.00	0.00
43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.38	0.00	24.04	0.00	0.00	0.00	0.00
44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	94.15	95.64	0.00	0.00	0.00	25.78	0.00	0.00
45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.96	49.60	30.21	0.00	0.00	0.00	0.00
46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.68	0.00	0.00	0.00	0.00	0.00	0.00
47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.25	0.00	88.96	0.00	0.00	0.00	0.00
48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	92.06	0.00	38.35	0.00	0.00
49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.87	0.00	12.55	0.00	0.00	0.00	61.10
50	0.00	0.00	0.00	4.56	0.00	0.00	0.00	0.00	17.76	0.00	26.14	0.00	0.00	0.00	0.00
52	0.00	0.00	0.00	3.46	0.00	0.00	0.00	0.00	39.54	0.00	0.00	0.00	0.00	0.00	0.00
53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	54.70	0.00	0.00	0.00	0.00	0.00	0.00
54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	69.33	0.00	28.88	0.00	0.00
55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.35	0.00	0.00	0.00	0.00

plant #	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.69	0.00	61.92	0.00	0.00	0.00	0.00
57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	87.76	0.00	36.56	0.00	0.00
58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.27	0.00	51.21	0.00	0.00	0.00	0.00
59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.20	0.00	14.58	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.63	0.00	0.00	0.00	0.00	0.00	0.00
63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.78	0.00	51.12	0.00	0.00	0.00	0.00
66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.87	0.00	21.44	0.00	0.00	0.00	0.00
69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.03	0.00	13.99	0.00	0.00	0.00	0.00
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.15	0.00	16.93	0.00	0.00	0.00	0.00
73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.11	0.00	0.00	0.00	0.00	0.00	0.00
74	0.00	0.00	0.00	0.00	23.35	0.00	89.20	0.00	53.65	76.37	35.01	0.00	0.00	0.00	0.00
77	0.00	0.00	0.00	0.00	21.92	0.00	83.76	0.00	33.94	71.71	32.87	0.00	0.00	0.00	0.00
78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.58	0.00	0.00	0.00	12.33	0.00	0.00
79	0.00	0.00	0.00	0.00	23.24	0.00	88.78	0.00	33.54	84.62	38.79	0.00	0.00	0.00	0.00
80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.68	40.65	24.77	0.00	0.00	0.00	0.00
81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.57	0.00	0.00	0.00	0.00	0.00	0.00
83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	31.58	0.00	20.40	0.00	0.00
84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.55	0.00	9.84	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.76	0.00	11.69	0.00	0.00	0.00	0.00
88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.82	0.00	27.83	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.14	0.00	0.00	0.00	0.00	0.00	0.00
91	0.00	0.00	0.00	13.07	0.00	0.00	0.00	16.30	77.47	0.00	0.00	0.00	13.18	0.00	0.00
92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.68	0.00	65.94	0.00	0.00	0.00	0.00
96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.40	0.00	0.00	0.00	0.00	0.00	0.00
100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.35	0.00	46.66	0.00	0.00	0.00	0.00
101	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.48	0.00	0.00	0.00	0.00	0.00	0.00
104	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.06	0.00	0.00	0.00	0.00	0.00	0.00
106	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	106.79	0.00	44.49	0.00	0.00
107	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	41.72	0.00	78.31	0.00	0.00	0.00	0.00
108	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.11	0.00	40.04	0.00	0.00	0.00	0.00
109	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.16	17.87	0.00	0.00	0.00
110	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.49	0.00	22.64	26.70	0.00	0.00	0.00
112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.09	0.00	0.00	0.00	0.00	0.00	0.00
113	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.15	63.66	25.55	0.00	0.00	0.00	0.00
115	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.10	0.00	18.04	0.00	0.00	0.00	87.86
116	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.39	0.00	14.15	0.00	0.00	0.00	0.00
117	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.75	0.00	46.95	0.00	0.00	0.00	0.00
121	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.90	0.00	72.19	0.00	14.40	0.00	0.00
127	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.60	0.00	0.00	0.00	53.83	0.00	0.00
137	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	51.42	0.00	0.00	0.00	12.84	0.00	0.00
143	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.96	0.00	54.75	0.00	0.00	0.00	0.00
146	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.47	0.00	22.91	0.00	0.00	0.00	0.00
148	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.09	0.00	0.00	0.00	0.00
149	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.44	0.00	45.00	0.00	55.43	0.00	0.00
152	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.39	0.00	0.00	0.00	0.00	0.00	0.00
154	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.89	0.00	26.69	0.00	0.00	0.00	0.00
155	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	105.32	0.00	0.00	0.00	0.00
158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.38	0.00	25.81	0.00	0.00	0.00	0.00
159	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	54.30	0.00	47.59	0.00	0.00	0.00	0.00
161	0.00	3.35	0.00	0.00	0.00	0.00	0.00	0.00	55.68	0.00	15.10	0.00	42.73	0.00	0.00
164	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.21	0.00	26.52	0.00	0.00	0.00	0.00
165	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.66	0.00	44.68	0.00	0.00	0.00	0.00
167	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.73	0.00	0.00	0.00	0.00	0.00	0.00
169	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.55	72.15	0.00	0.00	0.00	0.00	0.00	0.00
177	0.00	22.45	0.00	0.00	0.00	55.86	0.00	0.00	63.18	0.00	0.00	0.00	0.00	0.00	0.00

plant #	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
194	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.92	0.00	0.00	0.00	23.45	0.00	0.00
198	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	49.80	0.00	46.03	0.00	15.48	0.00	0.00
200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.77	75.19	0.00	0.00	0.00	48.26	0.00	0.00
204	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.58	0.00	31.19	0.00	0.00	0.00	0.00
205	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.37	91.56	0.00	0.00	0.00	21.18	0.00	0.00
207	11.79	38.31	0.00	0.00	0.00	85.49	0.00	0.00	65.32	0.00	0.00	0.00	0.00	0.00	0.00
211	0.00	0.00	0.00	0.00	0.00	0.00	0.00	46.81	79.90	0.00	69.65	0.00	0.00	0.00	0.00
212	0.00	0.00	0.00	0.00	0.00	0.00	0.00	63.09	87.67	0.00	13.51	0.00	0.00	0.00	0.00
219	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.32	0.00	0.00	0.00	0.00	0.00	0.00
220	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
225	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.27	0.00	34.57	0.00	0.00	0.00	0.00
226	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.07	0.00	0.00	0.00	0.00	0.00	0.00
227	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.36	0.00	25.43	0.00	0.00	0.00	0.00
230	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.27	0.00	39.49	0.00	0.00	0.00	0.00
236	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.26	0.00	0.00	0.00	0.00	0.00	0.00
238	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.32	0.00	84.87	0.00	0.00	0.00	0.00
240	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.27	0.00	24.35	0.00	0.00	0.00	0.00
241	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.43	0.00	0.00	0.00	0.00	0.00	0.00
242	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.14	0.00	0.00	0.00	0.00	0.00	0.00
243	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	44.57	0.00	0.00	0.00	0.00	0.00	0.00
244	0.00	0.00	0.00	0.00	0.00	0.00	0.00	61.12	81.88	0.00	0.00	0.00	16.74	0.00	0.00
245	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.76	0.00	18.42	0.00	52.14	0.00	0.00
246	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.11	0.00	78.89	0.00	0.00	0.00	0.00
247	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.93	0.00	0.00	0.00	0.00	0.00	0.00
248	0.00	0.00	40.12	0.00	0.00	0.00	0.00	0.00	26.97	0.00	41.16	0.00	0.00	0.00	0.00
249	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.11	0.00	0.00	0.00	0.00	0.00	0.00
250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.66	0.00	51.29	0.00	0.00	0.00	0.00
251	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.13	68.98	0.00	0.00	0.00	0.00	0.00	0.00
252	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.70	0.00	40.09	0.00	64.92	0.00	0.00
253	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.64	0.00	0.00	0.00	44.12	0.00	0.00
254	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.05	0.00	0.00	0.00	0.00	0.00	0.00
255	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	34.15	0.00	0.00	0.00	0.00
256	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.37	0.00	81.42	0.00	0.00	0.00	0.00
257	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	69.93	12.75	0.00
258	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.57	0.00	0.00	0.00	0.00	0.00	0.00
259	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.25	0.00	0.00	0.00	0.00	0.00	0.00
260	81.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.15	0.00	20.09	0.00	0.00	0.00	0.00
261	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.02	0.00	20.95	0.00	0.00	0.00	0.00
262	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.25	0.00	0.00	0.00	19.30	0.00	0.00
263	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	55.25	0.00	0.00
264	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	79.44	44.47	0.00
265	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.69	0.00	77.03	0.00	0.00	0.00	0.00
266	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.65	0.00	28.94	0.00	0.00	0.00	0.00
267	0.00	0.00	0.00	0.00	0.00	0.00	0.00	59.25	80.74	0.00	88.17	0.00	0.00	0.00	0.00
268	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.77	0.00	0.00	0.00	0.00	0.00	0.00
269	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.57	0.00	81.04	0.00	0.00	0.00	0.00
270	0.00	0.00	0.00	0.00	0.00	0.00	0.00	81.16	91.77	0.00	0.00	0.00	22.22	0.00	0.00
271	0.00	0.00	0.00	0.00	0.00	0.00	0.00	98.50	97.51	0.00	20.31	0.00	0.00	0.00	0.00
272	0.00	0.00	0.00	0.00	0.00	0.00	0.00	92.49	93.57	0.00	19.07	0.00	0.00	0.00	0.00
273	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.16	0.00	90.39	0.00	0.00	0.00	0.00
274	0.00	0.00	0.00	0.00	0.00	0.00	0.00	91.24	93.37	0.00	63.98	0.00	44.05	0.00	0.00
275	0.00	0.00	0.00	0.00	0.00	0.00	0.00	66.88	87.85	0.00	15.94	0.00	0.00	0.00	0.00
276	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.68	0.00	0.00	0.00	0.00	0.00	0.00
277	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.98	0.00	21.87	0.00	0.00	0.00	0.00
278	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.66	0.00	54.63	0.00	0.00	0.00	0.00

plant #	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
279	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.33	0.00	0.00	0.00	0.00	0.00	0.00
280	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.78	0.00	0.00	0.00	0.00	0.00	0.00
281	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
282	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.64	76.88	0.00	0.00	0.00	12.65	0.00	0.00
283	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.93	0.00	19.11	0.00	54.09	0.00	0.00
284	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.48	0.00	0.00	0.00	24.98	0.00	0.00
285	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.25	0.00	26.63	0.00	0.00	0.00	0.00
286	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.21	0.00	63.55	0.00	12.67	0.00	0.00
287	0.00	0.00	0.00	0.00	0.00	0.00	64.16	0.00	43.48	0.00	0.00	0.00	0.00	0.00	0.00
288	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.07	0.00	42.69	0.00	69.14	0.00	0.00
289	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.76	0.00	0.00	0.00	0.00	0.00	0.00
290	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.26	0.00	0.00	0.00	46.99	0.00	0.00
291	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.71	0.00	21.06	0.00	0.00	0.00	0.00
292	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.34	0.00	17.31	0.00	0.00	0.00	0.00
293	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.72	0.00	0.00	0.00	0.00	0.00	0.00
294	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.06	0.00	58.92	0.00	44.24	0.00	0.00
295	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
296	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.24	0.00	149.97	0.00	0.00	0.00	0.00
297	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.09	77.96	0.00	0.00	0.00	0.00	0.00	0.00
298	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.01	0.00	60.85	0.00	0.00	0.00	0.00
299	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.26	0.00	26.95	0.00	0.00	0.00	0.00
300	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.17	0.00	74.14	0.00	0.00	0.00	0.00
301	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.23	0.00	52.53	0.00	0.00	0.00	0.00
302	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	39.25	0.00	28.42	0.00	0.00	0.00	0.00
303	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.74	0.00	29.24	0.00	11.71	0.00	0.00
304	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.74	0.00	87.50	0.00	0.00	0.00	0.00
305	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.27	0.00	48.90	0.00	79.20	19.05	0.00
306	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.92	0.00	22.86	0.00	0.00	0.00	0.00
307	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.53	0.00	55.40	0.00	0.00	0.00	0.00
308	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.25	0.00	0.00	0.00	0.00
309	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
310	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.12	75.72	0.00	0.00	0.00	0.00	0.00	0.00
311	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.42	0.00	0.00	0.00	0.00	0.00	0.00
312	0.00	0.00	3.27	0.00	0.00	0.00	0.00	10.36	71.89	0.00	0.00	0.00	42.48	0.00	0.00
313	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	55.66	0.00	0.00	0.00	0.00	0.00	0.00
314	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.30	0.00	30858.0	0.00	0.00	0.00	0.00
315	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.57	0.00	0.00	0.00	0.00	0.00	0.00
316	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.45	0.00	23.49	0.00	67.38	0.00	0.00
317	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.68	0.00	66.16	0.00	0.00
318	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.07	0.00	0.00	0.00	0.00	0.00	0.00
319	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	50.20	0.00	10.01	0.00	0.00
320	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	92.71	0.00	0.00	0.00	0.00
321	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	73.24	0.00	0.00	0.00	0.00
322	0.00	0.00	0.00	0.00	0.00	0.00	0.00	95.09	97.09	0.00	66.68	0.00	45.90	0.00	0.00
323	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	62.58	0.00	0.00	0.00	0.00
324	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.62	0.00	0.00	0.00	0.00	0.00	0.00
325	0.00	0.00	0.00	0.00	0.00	0.00	0.00	112.83	103.40	0.00	23.26	0.00	0.00	0.00	0.00
326	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	42.94	0.00	22.54	0.00	64.65	0.00	0.00
327	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	49.02	0.00	0.00	0.00	0.00	0.00	0.00
328	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.14	0.00	30.43	0.00	0.00	0.00	0.00
329	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.53	0.00	45.81	0.00	15.41	0.00	0.00
330	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.14	0.00	17.28	0.00	0.00	0.00	0.00
331	0.00	0.00	0.00	0.00	0.00	0.00	0.00	67.19	89.30	0.00	14.38	0.00	0.00	0.00	0.00
332	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.67	0.00	31.42	0.00	0.00	0.00	0.00
333	72.34	0.00	0.00	0.00	0.00	0.00	0.00	62.16	85.68	0.00	92.49	0.00	0.00	0.00	0.00

plant #	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
334	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.38	0.00	54.51	0.00	0.00	0.00	0.00
335	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.83	66.35	26.63	0.00	0.00	0.00	0.00
336	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
337	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	42.62	0.00	18.73	0.00	0.00	0.00	0.00
338	0.00	0.00	0.00	0.00	0.00	0.00	0.00	72.10	89.99	0.00	107.29	0.00	0.00	0.00	0.00
339	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	35.59	0.00	21.64	0.00	0.00	0.00	0.00
340	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.86	0.00	0.00	0.00	0.00	0.00	0.00
341	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.49	0.00	0.00	0.00	0.00	0.00	0.00
342	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	50.37	0.00	32.91	0.00	21.26	0.00	0.00
343	0.00	0.00	44.57	0.00	0.00	0.00	0.00	0.00	16.90	0.00	31.29	0.00	0.00	0.00	0.00
344	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.14	0.00	39.42	0.00	0.00	0.00	0.00
345	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.26	0.00	31.96	0.00	0.00	0.00	45.99
346	0.00	0.00	2.35	0.00	0.00	0.00	0.00	0.00	11.63	0.00	36.30	0.00	0.00	0.00	0.00
347	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.33	0.00	21.04	0.00	0.00	0.00	0.00
348	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.73	0.00	74.58	0.00	56.00	0.00	0.00
349	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.14	0.00	0.00	0.00	0.00
350	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.21	70.98	0.00	0.00	0.00	0.00	0.00	0.00
351	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	29.18	0.00	0.00	0.00	0.00	0.00	0.00
352	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.85	0.00	0.00	0.00	0.00	0.00	0.00
353	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.26	0.00	44.16	0.00	14.85	0.00	0.00
354	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.71	0.00	57.72	0.00	0.00	0.00	0.00
355	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.02	0.00	52.27	0.00	0.00
356	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.66	75.14	0.00	0.00	0.00	0.00	0.00	0.00
357	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.41	0.00	84.73	0.00	63.62	0.00	0.00
358	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.24	72.09	0.00	0.00	0.00	0.00	0.00	0.00
359	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.24	0.00	82.22	0.00	0.00	0.00	0.00
360	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.19	0.00	29.00	0.00	0.00	0.00	0.00

plant #	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**
1	0.00	0.00	33.25	0.00	0.00	9.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	23.78	0.00	52.68	11.54	0.00	13.72	0.00	0.00	89.13	49.97	0.00	0.00	0.00	0.00	92.18
3	0.00	0.00	16.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	21.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	12.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	33.86	0.00	0.00	10.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	23.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	15.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	14.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	10.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.00	11.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	25.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	0.00	0.00	9.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	15.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	42.21	0.00	66.74	27.94	0.00	15.50	0.00	0.00	11.56	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	0.00	24.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	0.00	0.00	23.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28	0.00	0.00	18.06	0.00	0.00	0.00	0.00	0.00	9.18	0.00	0.00	0.00	0.00	0.00	0.00
29	0.00	0.00	34.18	0.00	0.00	10.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32	0.00	0.00	13.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
34	0.00	0.00	20.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35	0.00	0.00	34.40	0.00	0.00	10.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36	0.00	0.00	16.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39	0.00	50.70	13.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
41	0.00	0.00	18.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

plant #	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
42	11.81	0.00	39.77	11.11	19.91	25.51	0.00	0.00	80.06	36.46	0.00	0.00	0.00	16.94	87.37
43	0.00	0.00	14.70	0.00	40.09	51.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
44	12.87	0.00	41.58	24.02	23.96	35.86	0.00	0.00	30.04	0.00	0.00	0.00	0.00	0.00	33.90
45	0.00	0.00	16.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
46	0.00	0.00	19.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	0.00	0.00	23.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	0.00	0.00	30.79	0.00	0.00	7.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
49	0.00	40.05	12.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	15.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
52	0.00	0.00	23.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
53	0.00	0.00	24.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
54	0.00	0.00	29.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
55	0.00	0.00	10.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
56	0.00	0.00	15.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
57	0.00	0.00	30.09	0.00	0.00	17.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
58	0.00	0.00	14.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
59	0.00	0.00	15.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	18.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
63	0.00	0.00	22.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
66	0.00	0.00	15.61	0.00	12.82	20.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
69	0.00	0.00	20.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
72	0.00	0.00	14.26	0.00	10.13	18.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
73	0.00	0.00	13.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
74	0.00	0.00	24.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
77	0.00	0.00	22.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
78	0.00	0.00	26.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
79	0.00	0.00	22.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80	0.00	0.00	18.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
81	0.00	0.00	20.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
83	0.00	0.00	29.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
84	0.00	0.00	14.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
85	23.48	0.00	51.87	53.87	0.00	13.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
88	0.00	0.00	21.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	12.49	0.00	27.67	39.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
91	0.00	0.00	27.07	0.00	0.00	0.00	0.00	0.00	35.36	0.00	0.00	0.00	0.00	0.00	36.82
92	0.00	0.00	20.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96	0.00	0.00	21.23	0.00	22.13	31.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100	0.00	0.00	16.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
101	0.00	0.00	18.67	0.00	0.00	0.00	0.00	0.00	12.01	0.00	0.00	0.00	0.00	0.00	0.00
104	0.00	0.00	20.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
106	0.00	0.00	31.41	0.00	0.00	8.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
107	0.00	0.00	23.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
108	0.00	0.00	15.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
109	0.00	0.00	10.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
110	0.00	0.00	11.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
112	0.00	0.00	13.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
113	0.00	0.00	20.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
115	0.00	57.59	13.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
116	0.00	0.00	16.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
117	15.19	0.00	45.51	14.50	18.38	24.35	0.00	0.00	15.12	0.00	0.00	0.00	0.00	0.00	0.00
121	0.00	0.00	27.40	0.00	47.56	58.08	0.00	0.00	15.15	0.00	0.00	0.00	0.00	19.49	0.00
127	33.28	0.00	58.38	0.00	73.16	85.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
137	0.00	0.00	27.00	43.27	45.49	57.48	0.00	0.00	11.05	0.00	0.00	0.00	0.00	0.00	0.00
143	33.50	0.00	58.70	8.98	22.81	32.71	0.00	0.00	13.22	0.00	0.00	0.00	0.00	0.00	0.00
146	0.00	22.43	13.35	0.00	15.93	21.97	0.00	0.00	21.22	0.00	0.00	0.00	15.80	0.00	22.37

plant #	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
148	12.00	0.00	40.22	11.45	14.52	20.52	0.00	0.00	11.94	0.00	0.00	0.00	0.00	0.00	0.00
149	0.00	0.00	32.37	0.00	0.00	9.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
152	0.00	0.00	17.69	0.00	14.71	21.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
154	0.00	0.00	17.41	0.00	69.89	79.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
155	13.65	0.00	43.57	26.44	43.20	54.70	13.82	0.00	16.12	0.00	0.00	0.00	0.00	0.00	0.00
158	45.87	0.00	72.22	0.00	0.00	16.07	0.00	0.00	40.88	14.28	0.00	0.00	0.00	0.00	44.47
159	33.71	0.00	59.97	9.04	22.95	32.73	0.00	0.00	17.27	0.00	0.00	0.00	0.00	0.00	0.00
161	0.00	0.00	30.89	0.00	23.31	33.91	0.00	0.00	15.21	0.00	0.00	0.00	0.00	0.00	0.00
164	73.70	0.00	95.97	52.88	46.60	57.72	0.00	0.00	14.08	0.00	0.00	0.00	0.00	0.00	0.00
165	39.86	0.00	64.95	10.68	27.14	38.40	0.00	0.00	20.42	0.00	0.00	0.00	0.00	0.00	20.81
167	10.90	0.00	38.97	15.10	64.16	73.46	0.00	0.00	36.27	0.00	0.00	0.00	0.00	0.00	38.94
169	0.00	0.00	25.93	0.00	64.44	76.21	0.00	0.00	15.05	0.00	0.00	0.00	0.00	0.00	0.00
177	0.00	0.00	25.04	0.00	9.81	18.75	0.00	0.00	22.94	0.00	19.37	0.00	0.00	0.00	25.85
194	0.00	0.00	29.56	0.00	55.27	65.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
198	8.28	0.00	34.95	16.04	26.20	37.16	8.38	0.00	9.78	0.00	0.00	0.00	0.00	0.00	0.00
200	60.70	0.00	84.49	40.18	0.00	16.69	0.00	0.00	16.62	0.00	0.00	0.00	0.00	0.00	0.00
204	0.00	0.00	20.10	0.00	14.17	20.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
205	10.58	0.00	38.41	19.74	19.69	25.38	0.00	0.00	24.69	0.00	0.00	0.00	0.00	0.00	26.14
207	38.19	0.00	62.75	0.00	31.34	44.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
211	14.22	0.00	44.42	0.00	21.87	30.82	0.00	0.00	13.72	0.00	0.00	0.00	0.00	0.00	0.00
212	36.32	0.00	60.35	0.00	0.00	15.32	0.00	0.00	31.22	0.00	0.00	0.00	0.00	0.00	34.28
219	11.75	0.00	39.66	18.03	28.52	40.10	0.00	0.00	10.07	0.00	0.00	0.00	0.00	0.00	0.00
220	0.00	0.00	11.07	0.00	7.75	18.44	0.00	0.00	18.12	0.00	0.00	0.00	0.00	12.02	10.97
225	0.00	0.00	19.94	0.00	57.65	68.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
226	36.81	0.00	62.21	24.37	0.00	15.32	0.00	0.00	10.08	0.00	0.00	0.00	0.00	0.00	0.00
227	49.27	0.00	74.79	0.00	30.92	42.44	0.00	0.00	71.92	34.75	55.82	0.00	0.00	0.00	78.75
230	0.00	0.00	20.46	0.00	21.65	29.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
236	0.00	15.45	20.92	0.00	10.98	19.38	0.00	0.00	14.62	0.00	0.00	0.00	15.63	0.00	0.00
238	19.33	0.00	49.40	75.25	0.00	13.04	0.00	0.00	89.31	60.35	0.00	0.00	0.00	0.00	93.20
240	0.00	0.00	16.58	0.00	14.57	21.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
241	28.11	11.25	56.55	22.43	0.00	13.95	0.00	0.00	19.05	0.00	21.51	0.00	0.00	0.00	13.41
242	45.57	0.00	71.24	12.21	31.02	43.71	0.00	0.00	17.99	0.00	0.00	0.00	0.00	0.00	10.10
243	37.83	0.00	62.30	99.98	52.90	63.31	0.00	0.00	22.13	0.00	0.00	0.00	0.00	0.00	23.60
244	8.36	0.00	35.98	15.59	15.55	21.96	0.00	0.00	19.50	0.00	0.00	0.00	0.00	0.00	19.93
245	0.00	0.00	31.70	0.00	28.44	39.77	0.00	0.00	18.56	0.00	0.00	0.00	0.00	0.00	12.72
246	0.00	0.00	23.40	0.00	17.87	23.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.06	0.00
247	9.68	0.00	37.99	71.59	0.00	10.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
248	22.93	0.00	51.12	0.00	50.41	60.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
249	0.00	0.00	11.22	0.00	26.81	37.36	0.00	0.00	81.37	43.99	0.00	0.00	0.00	0.00	89.11
250	11.31	0.00	39.31	91.85	0.00	11.95	0.00	0.00	16.95	0.00	0.00	0.00	0.00	0.00	0.00
251	0.00	0.00	25.66	0.00	50.91	61.01	0.00	0.00	11.89	0.00	0.00	0.00	0.00	0.00	0.00
252	29.50	0.00	57.18	12.33	12.16	19.73	0.00	0.00	20.29	0.00	0.00	0.00	0.00	0.00	20.81
253	0.00	0.00	31.26	0.00	77.83	98.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
254	0.00	0.00	16.40	0.00	44.40	55.48	0.00	0.00	10.37	0.00	0.00	0.00	0.00	0.00	0.00
255	64.97	0.00	88.42	43.49	0.00	16.84	0.00	0.00	12.11	0.00	0.00	0.00	0.00	0.00	0.00
256	0.00	0.00	23.55	0.00	18.44	24.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.58	0.00
257	0.00	0.00	33.28	0.00	0.00	10.03	0.00	0.00	51.32	15.02	0.00	0.00	0.00	0.00	54.36
258	29.49	11.80	57.06	23.52	0.00	14.13	0.00	0.00	19.98	0.00	22.56	0.00	0.00	0.00	19.93
259	0.00	0.00	12.81	0.00	21.10	26.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
260	38.92	0.00	63.89	0.00	24.43	36.68	0.00	0.00	56.82	21.50	44.09	0.00	0.00	0.00	65.81
261	58.22	0.00	82.84	41.78	36.81	51.57	0.00	0.00	11.12	0.00	0.00	0.00	0.00	0.00	0.00
262	55.43	0.00	79.92	0.00	45.48	56.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
263	0.00	0.00	32.34	0.00	0.00	9.34	0.00	0.00	40.54	12.36	0.00	0.00	0.00	0.00	44.25
264	0.00	0.00	33.54	0.00	0.00	10.40	0.00	0.00	58.30	22.44	0.00	0.00	0.00	0.00	66.54
265	0.00	0.00	13.17	0.00	14.25	20.34	0.00	0.00	33.29	0.00	0.00	0.00	17.83	15.39	34.61

plant #	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
266	55.06	0.00	79.28	36.86	0.00	16.67	0.00	0.00	10.26	0.00	0.00	0.00	0.00	0.00	0.00
267	17.99	0.00	47.62	0.00	27.69	39.60	0.00	0.00	17.36	0.00	0.00	0.00	0.00	0.00	0.00
268	13.51	0.00	41.84	99.89	0.00	12.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
269	0.00	41.81	13.57	0.00	0.00	0.00	0.00	0.00	36.87	0.00	0.00	10.85	0.00	0.00	40.10
270	11.10	0.00	39.11	20.70	20.65	26.69	0.00	0.00	25.90	0.00	0.00	0.00	0.00	0.00	26.78
271	43.64	0.00	69.79	42.64	0.00	15.83	0.00	42.43	53.30	18.27	0.00	0.00	0.00	0.00	56.72
272	51.60	0.00	75.71	50.42	0.00	16.15	0.00	50.17	63.02	26.81	0.00	0.00	0.00	0.00	69.66
273	16.35	0.00	46.80	63.64	0.00	12.66	0.00	0.00	75.53	34.98	0.00	0.00	0.00	0.00	83.78
274	0.00	0.00	31.12	0.00	0.00	8.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
275	42.85	0.00	67.98	0.00	0.00	15.57	0.00	0.00	36.84	0.00	0.00	0.00	0.00	0.00	39.66
276	0.00	0.00	17.34	0.00	16.04	22.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
277	38.87	0.00	63.37	0.00	0.00	15.37	0.00	0.00	34.64	0.00	0.00	0.00	0.00	0.00	36.82
278	9.57	0.00	37.48	77.68	0.00	10.71	0.00	0.00	14.34	0.00	0.00	0.00	0.00	0.00	0.00
279	9.94	0.00	38.28	15.25	24.12	36.36	0.00	0.00	8.52	0.00	0.00	0.00	0.00	0.00	0.00
280	0.00	0.00	12.16	0.00	20.15	25.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
281	11.45	0.00	39.39	84.65	0.00	12.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
282	0.00	92.94	26.78	0.00	20.28	26.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
283	0.00	0.00	32.01	0.00	29.50	41.48	0.00	0.00	19.25	0.00	0.00	0.00	0.00	0.00	15.55
284	0.00	0.00	29.62	0.00	46.74	58.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
285	58.64	0.00	83.33	33.86	18.23	24.12	0.00	0.00	83.51	47.15	0.00	0.00	0.00	0.00	89.50
286	0.00	0.00	26.84	0.00	41.87	53.51	0.00	0.00	13.34	0.00	0.00	0.00	0.00	17.16	0.00
287	0.00	0.00	23.63	0.00	14.59	21.51	0.00	0.00	44.29	14.79	0.00	0.00	0.00	0.00	49.60
288	24.94	0.00	53.77	10.42	10.28	18.99	0.00	0.00	17.16	0.00	0.00	0.00	0.00	0.00	0.00
289	81.40	0.00	97.95	77.70	0.00	17.37	0.00	0.00	45.76	14.95	0.00	0.00	0.00	0.00	52.35
290	0.00	0.00	31.68	0.00	65.82	77.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
291	8.87	0.00	37.03	16.55	16.51	23.57	0.00	0.00	20.70	0.00	0.00	0.00	0.00	0.00	21.00
292	0.00	0.00	17.46	0.00	66.53	77.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
293	0.00	0.00	17.87	0.00	14.55	20.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
294	0.00	0.00	31.40	78.26	16.27	23.30	0.00	0.00	15.24	0.00	0.00	0.00	0.00	0.00	0.00
295	0.00	0.00	34.47	0.00	21.18	28.60	0.00	0.00	64.28	29.07	0.00	0.00	0.00	0.00	75.32
296	34.15	0.00	60.17	132.97	0.00	15.13	0.00	0.00	157.81	68.56	0.00	0.00	0.00	0.00	99.15
297	79.30	0.00	96.92	23.79	42.68	53.71	0.00	36.38	14.42	0.00	0.00	0.00	0.00	0.00	0.00
298	0.00	0.00	13.03	0.00	11.25	19.45	0.00	0.00	26.30	0.00	0.00	0.00	0.00	12.16	27.81
299	0.00	0.00	19.78	0.00	59.92	72.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
300	66.13	0.00	90.42	17.73	45.03	55.73	0.00	0.00	26.10	0.00	0.00	0.00	0.00	0.00	27.08
301	0.00	0.00	21.53	0.00	71.46	83.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
302	0.00	0.00	23.47	0.00	59.11	72.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
303	96.61	0.00	116.88	14.36	0.00	17.38	0.00	0.00	53.83	19.02	0.00	45.19	0.00	0.00	61.97
304	0.00	0.00	13.21	0.00	16.18	23.25	0.00	0.00	37.82	8.86	0.00	0.00	0.00	17.48	42.66
305	27.73	0.00	55.14	11.59	11.43	19.66	0.00	0.00	19.07	0.00	0.00	0.00	0.00	0.00	15.35
306	43.50	0.00	69.62	29.12	0.00	15.77	0.00	0.00	8.11	0.00	0.00	0.00	0.00	0.00	0.00
307	17.93	0.00	47.42	17.11	21.69	30.45	0.00	0.00	17.84	0.00	0.00	0.00	0.00	0.00	0.00
308	0.00	0.00	7.48	49.61	52.16	61.40	0.00	0.00	12.67	0.00	19.59	0.00	0.00	0.00	0.00
309	54.50	0.00	78.25	52.02	0.00	16.58	0.00	0.00	30.64	0.00	0.00	0.00	0.00	0.00	34.28
310	0.00	0.00	26.48	0.00	73.20	91.48	0.00	0.00	17.10	0.00	0.00	0.00	0.00	0.00	0.00
311	0.00	0.00	17.01	0.00	30.83	42.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
312	53.43	0.00	76.57	35.37	0.00	16.49	0.00	0.00	14.63	0.00	0.00	0.00	0.00	0.00	0.00
313	32.00	0.00	58.21	84.55	44.74	55.49	0.00	0.00	18.72	0.00	0.00	0.00	0.00	0.00	12.78
314	58.14	0.00	82.08	0.00	36.48	49.22	0.00	0.00	84.87	47.68	65.86	0.00	0.00	0.00	90.50
315	72.94	0.00	94.66	34.23	55.91	68.05	0.00	0.00	42.32	14.43	0.00	0.00	0.00	0.00	49.29
316	15.09	0.00	45.17	0.00	23.21	33.79	0.00	0.00	14.56	0.00	0.00	33.59	0.00	0.00	0.00
317	20.93	0.00	49.47	10.16	0.00	13.20	0.00	0.00	78.46	36.06	0.00	0.00	0.00	0.00	86.41
318	92.33	0.00	110.10	43.33	70.77	82.66	0.00	0.00	53.57	18.93	0.00	0.00	0.00	0.00	57.37
319	0.00	0.00	26.58	0.00	33.08	46.36	0.00	0.00	10.54	0.00	0.00	0.00	0.00	13.56	0.00
320	12.02	0.00	40.88	23.28	38.03	51.82	12.16	0.00	14.19	0.00	0.00	0.00	0.00	0.00	0.00

plant #	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
321	9.50	0.00	37.29	18.39	30.04	42.32	9.61	0.00	11.21	0.00	0.00	0.00	0.00	0.00	0.00
322	0.00	0.00	31.65	0.00	16.06	22.89	0.00	0.00	10.48	0.00	0.00	0.00	0.00	0.00	0.00
323	10.63	0.00	38.83	86.34	0.00	11.61	0.00	0.00	15.94	0.00	0.00	0.00	0.00	0.00	0.00
324	19.10	0.00	49.17	7.98	7.88	18.64	0.00	0.00	13.14	0.00	0.00	0.00	0.00	0.00	0.00
325	48.50	0.00	74.04	47.40	0.00	16.07	0.00	47.16	59.24	22.96	0.00	0.00	0.00	0.00	66.76
326	39.98	0.00	65.83	62.70	0.00	15.40	0.00	0.00	69.17	34.37	0.00	0.00	0.00	0.00	78.44
327	104.89	0.00	173.08	49.22	80.39	105.61	0.00	0.00	60.86	24.12	0.00	0.00	0.00	0.00	66.78
328	0.00	0.00	15.71	0.00	50.75	60.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
329	0.00	0.00	28.30	0.00	11.12	19.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
330	30.71	0.00	57.41	0.00	0.00	14.69	0.00	0.00	27.37	0.00	0.00	0.00	0.00	0.00	33.29
331	30.71	0.00	57.61	0.00	0.00	15.06	0.00	0.00	26.40	0.00	0.00	0.00	0.00	0.00	27.81
332	69.20	0.00	94.09	39.95	21.51	29.23	0.00	0.00	98.54	65.06	0.00	0.00	0.00	0.00	99.15
333	18.88	0.00	49.14	0.00	29.05	40.95	0.00	0.00	18.21	0.00	0.00	0.00	0.00	0.00	11.49
334	48.63	0.00	74.31	13.03	33.11	46.89	0.00	0.00	19.19	0.00	0.00	0.00	0.00	0.00	15.42
335	0.00	0.00	19.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
336	0.00	0.00	8.75	0.00	25.85	36.96	0.00	0.00	78.44	35.37	0.00	0.00	0.00	0.00	83.78
337	53.90	0.00	77.95	0.00	23.38	34.62	0.00	34.91	19.37	0.00	0.00	0.00	0.00	0.00	19.56
338	21.90	0.00	49.53	0.00	33.69	48.88	0.00	0.00	21.13	0.00	0.00	0.00	0.00	0.00	21.49
339	0.00	0.00	23.30	0.00	27.62	38.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
340	22.21	8.89	49.57	17.72	0.00	13.36	0.00	0.00	15.05	0.00	16.99	0.00	0.00	0.00	0.00
341	13.87	0.00	44.00	21.27	33.65	48.32	0.00	0.00	11.88	0.00	0.00	0.00	0.00	0.00	0.00
342	0.00	0.00	29.46	0.00	6.98	18.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
343	86.96	0.00	99.05	62.40	54.99	64.49	0.00	0.00	16.61	0.00	0.00	0.00	0.00	0.00	0.00
344	0.00	0.00	18.60	0.00	49.09	59.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
345	0.00	0.00	11.47	62.80	66.02	77.65	0.00	0.00	16.04	0.00	24.80	0.00	0.00	0.00	0.00
346	0.00	0.00	12.83	71.34	75.00	91.79	0.00	0.00	18.22	0.00	28.17	8.40	0.00	0.00	11.70
347	46.33	0.00	73.90	26.75	14.40	20.44	0.00	0.00	65.97	30.49	0.00	0.00	0.00	0.00	77.89
348	0.00	0.00	32.68	99.06	20.59	26.58	0.00	0.00	19.29	0.00	0.00	0.00	0.00	0.00	18.41
349	0.00	0.00	8.85	0.00	0.00	0.00	0.00	0.00	13.33	0.00	0.00	0.00	0.00	0.00	0.00
350	0.00	0.00	25.72	0.00	0.00	0.00	0.00	0.00	17.44	0.00	0.00	0.00	0.00	0.00	0.00
351	0.00	0.00	21.29	0.00	70.36	82.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
352	0.00	0.00	19.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
353	0.00	0.00	28.12	0.00	10.71	19.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
354	0.00	0.00	12.03	0.00	0.00	0.00	0.00	0.00	19.16	0.00	0.00	0.00	0.00	0.00	15.38
355	16.54	0.00	46.88	8.03	0.00	12.84	0.00	0.00	61.98	26.51	0.00	0.00	0.00	0.00	66.78
356	0.00	0.00	26.31	0.00	0.00	0.00	0.00	0.00	22.07	0.00	0.00	0.00	0.00	0.00	23.52
357	0.00	0.00	32.83	112.53	23.39	34.63	0.00	0.00	21.91	0.00	0.00	0.00	0.00	0.00	23.31
358	0.00	0.00	25.84	0.00	0.00	0.00	0.00	0.00	21.28	0.00	0.00	0.00	0.00	0.00	22.37
359	63.62	0.00	87.11	29.85	48.76	59.25	0.00	0.00	36.91	0.00	0.00	0.00	0.00	0.00	42.66
360	0.00	28.39	15.19	0.00	20.17	25.84	0.00	0.00	26.86	0.00	0.00	0.00	20.00	0.00	31.80

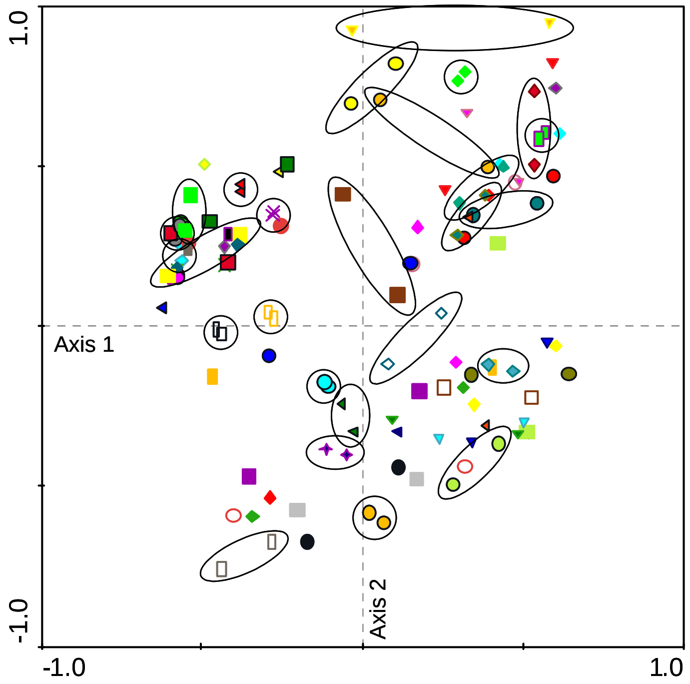

Figure A6 PCA based on the distribution of phenolic compound concentrations in samples of *Artemisia vulgaris* (plotted as symbols) harvested at 3 sites along a gradient of decreasing soil pollution (site 1 - 3). Samples collected from plants grown in a pair often are plotted close together. Axis 1 (EV = 0.268) and axis 2 (EV = 0.092) are presented.

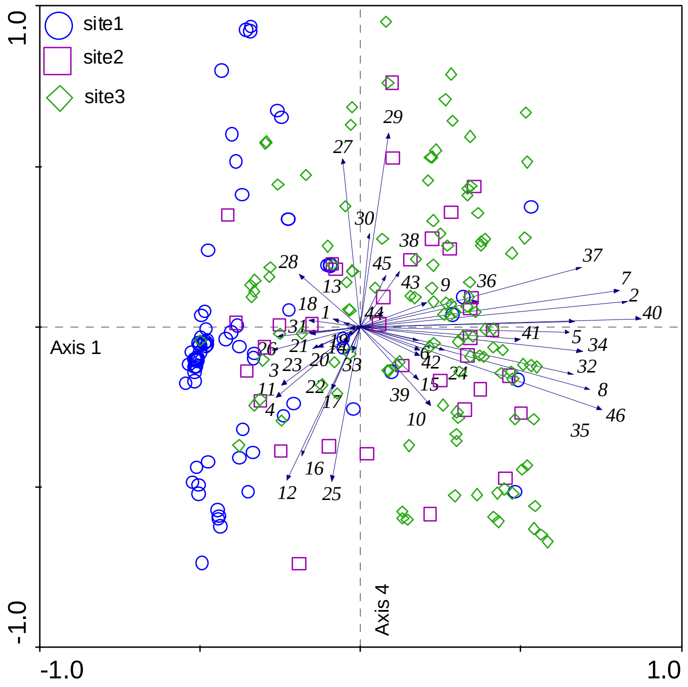

Figure A7 Distribution of phenolic compound concentrations (plotted as vectors) in samples of *Artemisia vulgaris* (plotted as symbols) harvested at 3 sites along a gradient of decreasing soil pollution (site 1 - 3) in the ordination plot of a PCA. Axis 1 (EV = 0.268) and axis 4 (EV = 0.063) are presented.

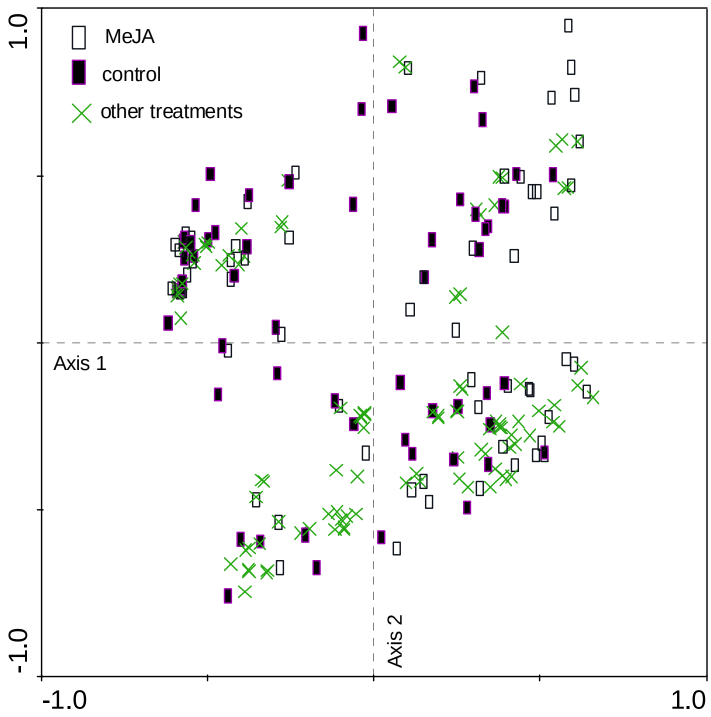

Figure A8 PCA based on the distribution of phenolic compound concentrations samples of *Artemisia vulgaris* (plotted as symbols) harvested at 3 sites along a gradient of decreasing soil pollution (site 1 - 3) that were elicited with methyl jasmonate (MeJA), treated with insecticide and/or herbivores (other treatments) or left untreated (con). Axis 1 (EV = 0.268) and axis 2 (EV = 0.092) are presented.