

Autecological studies of the competitiveness of transgenic sugar beets

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Synopsis

Das Ziel der Freilandexperimente war die Entwicklung eines Konkurrenzversuches zur Abschätzung des Verwildерungsrisikos von gentechnisch veränderten (transgenen) Zuckerrüben mit eingefügten BNYVV-Hüllprotein-, Phosphinothricin-Acetyl-Transferase- und NPTII-Genen. Aufgrund der schrittweisen Vorgehensweise bei Risikoabschätzungen wurde das Freilandexperiment 1993 zunächst mit einer transgenen Zuckerrüben-Inzuchtlinie durchgeführt. Zum Vergleich wurden die nichttransformierte Ausgangslinie sowie eine handelsübliche Kulturhybridsorte herangezogen. Der Versuchsaufbau orientiert sich an einer Brachlandsituation, wie sie im ersten Jahr nach Rübenanbau als wahrscheinliche Ausgangssituation für Verwilderingen auftreten könnte. Zu jeweils vier Zuckerrüben wurde das typische Hackkulturunkraut *Chenopodium album* in unterschiedlichen Dichten in 0,25 m² Parzellen gepflanzt. Nach 80-tägiger Kultur an einem BNYVV-Befallsstandort in Oberviehhausen (Bayern) bzw. 87-tägiger Kultur aus einer befallsfreien Fläche in Wetze (Niedersachsen) wurde die Biomasseproduktion von Sproß und Rübenkörper auf Frischgewichtsbasis festgestellt um Konkurrenzphänomene darzustellen. Erwartungsgemäß erwies sich die Kultursorte durch den züchterischen Hybrideffekt als konkurrenzkräftiger im Vergleich zu den beiden Zuchlinien mit ihrem hohen Inzuchtgrad. Die transgene Zuchlinie zeigte sich konkurrenzschwächer als die nichttransformierte Ausgangslinie. Innerhalb der kurzen Versuchszeit und der gewählten Kulturbedingungen konnte kein Konkurrenzvorteil der resistenten (transgenen) Zuchline auf dem Virusbefallsstandort in Bayern festgestellt werden. Die Biomasseproduktion von *C. album* wurde nur in einigen wenigen Dichtevarianten von nichttransgenen Zuckerrüben beeinträchtigt. Die transgene Zuckerrübenlinie zeigte keinen signifikanten Einfluß auf das Wachstum von *C. album*. Der hier vorgestellte Versuchsaufbau ist geeignet, das Konkurrenzverhalten von jungen Zuckerrüben auf Brachflächen darzustellen.

Modellversuch, Konkurrenzverhalten, Transgene Zuckerrüben, Beta vulgaris, Chenopodium album, BNYVV-Resistenz, Freilandversuch, Ökologische Begleitforschung.

Model experiments, competitiveness, transgenic sugar beet, Beta vulgaris, Chenopodium album, BNYVV-resistance, field test, ecological risk research.

1. Overall Goal

This project is intended to improve our understanding of aspects of biological security and ecological risk assessment used for the environmental release of genetically engineered plants. Autecological model tests will be developed to predict the interaction of transgenic plants in the environment, and experimental release will be carried out to assess the impact of particular transgenic plants on different ecosystems.

The rules governing the interaction between transgenic organisms and their environment are no different from those governing other living organisms (CRAWLEY 1990). Risk scenarios concerning the introduction of transgenic organisms into the environment (environmental release) involve the naturalization and dispersal of the organisms and/or the new genes, and the development of undesirable characteristics. The acclimatization and dispersal of new organisms or genotypes are subject to the law of natural selection. Experience has shown that selection acts quickly against introduced genotypes and prevents the establishment of most genotypes in new areas. Even so, several examples demonstrate that the acclimatization and dispersal of genotypes in foreign ecosystems can lead to undesirable results (SUKOPP 1987, DRAKE & al. 1989, KOWARIK 1990, 1992). The number of genes newly introduced into an area during the release of transgenic plants, as well as during the introduction of non-native plant species, does not serve as a reliable ecological measure for estimating risk (SUKOPP & SUKOPP 1993). Due to the uncertainty about the likelihood of subsequent risks, and hence the ensuing events, many ecologists consider transgenic organisms to be potential pests per se (LUBCHENKO & al. 1991). Therefore, the research on each individual release of transgenic organisms, must include a thorough assessment of the potential ecological risks.

The starting point for the project was a BNYV-Virus resistance in sugar beets created by the company PLANTA in Einbeck (Germany) using an *Agrobacterium tumefaciens* mediated gene transfer. Besides containing the coat protein of the BNYVV, the transgenic sugar beets also contain two genetical markers: The Phosphinothricine-Acetyl-transferase (bar) -gene determines resistance to the herbicide BASTA (from the company Hoechst, Frankfurt). The NPTII gene enables plant growth with the antibiotica Kanamycin. PLANTA has made this plant material (sugar beet, *Beta vulgaris* L. subsp. *rapacea* var. *altissima* (KOCHE) DÖLL, ROTHMALER 1990) available to interested scientists.

It is generally accepted that the culture varieties we have today are descended from the highly variable and adaptive wild beet, *Beta vulgaris* L. subsp. *maritima* ARCANG. There are no barriers to crosses between wild and cultured varieties, so wild beets provide an important gene reservoir for plant breeding (BAROCKA 1985). A reverse development of cultured varieties back to wild forms occurs naturally (HORNSEY & ARNOLD 1979). It is also possible for sugar beets to show weed-like characteristics (LONGDEN 1974, BOUDRY & al. 1993). Transgenic sugar beets are predicted to develop the ability to have an ecological impact through gene transfer on crossable relatives (TIEDJE & al. 1989, HOFFMAN 1990, De VRIES & al. 1992, BARTSCH & al. 1993, KAPTEIJNS 1993, RAYBOULD & GRAY 1993, SUKOPP & SUKOPP 1993).

Available data and a theoretical assessment of the chances of unwanted dissemination, propagation, and of *Beta vulgaris* running wild in agro-ecosystems and non-agro-ecosystems, are given in an overview in Fig. 1.

Ecological impacts could arise through factors which improve the acclimatisation and dispersal of new genotypes. These factors are, according to CRAWLEY (1990):

- a) high inner growth rate (eg. use of nutrients, seed production)
- b) tolerance of close proximity to other plants (eg. allelopathy, tolerance of competitors and abiotic environmental factors).
- c) low sensitivity to natural enemies and/or
- d) large number of symbiotic relationships.

Possession of the above qualities leads to a high intra-specific and inter-specific competitive ability. In addition, the risk of undesirable ecological effects of transgenic plants is increased by:

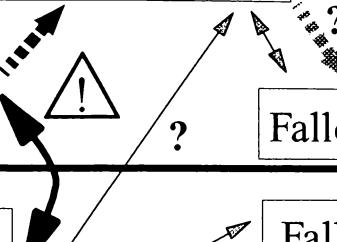
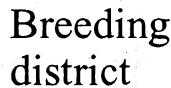
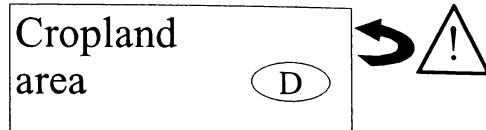
- e) a qualitative and quantitative increase in ecological niches.
- f) a high dispersal rate.

Experimental research should place equal emphasis on ecological and genetic studies of plants which should be introduced into agricultural practice in a step-by-step fashion.

In 1993, a first field experiment was conducted in order to evaluate a test system describing the competitiveness phenomena of sugar beet in young fallow. The competitiveness was determined in degrees of biomass production in a planting system of sugar beet with different densities of *Chenopodium album*. The experimental programme focusses on the investigation of the ecological interaction of sugar beet in the target ecosystem. Young fallow is one of the most interesting area in the target agro-ecosystem where an unwanted survival of sugar beets could realistically occur. Sugar beet is a relatively weak competitor and, at the same time, is dependent on the high nutrient levels present in the test field. We assume that a small number of overwintering beets (e.g. dormant seed) will have to struggle with an upcomming competitor. The growth of the sugar beet was assessed in a situation where it was competing with *Chenopodium album*. The genus *Chenopodium* was chosen on the basis of the following criteria:

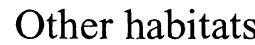
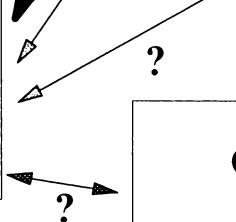
- weed properties in pure sugar beet culture (common habitat exists)
- occurrence in young fallow
- similar ecological niches to the cultured plants (common adaptation strategies in disturbed habitats with possibly similar alternative habitats outside of agricultural cultures).

Agro-ecosystem



Moist site,
occasionally flooded
Moderate chloride content
Full light conditions
Rich available nitrogen
Mild winter

DK
I
NL



Other habitats

Non-agro-ecosystems

Fig. 1: Chances of *Beta vulgaris* running wild in agro-ecosystems and non-agro-ecosystems. Available data are marked with !, unknown/unpublished with ?. The symbols represent the countries Germany (D), Italy (I), Denmark (DK) and the Netherlands (NL). In breeding districts close to wild beet habitats, gene transfer occurs naturally. Hybrids of wild beets/cultivars are sometimes taken by plant breeders to other cropland areas, even to other countries. In cropland, weed varieties form stable populations. An escape to non-agro-ecosystems, however, can not be ruled out. Beet seeds can remain dormant in the fallow for a number of years before germination.

Abb. 1: Verwilderungsmöglichkeiten von *Beta vulgaris* in Agrarökosystemen und Nichtagrarökosystemen. Verfügbare Daten sind mit !, unbekannte oder nicht publizierte mit ? bezeichnet. Die Ländersymbole kennzeichnen Deutschland (D), Italien (I), Dänemark (DK) und die Niederlande (NL). In Gebieten zur Saatguterzeugung (I) kommt es zu einem natürlichen Genaustausch zwischen Zuckerrüben und nahegelegenen Unkraut-Wildrübenpopulationen. Ein Teil der entstehenden Hybriden wird zusammen mit dem erzeugten Saatgut über Ländergrenzen in Rübenanbaugebiete verbracht. Dort kann es zu einer Verwilderung und/oder einem Genaustausch mit lokalen Unkrautpopulationen kommen. Auch auf Brachflächen können die Samen über mehrere Jahre im Boden überdauern.

2. Material and methods

Young sugar beet plants were precultured and exposed to the conditions that occur in the first year of fallow. Seeds of *Chenopodium album* L. var. *album* (ROTHMALER 1990) were sampled at the New Botanical Garden, University of Göttingen in 1992. After germination and pre-cultivation, the seedlings were planted in a 16 x 24 m field plot in different densities with the sugar beet. The experimental design is shown in Fig. 2.

Each *Beta* genotype to be tested was planted with different densities of *Chenopodium album* (0/1/2/4), with 10 replications for each density (ie. 10 experimental subplots for each). Controls were established for

- the growth of *Chenopodium album* and
- the accompanying herbaceous plants from the seed bank.

Subplots with pure cultures were also set up. Each subplot consisted of an area of 50 x 50 cm (0.25 m²). The following three Beta genotypes were tested:

L3 = transgenic breeding line of the type described in chapter 1 (with 98.4% homozygosity, double selfing of L5)

L5 = unaltered original breeding line (parent with 96.9% homozygosity)

L6 = conventional hybrid "rizor".

The sugar beet genotypes were precultivated in the greenhouse. They originated from in-vitro cloning. The plant size was equal for each genus: *Beta* plants were precultivated for four weeks, after having grown for six weeks on agar plates with rooting hormones (resulting in eight leaves, total biomass of leaves and beet: 4.4 g); *Chenopodium* plants were precultivated for eight weeks after germination (total biomass of shoot: 4.0 g).

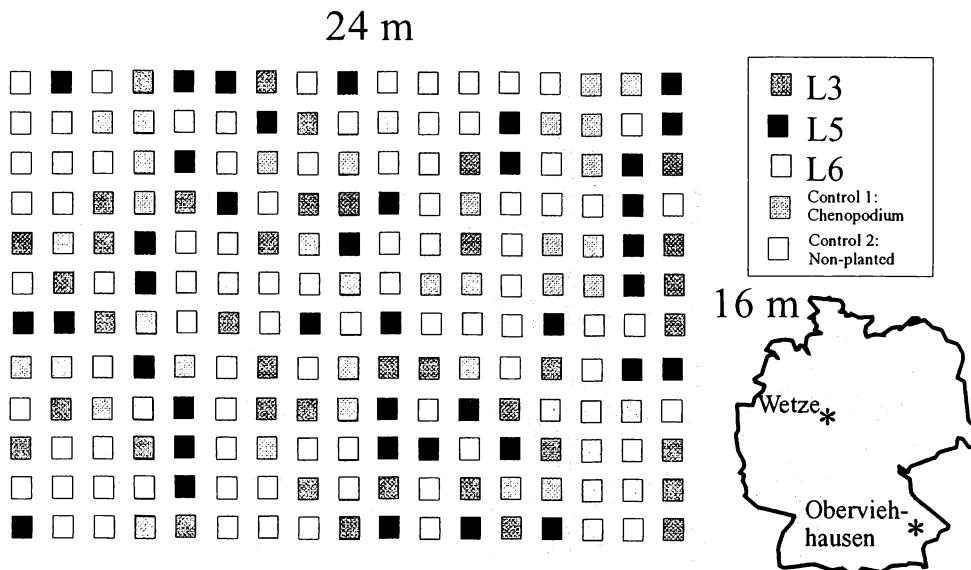


Fig. 2: Random distribution of the sugar beet genotypes in the field plot. The density of *C. album* planting is not represented. The experimental design includes 204 subplots (50 x 50 cm each) on a 16 x 24 m field plot. Germinating plants between the subplots, which are 80 cm apart, were removed. The sugar beet genotypes used are L3 (transgenic line with BNYV virus-resistance and bar/nptII gene markers), L5 (conventional parental line) and L6 (conventional hybrid cultivar with BNYV virus-tolerance).

Abb. 2: Randomisierte Verteilung der Zuckerrüben genotypen im Feldversuch. Die Pflanzendichte von *C. album* ist nicht dargestellt. Der Versuchsaufbau umfaßte 204 Teilparzellen (Einzelgröße 50 x 50 cm) auf einer Gesamtfläche von 16 x 24 m. Keimende Pflanzen wurden in den 80 cm breiten Zwischenräumen und in allen Parzellen mit Ausnahme von unbepflanzten Kontrollen kontinuierlich entfernt. Die verwendeten Zuckerrüben genotypen sind mit L3 (transgenes Zuchtlinienmaterial mit BNYV-Virusresistenz und bar/nptII-Genmarkern), L5 (konventionelles Eltern-Zuchtlinienmaterial) und L6 (konventionelle Hybridsorte mit BNYV-Virustoleranz) bezeichnet.

The plots were set up at two sites: Wetze, in Lower Saxony, a location without virus infection, and Oberviehhäusen, in Bavaria, a location with virus infestation. Both sites are typical cropland areas for sugar beet, with a clay-loam soil, high in nutrient supply. In order to build up reproducible competition densities the subplots were planted as shown in Fig. 3.

The experimental sites were ploughed in early spring and milled immediately before planting in the first week of May 1993. With the exception of *C. album*, which was planted by us, all other weed varieties were unwanted due to the risk of an uncontrolled development of different species compositions and densities. Therefore, germinating weeds were removed from all the subplots every two weeks, apart from non-planted subplots (control

of actual weed flora). The experiment was concluded when the competitiveness of *C. album* began to decline, due to the loss of seed and leaf at the end of its life cycle in July. Therefore, after growing for 80 and 87 days, in Oberviehhhausen and Wetze respectively, the plants were harvested. The fresh weight of the beet, and the foliage was measured.

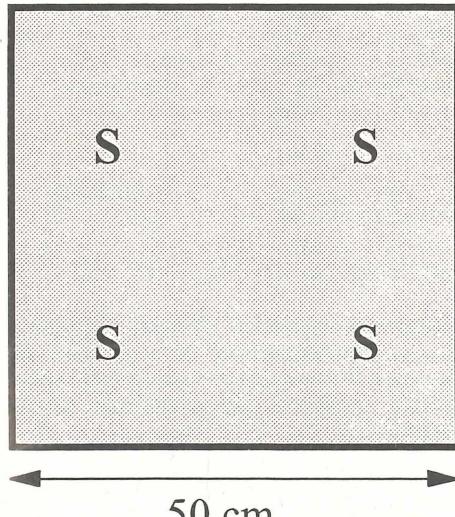
The start factors (climate, planting time, soil cultivation) of the experiments were the same at both test sites. Also during the experimental period in 1993 the climate was equally warm and dry in spring and wet and cool in summer.

Competition is indicated both by De WIT's displacement coefficient (De WIT 1960) and the absolute biomass production. The displacement coefficient is given by the coefficient biomass (species A in mixed culture)/biomass (species A in pure culture). Instead of De WIT (1960) we used a test system with an equal number of the main interesting plant species (sugar beet), and different numbers/densities of a competitor (*C. album*). An increase in plant density results in this case in a higher absolute number of plants. De WIT's experimental replacement design evaluated competitiveness within an equal absolute number of plants focussing on both competing plants. Our design was conducted to compare interspecific competitiveness of different genotypes of the same plant species.

Raw data was analysed by means of the approximation of the Wilcoxon-Mann-Whitney U-test at a significance level $\alpha = 0.05$.

Number of plants per sub-plot							
Sugar beet	4		4		4		4
<i>Ch. album</i>	0		4		8		16

Sugar beet (S)



Chenopodium album (C)

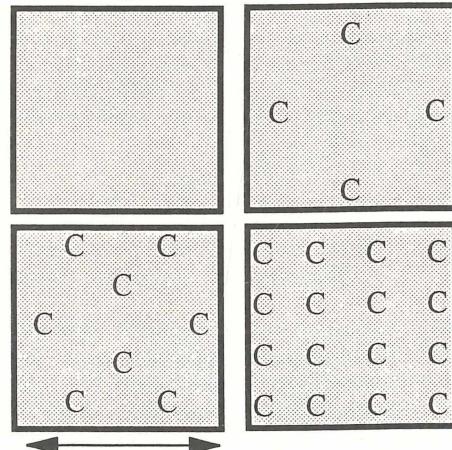


Fig. 3: Planting system for sugar beet (S) and *C. album* (C) in the subplots according to the competition density.

Abb. 3: Auspflanzsystem von Zuckerrüben (S) und *C. album* (C) in den jeweiligen Konkurrenzdichten der Einzelparzellen.

3. Results

The vegetative biomass production of the sugar beet genotypes is shown in Fig. 4 and De WIT's displacement coefficient in Fig. 5.

At the same competition level, the sugar beet genotypes differ significantly in their biomass production on a fresh weight basis at the same experimental site. The highest biomass production is exhibited by the hybrid cultivar L6, the lowest, by the transgenic breeding line L3. No difference is seen at the two test sites when comparing the same sugar beet breeding line. The hybrid cultivar, however, had a significantly higher productivity at the Oberviehhause test site.

The vegetative biomass production of the *C. album* is shown in Fig. 6 and DE WIT's displacement coefficient in Fig. 7. The observation of *C. album*, at the lowest density level (4/4), revealed a decrease in the absolute and relative biomass production in the sugar beet line L6, at both sites, and in L5 in Oberviehhause. A doubling in the number of plants per subplot in the control variant, grown without sugar beet, only marginally increased the total productivity of *C. album*. Other differences were statistically insignificant.

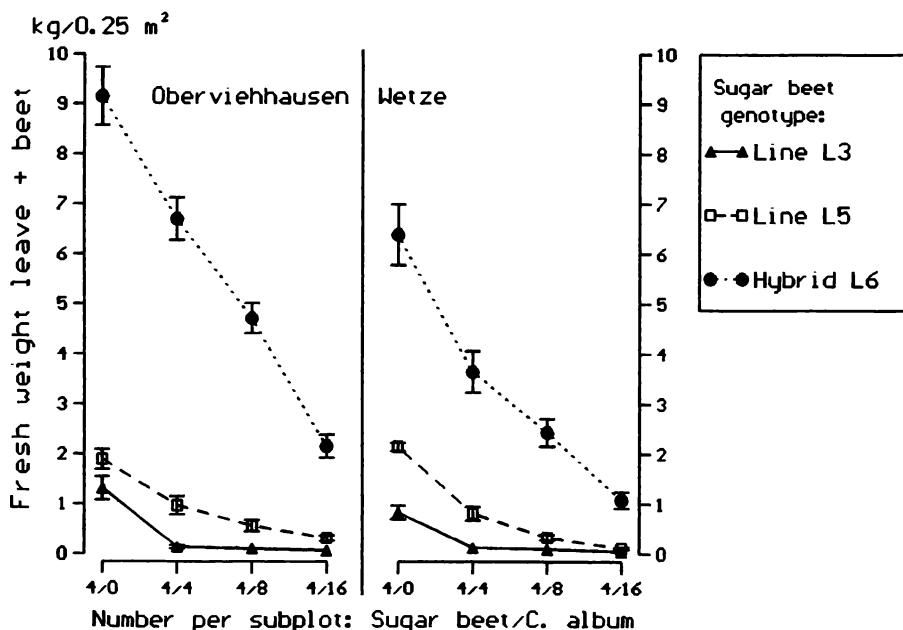


Fig. 4: Vegetative biomass production [kg fresh weight/subplot] of sugar beet genotypes (L3 = transgenic virus-resistant breeding line, L5 = conventional parental breeding line, L6 = conventional virus-tolerant hybrid cultivar) grown in four different competition densities with *Chenopodium album* at a site with (Oberviehhause) and a site without BNYV-Virus infection (Wetze). Bar lines represent the standard deviation from the mean ($n = 10$).

Abb. 4: Vegetative Biomasseproduktion [kg Frischgewicht/Einzelparzelle] der Zuckerrübenarten (L3 = transgene virusresistente Zuchlinie, L5 = konventionelle Elternzuchlinie, L6 = konventionelle virustolerante Hybridsorte) in Abhängigkeit der Konkurrenzdicthe von *Chenopodium album* an einem Versuchsstandort mit (Oberviehhause) und einem Versuchsstandort ohne BNYV-Virusbefall (Wetze). Die Balkenintervalle stellen den Standardfehler des Mittelwertes bei $n = 10$ dar.

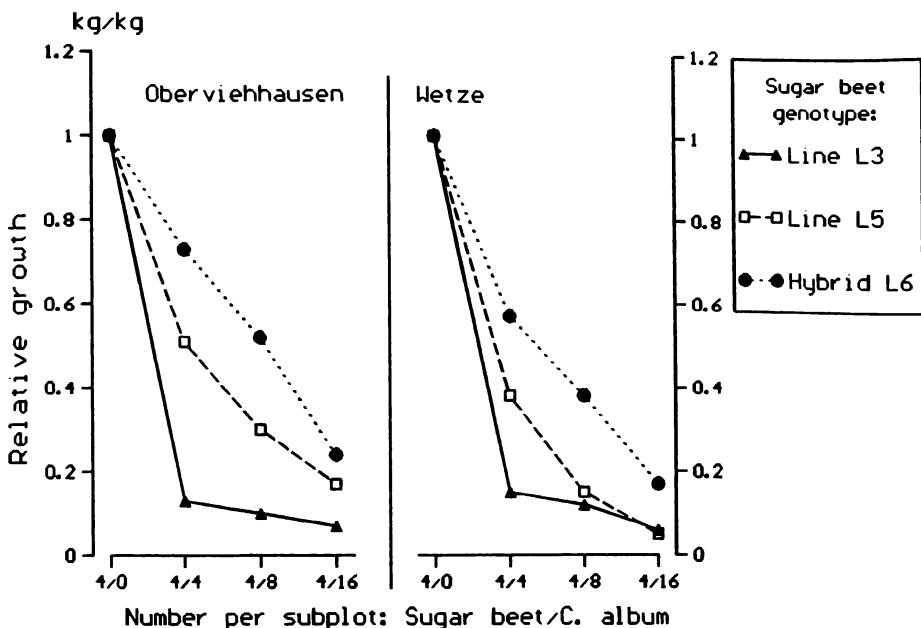


Fig. 5: Relative biomass production (De WIT's displacement coefficient) of sugar beet genotypes (L3 = transgenic virus-resistant breeding line, L5 = conventional parental breeding line, L6 = conventional virus-tolerant hybrid cultivar "rizor") grown in four different competition densities with *Chenopodium album* at a site with (Oberviehhause) and a site without BNYV-Virus infection (Wetze).

Abb. 5: Relative Biomasseproduktion (Verdrängungskoeffizient nach De WIT) der Zuckerrübengenotypen (L3 = transgene virusresistente Zuchtlinie, L5 = konventionelle Elternzuchtlinie, L6 = konventionelle virustolerante Hybridsorte) in Abhängigkeit der Konkurrenzdichte von *Chenopodium album* an einem Versuchsstandort mit (Oberviehhause) und einem Versuchsstandort ohne BNYV-Virusbefall (Wetze).

4. Discussion

As seen in Fig. 4 and 5, competitiveness of vegetative sugar beets can be clearly demonstrated in growth experiments with *Chenopodium album*. With an increase of intra-specific competition the biomass production decreases. The experimental design reflects the competitive situations from a moderate to high degree.

A comparison between the test-genotypes reveals no unexpected results. Due to the hybridization effect, the cultivar has the highest biomass production of sugar beet in each competitive density. Through selfing and inbreeding effects, the breeding lines show very low absolute and relative productivity both in general and when under competitive stress. The decrease in biomass production from the L5 to the L3 genotype may be due to the genetic modification, a single increase in the selfing rate or a combination of both. Although the transgenic line L3 should be resistant to virus infection, the sensitive parental line L5 shows a higher absolute and relative competitiveness, both at the virus-infected site at Oberviehhause, as well as at the virus-free site at Wetze. This phenomenon could be attributed to the short experimental period where an ecological advantage was not noticeable and/or covered by secondary effects like inbreeding or the use of sugar beet planting instead of seeding. The results in Fig. 6 and 7 suggest that intra-specific competition is most important for *C. album*. Inter-specific competition with sugar beet takes place only in growing situations where *C. album* has to compete with an equal number of well developed sugar beet genotypes. *C. album* seems to be a more powerful competitor due to the relatively slight decline of productivity under competitive sugar beet stress.

In this field study the influence of the ecologically relevant factors (according to CRAWLEY 1990), internal growth rate (a) and density tolerance (b_1), has been clarified. There is inadequate data on the following factors: density tolerance for the natural flora present in the study area (b_2), sensitivity to natural enemies (c) and the

number of symbiotic relationships (d). Changing plant attributes of the factors a-d can lead to a high intra-specific or inter-specific competitive ability. Further research is planned.

This project includes a monitoring programme at the release sites, as will all our future studies. If increased dispersal properties of the plants should be observed, further studies could help to determine the possible causes of changed plant and environmental attributes. A qualitative and quantitative increase in ecological niches (e) as well as in an increased dispersal rate (f) may be observed, dependent on the release site. The influence of factors (e) and (d) are not easily predictable due to the large number of variable factors. In these cases, the field study must also act as a screening method, using appropriate safety precautions. Depending on the results further studies may be necessary. We plan to continue competition experiments to analyse data on the following parameters: generative biomass development, seed dormancy, germinative ability of seed, and plant dispersal.

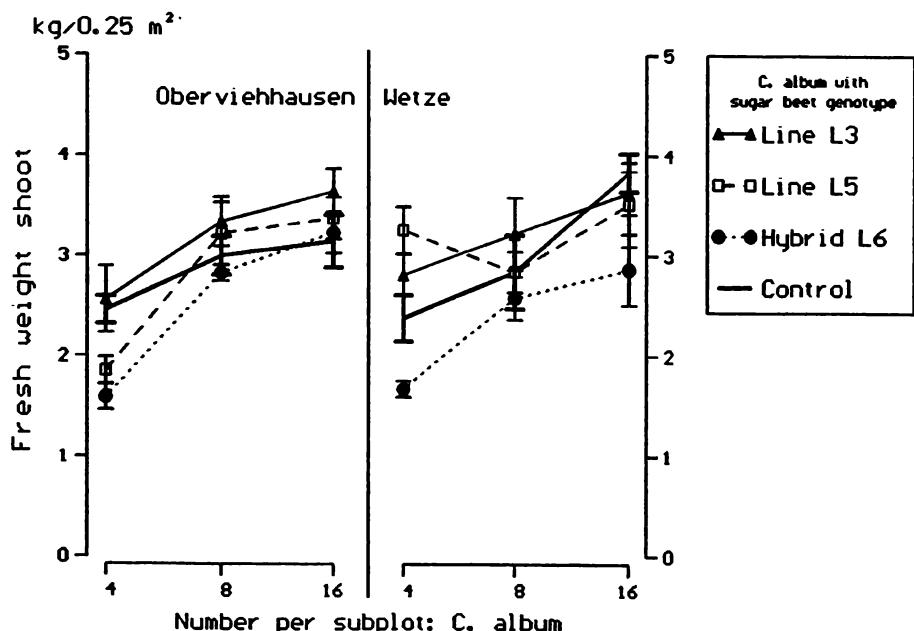


Fig. 6: Vegetative biomass production of *Chenopodium album* [kg fresh weight subplot] grown with different sugar beet genotypes (L3 = transgenic virus-resistant breeding line, L5 = unaltered parental breeding line, L6 = conventional virus-tolerant hybrid cultivar "izor") at two sites (Oberviehhhausen and Wetze). The *C. album* plants were grown in three different densities (4, 8, 16) without (= control) or with 4 sugar beet plants. Bar lines represent the standard deviation from the mean ($n = 10$).

Abb. 6: Vegetative Biomasseproduktion von *Chenopodium album* [kg Frischgewicht/Einzelparzelle] in Abhängigkeit des Wachstums mit unterschiedlichen Zuckerrüben genotypen (L3 = transgene virusresistente Zuchtlinie, L5 = konventionelle Elternzuchtlinie, L6 = konventionelle virustolerante Hybridsorten) an zwei Versuchsstandorten (Oberviehhhausen, Wetze). *C. album* wuchs in drei unterschiedlichen Dichten (4, 8, 16) ohne (= Kontrolle) oder mit 4 Zuckerrübenpflanzen. Die Balkenintervalle stellen den Standardfehler des Mittelwertes bei $n = 10$ dar.

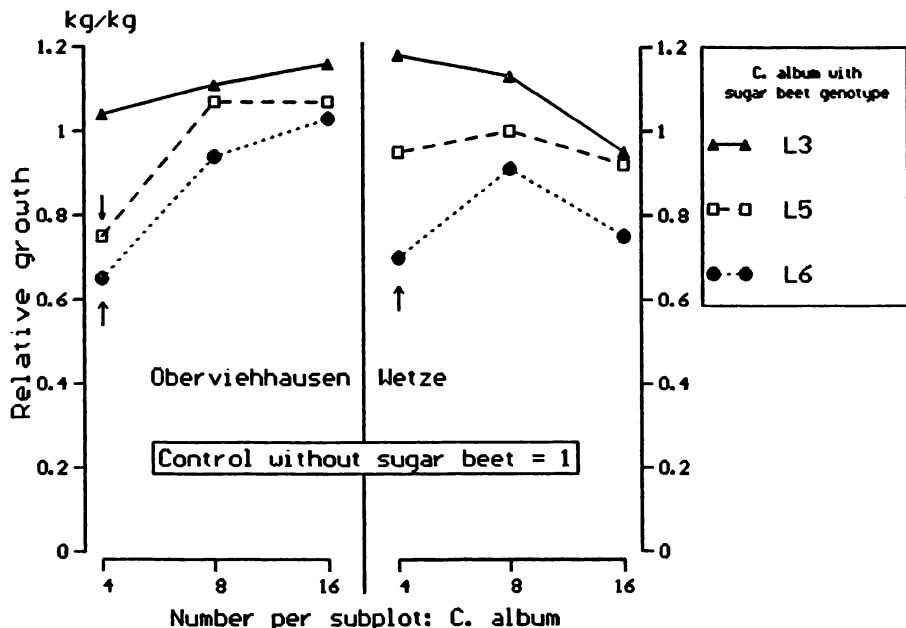


Fig. 7: Relative biomass production (De WIT's displacement coefficient) of *C. album* [kg/kg] grown with different sugar beet genotypes (L3 = transgenic virus-resistant breeding line, L5 = unaltered parental breeding line, L6 = conventional virus-tolerant hybrid cultivar "rizor") at two sites (Oberviehhauen, Wetze). The *C. album* plants were grown in 0,25 m² subplots in three different densities (4, 8, 16) without (= control) or with 4 sugar beet plants. Arrows symbolize a significant difference to the control.

Abb. 7: Relative Biomasseproduktion (Verdrängungskoeffizient nach De WIT) von *C. album* in Abhängigkeit des Wachstums mit unterschiedlichen Zuckerrüben genotypen (L3 = transgene virusresistente Zuchlinie, L5 = konventionelle Elternzuchlinie, L6 = konventionelle virustolerante Hybridsorte) an zwei Versuchsstandorten (Oberviehhauen, Wetze). *C. album* wuchs in drei unterschiedlichen Dichten (4, 8, 16) ohne (= Kontrolle) oder mit 4 Zuckerrübenpflanzen. Die Pfeile symbolisieren statistisch signifikante Unterschiede im Vergleich zur Kontrolle.

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