Gene flow and establishment of transgenes in natural plant populations

L. W. D. VAN RAAMSDONK and H. J. SCHOUTEN

Centre for Plant Breeding and Reproduction Research CPRO-DLO, PO Box 16, 6700 AA Wageningen, The Netherlands
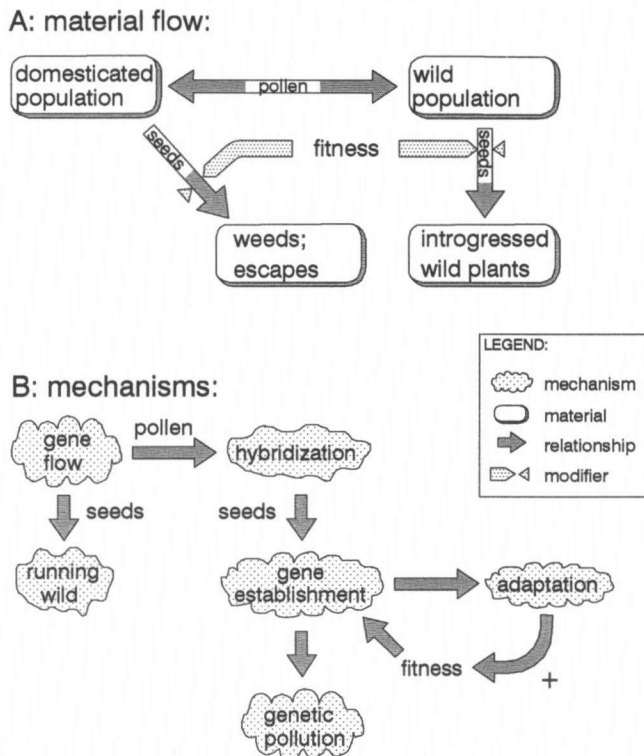
SUMMARY

In this paper the possibilities are indicated to model the introgression of a domestication gene, especially a transgene, into a wild plant population. The entire process of introgression consists of two phases, i.e. gene flow by pollen (hybridization) or by seed (escape), and establishment. A literature survey demonstrated that a lot of information is available on the first phase (gene flow), although seed dispersal is less studied than pollen dispersal. The second phase of introgression (establishment) is hardly studied in a quantitative way. It is important to focus further research on fitness effects of establishment, because even at low levels of gene flow a (trans)gene will finally succeed in entering a wild population. In this paper several parts of a simulation model are described. In case of small wild populations a stochastic model should be used in order to account for genetic drift.

Key-words: biosafety, transgenic plants, introgression, gene flow, establishment, population size, fitness, *Beta*, *Daucus*.

INTROGRESSION: GENE FLOW AND ESTABLISHMENT

The process of introgression can be divided into two phases: gene flow and establishment. Pollen and seeds as two different vectors are involved in this process (Fig. 1). In biosafety studies emphasis was predominantly on the first phase, i.e. gene flow from a transgenic or, in general a domesticated, cultivar to a wild relative. However, the spread of a novel (trans)gene does not depend exclusively on the rate of hybridization. The success of a gene in a natural environment depends greatly on the selective advantage of the individuals carrying the gene.

In order to acquire information on the relative importance of gene flow, either by pollen (hybridization) or by seed (escape), and of establishment (naturalizing), an analysis was carried out (Van Raamsdonk 1995), based primarily on the relevant data from De Vries *et al.* (1992) with respect to 36 crop species and some genera. In the study of De Vries *et al.* (1992) hybridization frequency was estimated by checking intermediate plants among herbarium material and in literature reports, i.e. hybridization was used to refer to pollen flow. Escape events were traced by checking for domesticated plants in the mentioned sources, which were collected under non-cultivated circumstances (De Vries *et al.* 1992). Their data, supplemented with some data obtained from other sources, were subjected to a principal component analysis in order to find the correlation between the parameters frequency of hybridization, escape frequency,

Fig. 1. A: Relationship between the two phases of introgression (gene flow, establishment) and the vectors involved (seed, pollen). B: Brief summary of the most important mechanisms for ecological biosafety matters (network display after Van Raamsdonk, 1993).

possibility of establishment of hybrids, occurrence of wild relatives in The Netherlands, and possibility of naturalizing (Van Raamsdonk 1995). The possibility of hybridization (i.e. pollen transfer) showed higher correlation coefficients with the other parameters than the possibility of escape (i.e. seed transfer). As a result of these correlations, the distribution of the 36 crop species in the principal component plot was predominantly determined by the hybridization frequency, indicating that pollen transfer plays a more important role than seed transfer (Van Raamsdonk 1995).

In a simulation study on establishment of a herbicide resistance gene, Reboud (1992) paid special attention to the hybridization barrier between the crop and its weedy relative, leading to, for example, sterility of the hybrids. He concluded that the hybridization barrier plays a major role in limiting the gene transfer from the crop to the weed. Hybridization can result in the establishment of a novel gene in a recipient population and in the development of weedy populations. Intermediate weeds occur in relation to a range of different crops and weedy populations can act as a gene pool for further backcrossing to wild relatives of a particular crop (Van Raamsdonk & Van der Maesen 1996). Hybridization will not necessarily lead to introduction of novel genes in the recipient population. The introduction depends on the fitness effect of each novel gene (Fig. 1).

In this paper we will discuss both stages of introgression. Attention will be paid primarily to two crop examples: *Beta vulgaris* (beet) and *Daucus carota* (carrot). After

a brief introduction to the domestication background of these crops, gene flow, gene establishment and the design of fitness measuring experiments will be discussed.

SURVEY OF CROP CHARACTERISTICS

Several characteristics make a crop suitable for research on introgression and establishment in the framework of biosafety studies. Data on gene flow (first phase of introgression, Fig. 1) are already available in many crops. Since restricted gene flow between crops and their wild relatives will limit the possibility of studying gene establishment (Lavigne 1994), unrestricted gene flow will allow us to focus on the second phase: spread and establishment of a transgene within a population. First-year flowering may enhance the rate of establishment of genes in a population. Finally, a case study based on a crop of which transgenic varieties are already available will result in conclusions which can be directly applied for support of decisions on transgenic variety release. So, the following prerequisites should be met:

- predominantly outcrossing;
- absence of reproductive barriers between crop and wild relative;
- first-year flowering (whether or not after vernalization); and
- foreseen releases of transgenic plants.

Two of the crops which confirm to these requirements are *Beta vulgaris* (beet, cross-pollinated by wind) and *Daucus carota* (carrot, cross-pollinated by insects).

DOMESTICATION BACKGROUND OF BEET AND CARROT

In order to properly evaluate establishment of genes in either weedy or wild populations, some information on the domestication background of beet and carrot will be given.

The cultivation and introduction of beet (red beet or beetroot) to new areas in northern Europe was accompanied by artificial selection, which resulted in the types fodder beet, beet root and sugar beet (Campbell 1976; Fig. 2). It is not known whether the more modern sugar beet cultivars originated from crosses between Silesian sugar beet varieties and types of *B. maritima* or from selection within the Silesian beets (Bosemark 1993). All species and varieties of section *Beta*, i.e. all close relatives of beet, are interfertile with each other (Eijlander 1989). A system of self-incompatibility is based on four interacting S-loci with two alleles each (Bosemark 1993). An experiment with one beet plant in a circle of six other plants each carrying a homozygous dominant marker (anthocyanin in the stem) resulted in 100% F_1 seedlings from the centre plant, i.e. in complete cross pollination (Wellensiek 1943). Beet is grown as a biennial crop. This is achieved by using exclusively cultivars with a high vernalization requirement. Bolters, i.e. plants which flower in their first year, can occur, but modern cultivars have a fairly low bolting incidence. Several reasons for bolting are assumed. It can be caused either by early sowing in cold springs of plants, therewith vernalizing the plants (Bosemark 1993), or by hybridization with weedy or wild relatives with a low vernalization requirement (Ford-Lloyd & Hawkes 1986). Two different genetic systems of vernalization requirement are currently recognized, i.e. one gene with two alleles (qualitative system with Mendelian segregation; Letschert 1993), or quantitative regulation based on a set of genes (Abegg 1936; Boudry *et al.* 1993). At least a major
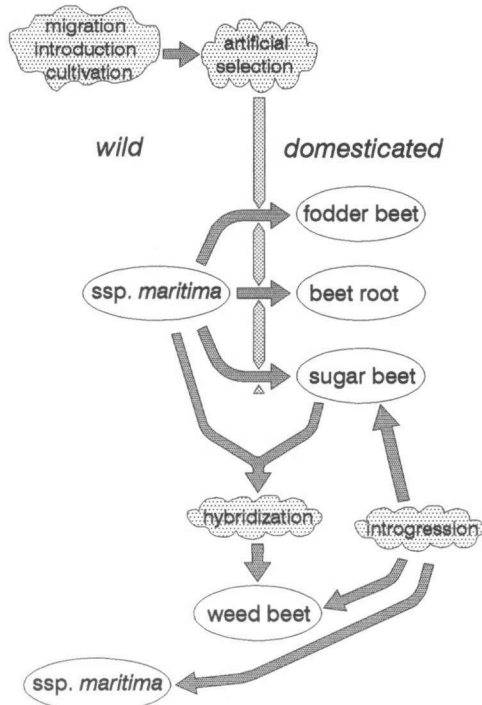
Fig. 2. Domestication history of beet, *Beta vulgaris* ssp. *vulgaris* (network display after Van Raamsdonk, 1995).

gene *B* is available, where the genotypes *BB* and *Bb* flower in the first year without vernalization. Wild beets in the Mediterranean area flower without vernalization and are therefore supposed to carry the *B* allele.

In *Daucus*, the most common wild subspecies ssp. *carota* and the domesticated ssp. *sativus* are completely interfertile. The species is outbreeding and self-fertilization is prevented by protandry (Banga 1976). Hybridization experiments were carried out successfully (Brandenburg, 1981). Intensive unintentional crossing and introgression of wild and cultivated carrots have been reported (Dale 1974; Brandenburg 1981; Small 1978, 1984; Wijnheimer *et al.* 1989). In eastern Europe introgression of domesticated traits from purple-rooted carrots is observed. The possibility of observing this introgression may be due to the very primitive background of this purple-coloured carrot, referred to as 'eastern carrot' (Small 1984). On the other hand, domesticated characters are hardly found in Canadian weed and wild populations (Small 1978). Hybrid plants in a wild population in The Netherlands had difficulties establishing, and second generation offspring hardly survived (Brandenburg 1981). The existence of wild, but presumably F$_1$ populations as may be deduced from an experiment of Lévêque de Vilmorin (1886) also points to successful introgression. Lévêque de Vilmorin (1886) were able to breed domesticated carrots from a wild population in a few generations. Similar results have not been achieved since then in similar experiments. The most likely explanation is that Lévêque de Vilmorin used the segregating offspring of a contaminated (i.e. F$_1$) population (Brandenburg 1981; Van Raamsdonk 1986).
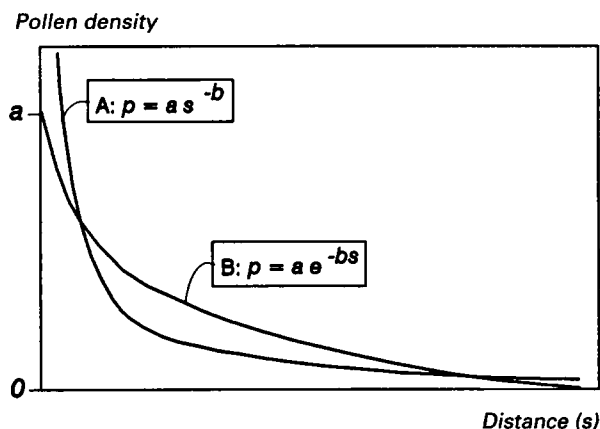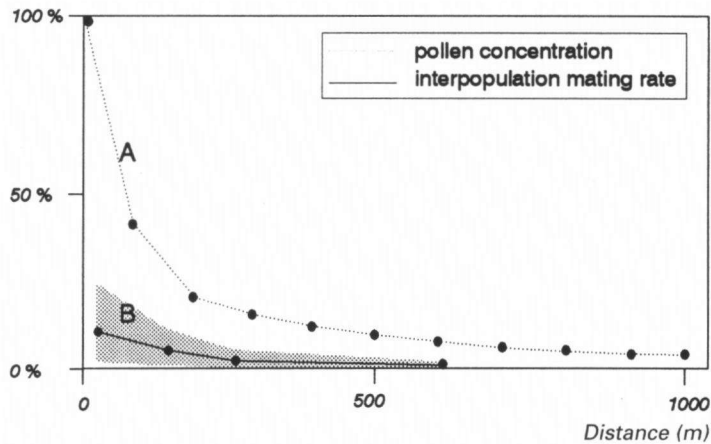
Pollen density

Fig. 3. Pollen density in dependence on distance to the pollen source. A: Inverse power model; B: negative exponential model. *a* and *b* have arbitrary values.
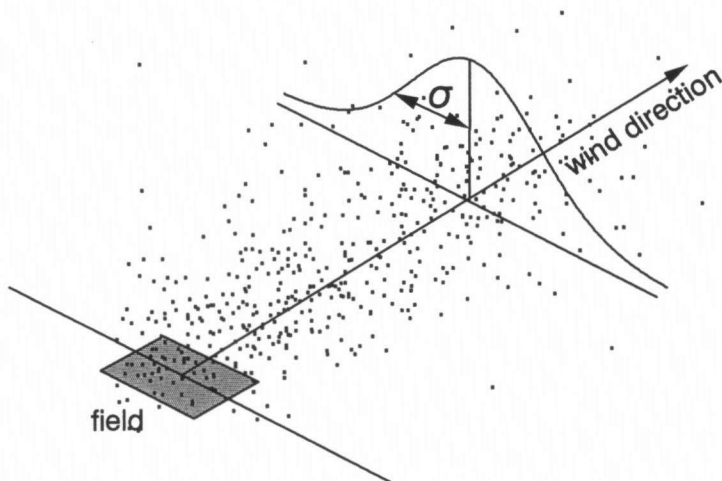
GENE FLOW

Two vectors are of importance for gene flow: seed and pollen (Fig. 1). Major differences between seed and pollen as carriers of genetic information are the size, which is one of the determinants of dispersal distance, and the genetic content. Seeds, being diploid, are independent propagules for establishing new plants, whereas pollen is normally haploid, which implies that transfer is successful only when an appropriate receptor plant is available. A difference between gene flow either by pollen or seeds, is that cytoplasmic inherited genes are usually transferred by seeds only (Rieseberg & Brunsfeld 1992). The occurrence of, for instance, cytoplasmic male sterility can therefore be used as indicator for the direction of gene flow. Gene flow by pollen accounts for considerable gene flow as is indicated in a number of studies (reviewed in Ellstrand & Hoffman 1990; Klinger *et al.* 1991; Van Raamsdonk 1995). In several papers pollen is implicitly assumed as vector for gene flow (e.g. Ellstrand & Hoffman 1990; Wilson 1990). Nonetheless this information, seed dispersal, including dispersal by artificial means (trade, soil transport), also needs significant attention when discussing gene flow, but quantitative data on seed dispersal is scarce. In *Beta vulgaris* in northern France seed dispersal appeared to occur within distances less than 100 metres. Calculations indicated that pollen dispersal is 60–90 times as large as natural seed dispersal (Cuguen, pers. comm.). Seed dispersal by man can occur at much larger distances than natural seed dispersal, since northern France weed beet populations originated from hybridization in south west France seed production fields (Boudry *et al.* 1993).

Pollen flow by wind

The dispersal of pollen by wind can formally be described in several ways. Two simple equations that may be used for curve fitting to describe pollen dispersion are the inverse power model and the negative exponential model (Fig. 3). Comparison of the success of these models in fitting data does not indicate a clear preference for either equation (Fitt & McCartney 1986). The curves of *Beta vulgaris* as displayed in Fig. 4 fit better to the negative exponential model.

Fig. 4. A: Dispersal of wind-borne pollen of beet and grasses. Percentages indicate the amount of pollen found at regular intervals outside the population relative to the amount of pollen within the blooming population (Jensen & Bogh, 1941). B: Interpopulational mating in beet. The percentages indicate the part of the total seed lot produced by a population after pollination with marker containing pollen of a specially designed donor population at several distances. Shaded area: variation caused by different wind directions (Schneider, 1944).

Fig. 5. Illustration of the Gaussian plume model, describing wind dispersal of pollen.

A mechanistic approach that is used most widely is provided by the Gaussian plume model (Fig. 5). This model has been used for describing the dispersion of air pollutants around smokestacks, but they have also been applied to spore dispersal (e.g. Aylor 1986; De Jong *et al.* 1990, 1991). The Gaussian plume model is based on the normal probability distribution. The normal probability distribution is given by a bell-shaped curve whose equation is:

$$P = \frac{1}{\sqrt{2\pi}\,\sigma}\,e^{-\frac{(\bar{x}-x)^2}{2\sigma^2}} \tag{1}$$

where P is the probability density function, σ^2 is the variance of P and x is the spread at a given distance s from the source. The factor $1/\sqrt{2\pi}$ included so that the total area under the curve from $x = -\infty$ to $x = +\infty$ will be equal to unity. Although the Gaussian plume model has several restrictions (see KNMI 1979, pp. 166–168), it appears to be the most widely used model for wind dispersal. The Gaussian plume model deals here with dilution and spread of a pollen cloud. For estimations of gene flow from crops to wild vegetations, estimations are needed on the number of pollen that depart from the crops relatively to that number from the wild plants, and the spatial distribution of the wild plants. If differences exist in timing of pollen release by crops and wild populations a correction may be needed when simulating fertilization competition by pollen from crops and pollen from wild plants.

At a distance of 1000 metres from the source windborne pollen of beet and grasses could still be detected (Fig. 5). Effective interpopulational mating in beet, expressed as percentage of seeds with a paternal parent from outside the population, was found at distances up to 606 metres (Fig. 5). Madsen (1994) carried out an experiment with a donor population of *B. vulgaris* var. *conditiva* (red beet) and receptor populations of *B. vulgaris* ssp. *maritima* in the directions E, ENE and ESE at several distances between 9.4 m and 75 m. Westerly wind was predominant. The interpopulational mating rate ranged from 3·6% at 9·4 m to 0·06–0·31% at 75 m. These interpopulational mating rates are surprisingly low compared to other wind pollinators (reviewed in Van Raamsdonk 1995). Interpopulation mating rates at distances between 50 m and 122 m ranged from 8% to 88% in the case of *Phlox* and *Pinus* (Ellstrand & Hoffman 1990). Pollen flow over distances of several kilometres has been traced around seed production fields of beet (van Dijk, pers. comm.). The comparability of the experimental results depends on the set-up of the experiment, on the absolute numbers of the donor and receptor plants, and on the ratio between these two numbers.

Bidirectional introgression was traced between wild and domesticated beets (Santoni & Berville 1992). A specific ribosomal DNA marker was present in 12 of 13 *B. vulgaris* var. *maritima* plants, whereas bolters in four of five production fields showed rDNA markers of *B. vulgaris* var. *maritima*. The absence of these markers in the bolters of the fifth field was explained by assuming a hybrid origin of the '*B. vulgaris* var. *maritima*' pollen donors surrounding this field (Santoni & Berville 1992). The isolation requirement for the production of basic seed is 1000 m, and for the production of certified seed between 300 m and 1000 m depending on the circumstances and ploidy level (Reheul 1987a; Santoni & Berville 1992; Bosemark 1993). Raybould & Gray (1993) stated an isolation requirement of 3200 m in beet.

Pollen flow by insects

Manasse (1992) used a special case of the negative exponential model as a frequency distribution for gene flow via pollen dispersal by insects:

$$P = \frac{1}{b} e^{-\frac{s}{b}} \tag{2}$$

Manasse (1992) estimated the factor b of the curve fitting models (Fig. 3) for dispersal of a marker gene in *Brassica campestris*. The pollen that contained the marker gene were spread by insects, mostly honey bees. He showed that at high plant densities the gene

flow was on average less far than at low densities. Apparently, the insects flew further if less flowers were available. Manasse (1992) also tested less parsimonious equations in the exponential family containing more parameters, but obtained no significantly better fits to his gene flow data. Kareiva et al. (1994) tested a Weibull probability function. In case of the negative exponential model the probability of a pollen of being deposited is constant when carried away from its source, whereas in the Weibull function the probability of deposition varies with distance away from the pollen source. A more explanatory model for honeybee movement is the 'advection–diffusion equation' (Okubo & Levin 1980). This model breaks movement into two components: random movement ('diffusion' similar to Equation 2) and directed movement because of attraction by flowers (advection). Kareiva et al. (1991) combined this model for bee movement with pollen deposition at a constant relative rate according to the negative exponential model. This combined model predicted that with declining distances between plants, bee movement and gene flow would also decline. This corresponded with their experimental results.

From experiments with carrot (Gary et al. 1972, 1977) it appeared that the advection component is important. Bees from apiaries at the borders of a carrot field (17 ha) and an adjacent onion (*Allium cepa*) field (2 ha) showed a slight tendency to forage more frequently on onion flowers, but bees from more distantly located apiaries (up to 6117 m) visited carrot flowers much more frequently. The mean foraging distance of bees on carrot was 1663 m (range: 121–6117 m), which was much higher than on onion (557 m; range: 41–4346 m; Gary et al. 1972). Carrot appeared to have the most attractive flowers compared to onion and safflower (*Carthamus tinctorius*), as far as may be concluded from the foraging distances of bees (Gary et al. 1977). Gary et al. (1972) found a considerable inter-field foraging of bees of the same colony. The minimal isolation distance for fodder carrot in The Netherlands is 500 m for basic seed and 300 m for certified seed (Reheul 1987b). Raybould & Gray (1993) stated an isolation requirement of 900 m in carrot.

GENE ESTABLISHMENT AND SPREAD

Even at relatively low hybridization rates a domestication gene will finally enter a population of a wild relative. In the second phase of introgression, i.e. establishment and spread of the gene, the effect on the fitness of the individuals carrying the gene is very important (Fig. 1). The gene will gradually be lost in the population when it has a negative fitness effect. A domestication gene with a fitness enhancing effect will generally suffer from a domesticated genetic background, which may have a decreasing fitness effect (Regal 1994; Dale 1994). However, if the hybrid can produce some offspring after backcrossing with wild representatives in the population, segregation and recombination will result in a loss of the fitness decreasing genetic background. This process will increase the total fitness of the plants carrying the gene in subsequent generations (Gregorius & Steiner 1993; Regal 1994; Kareiva et al. 1994) resulting in increased adaptation to new environments or in genetic pollution (Fig. 1b). Even a limited selective disadvantage of a gene can be compensated by recurrent gene flow or genetic drift (Dobzhansky et al. 1977; Hancock 1992; Tomiuk & Loeschke 1993). Recurrent gene flow can even result in such a serious fitness decrease that it can cause extinction of wild populations (Williamson 1993). The contribution to the seed bank of seeds
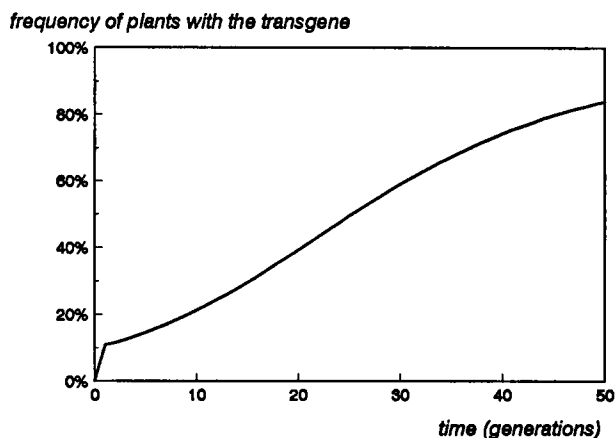
frequency of plants with the transgene

time (generations)

Fig. 6. An example of the establishment of a fitness-enhancing transgene in a wild population after growing a transgenic crop during one growing season only at $t=0$. The curve is a continuation of the numerical example given in the text.

containing transgenes can cause effects after years due to seed dormancy and persistence (Linder & Schmit 1994; Dale 1994).

Models of establishment

Although there is a vast amount of population genetic theory on modelling natural selection, there are only a few publications on modelling establishment of transgenes. A combined approach which provides information on changes in both allele frequency and population size was not published. Therefore, such a model has been developed by applying parts of population genetic theory on modelling establishment of transgenes.

Genotype frequencies in the course of time can be calculated by using the equation for the Hardy–Weinberg equilibrium, if several parameters values are known. Several underlying assumptions for the model are: a diploid organism which is reproducing sexually with non-overlapping generations, absence of genetic drift and mutation and presence of mendelian segregation. The selection coefficient will be applied to the plant stage, i.e. gamete selection is excluded.

Consider in a numerical example a pollen flow from a transgenic crop to a fully inter-fertile wild population of flowering plants, with 50% of the pollen from the crop containing one copy of a transgene A. Further it is assumed here that 20% of the pollen that fertilize the wild plants are from the transgenic crop. Then the initial frequency of the transgene A among the male gametes $p_{\male}$ equals $(0\cdot5)(0\cdot2)=0\cdot1$. The frequency of male gametes without the transgene, $q_{\male}$ equals $(1-p_{\male})=1-0\cdot1=0\cdot9$ (Fig. 6). After this single gene flow event the crop is removed and, hence, the establishment of the transgene depends on its selective advantage. Assuming that A behaves as a dominant gene with a selective advantage $\omega=0\cdot1$, the ratio $AA{:}Aa{:}aa$ changes from $0{:}0\cdot1{:}0\cdot9$ at the beginning of the generation time, into $(0\times1\cdot1){:}(0\cdot1\times1\cdot1){:}0\cdot9$ at the end of the generation time. These latter data can be normalized to frequencies by dividing by the sum: $P(AA)=0$, $P(Aa)=0\cdot109$, and $P(aa)=0\cdot891$. From the genotype frequencies the gene frequencies of the new gametes can be derived: $p_{\female}=P(AA)+0\cdot5\ P(Aa)=0\cdot0545$, and $q_{\female}=1-p_{\female}=0\cdot9455$ and the share of A in the next generation can be calculated.

In Fig. 6 the frequency of plants that harbour the transgene is given in case of presence of the transgenic crop during the first year only. Note that the frequency of the transgene continues to increase although the crop has been removed. Fitness enhancing genes have a permanent effect on the wild population. Fitness reducing genes of the domestication background of the hybrids are removed by recombination and selection, therewith having a temporal effect only.

The model can be run for different levels of gene flow, different fitness-values, etc. giving quantitative insight. Recurrent gene flow, mutation and genetic drift can be added to this model.

Genetic drift

In the above-described selection model an infinite population size is assumed. If the wild population is small, 'chance' factors may play a significant role. This may give rise to remarkable deviations from expected gene frequencies. To account for this phenomenon, called random genetic drift, calculations for small populations should be executed at the level of individual plants, rather than at the population level. By repeating the calculations for all plants separately, the stochastic behaviour of the small population can be studied. This is called a *stochastic model*, whereas the model described in the previous section is a *deterministic model*. For stochastic simulations random numbers can be used. This procedure is also applied for other processes during which chance plays a role, such as probability of survival and reproduction in dependence of fitness. The calculations are repeated for all plants in the population, and average frequencies are subsequently calculated. Each new run for all plants provides a result that deviates to some extent from the expected result, because of chance. By running the model a number of times, using the same parameter values, insight is gained into the extent of fluctuations caused by random genetic drift.

Modelling population size

Andow (1994) provided several examples of pest introductions that dramatically influenced local plant communities by nearly eradicating a plant species in the introduction area. Analogously, introduction of a novel resistance gene may dramatically change a plant community. If primary consumers strongly suppress the population size of a plant species in a wild vegetation, then the introduction of an effective resistance gene might enhance the fitness of the plants strongly, therewith increasing the size of the plants' population significantly. The effects of fitness alterations on population size can be incorporated in the above described simulation model. Figure 7 shows an expectation of the course of the size of a wild population after a gene flow from a transgenic crop during one season. Stochastic fluctuations are left aside. In Fig. 7 it is assumed that a number of domestication genes enter the wild population after a pollen flow. The majority of these genes are fitness-reducing or fitness-neutral, resulting into a decrease of the population size. Some of the genes, however, are fitness-enhancing, such as a gene that provide resistance to a pest or disease that suppresses the population size. During the first generations after the gene flow, the fitness-reducing genes will be removed by selection, but the frequency of fitness-enhancing genes will increase. Finally the average fitness and population size will be higher than before the occurrence of the gene flow. If a fitness-enhancing gene is linked to one or more fitness-reducing genes because of location on the same chromosome, then increase of the population size will
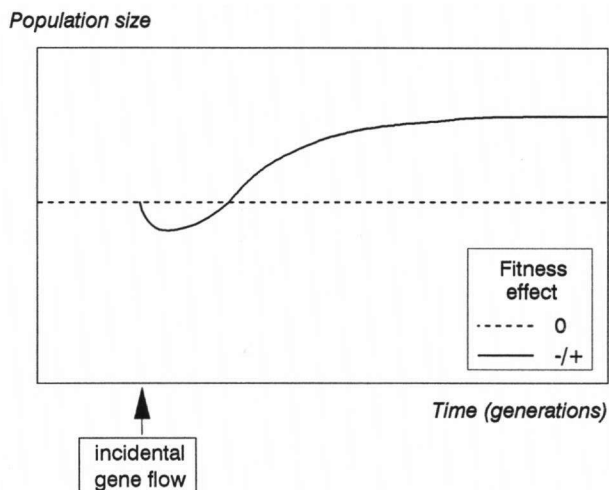
Population size

Fig. 7. Expected course of the size of a wild population after entrance of a series of domestication genes from a crop (see text).

be delayed. All these effects can very well be simulated. In this approach population genetics are linked to population dynamics. To the authors' knowledge this is new to risk assessment studies of transgenic plants.

Spread

A novel and fitness-enhancing gene that is introduced into a plant population will increase in frequency and will spread through the population. Both gene flow and fitness effects influence the velocity of spread through a population. The velocity of the invasion front is expressed in distance per time unit. Kareiva (1990) and Manasse & Kareiva (1991) reviewed and used theoretical equations from Fisher, Weinberger and others in order to obtain an equation that describes the speed of the invasion front. Fisher (1937) used a model in which spread is related to gene diffusion and to selection rate. Fisher's 'diffusion and selection' equation predicts an asymptotic spread of the introduced gene in the form of a travelling wave with an asymptotic rate of spread. Manasse & Kareiva (1991) preferred a later-developed model by Weinberger that required less strict assumptions, and elaborated that model numerically. They concluded that virtually all spread models predict that at first the rate of spread will be slow and will gradually accelerate to some asymptotic maximum rate in a uniform environment. Van den Bosch *et al.* (1988) discovered the same phenomenon for focus expansion in plant diseases.

Establishment in weedy populations

Reboud (1992) made a simulation model for the dynamics of a herbicide resistance gene that escaped from a crop to a weed in a verge along the crop. He did not focus on wild populations. Reboud (1992) assumed in his model that the weedy plants that are closely related to the crop are very numerous in the verge (infinitive), and therefore he ignored random genetic drift. He simulated the frequency of the herbicide resistance genotypes in the verge at equilibrium, which was assumed to be attained after 200 generations. The

model was not tailored to a specific plant species. Although reproductive isolation appeared to be important, in some situations even a poor compatibility results in a high frequency of resistant weeds after 200 generations. According to his model this is the case if the plant is an outbreeder, if there is a strong pollen flow and seed flow, if resistant individuals show no decrease in fitness when the herbicide is not used, and if segregation in the F_2 generation restores phenotypically wild plants (Reboud 1992). Lavigne (1994) concluded that 'a nuclear transgene would be more of a problem than a recessive or cytoplasmic one if it escapes from the crop'. Her models also indicated that interactions between parameters may play an important role, even on a short term. This would hamper simple predictions. In both beet and carrot weedy populations have been established, although with a different origin and genetic background. Some information is available on the genetics of these populations. The origin and evolution of weed beets are extensively studied in north western Europe, because of their economic problems (Hornsey & Arnold 1979; Evans & Weir 1981; Ford-Lloyd & Hawkes 1986; Boudry *et al.* 1993; Gliddon 1994). Ford-Lloyd & Hawkes (1986) recognized three categories of weed beets, of which two are of importance for biosafety studies: (a) so-called volunteer beets or bolters, genetically identical to domesticated beet, but with a relatively low vernalization requirement, and (b) conspecific wild forms (ssp. *maritima*), including their hybrids with domesticated beet. In both categories (a) and (b) the bolting allele *B*, i.e. the ability to flower in the first year of growing, is abundant.

Carrot is not indigenous in the New World. The escape from cultivation and establishment of weedy carrots in Canada with wild characters such as colourless, non-edible roots and bolting (the absence of vernalization requirement; Small 1978), indicates that wild type alleles remained in the domesticated carrot, presumably as recessive alleles in heterozygous plants. A high level of heterozygosity was indeed demonstrated in four common cultivars (St Pierre & Bayer 1991). The wild type allele of the bolting gene appeared to be recessive (Wijnheimer *et al.* 1989). Carrot is grown as a biennial crop. The biennial habit is dominant in carrot, which is in contrast to the dominant annual habit in beet. Therefore, heterozygous (i.e. hybrid) plants are unrecognizable in the carrot field and seed lots can contain unknown frequencies of the unwanted recessive, 'annual' allele. The dominance of the absence of vernalization requirement can be one of the causes for the difficulty to recognize weedy carrot populations, contrary to the abundance in beet.

MEASURING FITNESS

Good estimations of fitness effects of domestication genes and of linkage between genes that affect fitness are required in order to validate a simulation model. Transgenic plants have often been tested, but unfortunately these experiments are not suited for fitness estimations as needed for a simulation model. Until now, only a few experiments were reported of which certain aspects are useful for this goal. The most elaborate experiment was carried out by Crawley *et al.* (1993). They used experiment with a herbicide resistance line (Basta® resistance) and a kanamycin resistance line of *Brassica napus* ssp. *oleifera* (oilseed rape 'Westar') in three subsequent years (1990, 1991 and 1992) in 12 different habitats at three different sites in England, with four replicates per site/habitat. In absence of selective pressure, i.e. without applying the appropriate herbicide, transgenic rape seed is not more invasive or more persistent in natural habitats than conventionally bred lines. This experiment has been the object of

much debate (Kareiva 1993; Miller *et al.* 1993, 1994; Crawley 1993, 1994), but the experimental design can be used as basis for improved tests.

In an experiment by Fredshavn *et al.* (1995), attempting to estimate inter-specific competitive ability, plants of *Brassica napus* ssp. *oleifera* (oilseed rape) transgenic and non-transgenic lines were planted in mixed plots of different densities with the more aggressive crucifer (*Sinapis alba*). It was concluded that the relationship between yield of the oilseed rape and density was not different between the transgenic and non-transgenic varieties.

An experiment comparable to that of Crawley *et al.* (1993) was carried out by Madsen (1994) using three different types of *Beta* plants: hybrids between a glyphosate tolerant transgenic sugarbeet line and *B. vulgaris* ssp. *maritima* (sea beet), a non-transgenic sugarbeet line and *B. vulgaris* ssp. *maritima* itself. The plants were planted in monocultures in a series of mixed plots (plant ratios of 1:1, 1:4, 4:1) with three densities. The above-ground biomass (fresh weight) was used as measurement for competitive ability. Both sugar beet and the hybrid seem to show a somewhat higher competitive ability than sea beet in the sea beet population plots. An important drawback of this experiment is that the fresh root weight was not measured, while the rate of investment in root biomass is a major difference between sugar beet and sea beet (Madsen 1994).

Experiments for measuring interspecific competition with *Beta vulgaris* var. *altissima* (sugar beet) resistant to Beet Necrotic Yellow Vein Virus (BNYVV) were carried out by Bartsch *et al.* (1995, 1996). Competitive ability was measured by using mixed plots with *Chenopodium album*. Three types of beet plants were used: a transgenic test line with BNYVV resistance, a genetically comparable control line, and a conventionally bred BNYVV tolerant cultivar. Test plots were sown on a BNYVV infested field and on a non-infested field. In the 1993 experiment at the infested field, i.e. with selection, the fresh weight production of the transgenic resistant line was comparable to that of the susceptible control. However, in the 1994 experiment the fresh weight production was intermediate between the control and the conventionally bred tolerant cultivar. According to Bartsch *et al.* (1996), an increasingly additive ecological advantage of the transgenic test line can be noticed. The conventionally bred cultivar showed the highest production in all experiments due to an optimal breeding result. No significant differences were found between the three lines at the non-infested field (Bartsch *et al.* 1995, 1996).

It is apparent that in the experiment with selective pressure by Bartsch *et al.* (1996) the transgene had a fitness-enhancing effect. On the other hand, in the experiments without selective advantage of the transgenic lines (Crawley *et al.* 1993; Madsen 1994; Fredshavn *et al.* 1995; Bartsch *et al.* 1996) it is not surprising to discover that there is no enhancing effect on fitness of a character when the selective pressure is absent.

Reboud & Till-Bottraud (1991) found a fitness reduction between nearly isogenic lines of *Setaria italica* (foxtail millet) with and without a herbicide resistance gene. This fitness reduction of the resistant plants appeared to be density dependent and increased with density in a competition experiment. In this experiment selective pressure was not applied by using the herbicide (Reboud & Till-Bottraud 1991) and the fitness reduction can be due to genetic load. Small negative effects of a transgene on fitness by the absence of the selective agent can be compensated by a very small continuous gene flow from other populations which contain the transgene (i.e. the domesticated relative of a wild population; Dobzhansky *et al.* 1977) or by correlated response (hitch-hiking by linkage to fitness-enhancing genes; Price & Langen 1992).

CONCLUDING REMARKS

A computer model for simulating gene flow and establishment, including the effect of fitness on allele frequencies and population size, can be developed. A stochastic approach is preferred in order to be able to include genetic drift since wild populations are often small. The few risk assessment models available usually focus on effects of fitness on genotype frequencies, but never calculate the effects on the size of the wild population. The effects of fitness changes on population size particularly may be of more interest to decision makers than changes in genotype frequencies.

ACKNOWLEDGEMENTS

REFERENCES

Abegg, F.A. (1936): A genetic factor for the annual habit in beets and linkage relationship. *J. Agr. Res.* **53**: 493–511.

Andow, D.A. (1994): Community response to transgenic plant release: using mathematical theory to predict effects of transgenic plants. *Mol. Ecol.* **3**: 65–70.

Aylor, D.E. (1986): A framework for examining inter-regional aerial transport of fungal spores. *Agric. For. Meteorol.* **38**: 263–288.

Banga, O. (1976): Carrot, *Daucus carota* (Umbelliferae). IN: Simmonds, N.W. (ed.): *Evolution of Crop Plants*, pp. 291–293, Longman, London and New York.

Bartsch, D., Pohl-Orf, M., Schmidt, M. & Schuphan, I. (1995): Naturalization of transgenic (BNYV-virus resistant) sugar beets in agricultural and non-agricultural areas. *Paper presented at the Symposium for Risk Assessment of Transgenic Plants*, Monterey, California.

Bartsch, D., Schmidt, M., Pohl-Orf, M., Haag, C. & Schuphan, I. (1996): Competitiveness of transgenic sugar beet resistant to beet necrotic yellow vein virus and potential impact on wild beet populations. *Mol. Ecol.* **5**: 199–205.

Bosemark, N.O. (1993): Sugar beet. In: *Traditional crop breeding practices: an historical review to serve as a baseline for assessing the role of modern biotechnology*, pp. 123–136, Organization for Economic Cooperation and Development, Paris.

Boudry, P., Mörchen, M., Saumitou-Laprade, P., Vernet, Ph. & Dijk, H. van (1993): The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. *Theor. Appl. Gen.* **87**: 471–478.

Brandenburg, W.A. (1981): Possible relationships between wild and cultivated carrots (*Daucus carota* L.) in the Netherlands. *Kulturpflanze* **29**: 369–375.

Campbell, G.K.G. (1976): Sugar beet, *Beta vulgaris* (Chenopodiaceae). In: Simmonds, N.W. (ed.): *Evolution of Crop Plants*, pp. 25–28, Longman, London and New York.

Crawley, M.J. (1993): Arm-chair risk assessment [Correspondence]. *Bio/Technology* **11**: 1496.

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D. & Buxton, J. (1993): Ecology of transgenic oilseed rape in natural habitats. *Nature* **363**: 620–623.

Crawley, M.J. (1994): Risk assessment redux—reply. *Bio/Technology* **12**: 217.

Dale, J.M. (1974): The biology of Canadian weeds. 5. *Daucus carota.* Can. *J. Plant Sci.* **54**: 673–685.

Dale, P. (1994): The impact of hybrids between genetically modified crop plants and their related species: general considerations. *Mol. Ecol.* **3**: 31–36.

De Jong, M.D., Scheepens, P.C. & Zadoks, J.C. (1990): Risk analysis applied to biological control of a forest weed, using a Gaussian plume model. *Grana* **29**: 139–145.

De Jong, M.D., Wagenmakers, P.S. & Goudriaan, J. (1991): Modelling the escape of *Chondrostereum purpureum* spores from a larch forest with biological control of *Prunus serotina. Neth. J. Plant Pathol.* **97**: 55–61.

De Vries, F.T., Meijden, R. van der & Brandenburg, W.A. (1992): Botanical files: a study of the real chances for spontaneous gene flow from cultivated plants to the wild flora of the Netherlands. *Gorteria suppl* 1, Rijksherbarium/Hortus Botanicus, Leiden.

Dobzhansky, Th., Ayala, F.J., Stebbins, G.L. & Valentine, J.W. (1977): *Evolution.* W.H. Freeman and Co., San Francisco.

Eijlander, R. (1989): Transgene biet, ja of nee. In: de Gier, A.M. & Van Raamsdonk, L.W.D. (eds.): Risico-analyse van transgene planten in de praktijk. *Supplement Prophyta* **43**: 232–233.

Ellstrand, N.C. & Hoffman, C.A. (1990): Hybridization as an avenue of escape for engineered genes. Strategies for risk reduction. *Bioscience* **40**: 438–442.

Evans, A. & Weir, J. (1981): The evolution of weed beet in sugar beet crops. *Kulturpflanze* **29**: 301–310.

Fisher, R. (1937): The wave of advance of advantageous genes. *Ann. Eugenics* **7**: 355–369.

Fitt, B.D.L. & McCartney, H.A. (1986): Spore dispersal in relation to epidemic models. In: Leonard, K.J. & Fry, W.E. (eds.): *Plant Disease Epidemiology,* Vol. 1, pp. 311–345, Macmillan, New York, USA.

Ford-Lloyd, B.V. & Hawkes, J.G. (1986): Weed beets: their origin and classification. *Acta Hort.* **182**: 399–402.

Fredshavn, J.R., Poulsen, G.S., Huybrechts, I. & Rüdelsheim, P. (1995): Competitiveness of transgenic oilseed rape. *Transg. Res.* **4**: 142–148.

Gary, N.E., Witherell, P.C., Lorenzen, K. & Marston, J.M. (1977): The interfield distribution of honey bees on carrots, onions and safflower. *Environm. Entomol.* **6**: 637–640.

Gary, N.E., Witherell, P.C. & Marston, J.M. (1972): Foraging and distribution of honey bees used for carrot and onion pollination. *Environm. Entomol.* **1**: 71–78.

Gliddon, C. (1994): The impact of hybrids between genetically modified crop plants and their related species: biological models and theoretical perspectives. *Mol. Ecol.* **3**: 41–44.

Gregorius, H.-R. & Steiner, W. (1993): Gene transfer in plants as a potential agent of introgression. In: Wöhrmann, K. & Tomiuk, J. (eds.): *Transgenic Organisms, Risk Assessment of Deliberate Release,* pp. 83–107, Birkhäuser Verlag, Basel.

Hancock, J.F. (1992): *Plant Evolution and the Origin of Crop Species,* Prentice Hall, New Jersey.

Hornsey, K.G. & Arnold, M.H. (1979): The origins of weed beet. *Ann. Appl. Biol.* **92**: 279–285.

Jensen, I. & Bogh, H. (1941): Om forhold der har Indflydelse paa Krydsningsfarenhos vindbestøvende Kulturplanter. *Tidsskr. Planteavl.* **46**: 238–268.

Kareiva, P. (1990): Using models of population spread to analyze the results of field releases. In: *Risk Assessment in Agricultural Biotechnology,* pp. 168–174. University of California, Oakland.

Kareiva, P. (1993): Editorial comment. *Nature* **363**: 580–581.

Kareiva, P., Manasse, R. & Morris, W. (1991): Using models to integrate data from field trials and estimate risks of gene escape and gene spread. In: MacKenzie, D.R. & Henry, S.C. (eds.): *Biological Monitoring of Genetically Engineered Plants and Microbes,* pp. 168–174, Agric. Res. Inst. Bethesda, USA.

Kareiva, P., Morris, W. & Jacobi, C.M. (1994): Studying and managing the risk of cross-fertilization between transgenic crops and wild relatives. *Mol. Ecol.* **3**: 15–21.

Klinger, T., Elam, D.R. & Ellstrand, N.C. (1991): Radish as a model system for the study of engineered gene escape rates via crop–weed mating. *Cons. Biol.* **5**: 531–535.

KNMI (1979): *Luchtverontreiniging en weer.* De Bilt, Staatsuitgeverij 's-Gravenhage.

Lavigne, C. (1994): *Les risques associés à la culture de plantes transgébiques résistantes aux herbicides,* PhD Thesis, Institut National Agronomique Paris-Grignon, France.

Letschert, J.P.W. (1993): *Beta* section *Beta:* biogeographical patterns of variation and taxonomy. Thesis, *Wageningen Agricultural University Papers* **93-1**: 1–155.

Lévêque de Vilmorin, P.P.A. (1886): Notice sur l'amelioration de la carotte sauvage. In: *Notices sur l'amelioration des plantes,* pp. 5–14. Vilmorin-Andrieux et cie., Paris.

Linder, C.R. & Schmitt, J. (1994): Assessing the risks of transgene escape through time and crop-wild hybrid persistence. *Mol. Ecol.* **3**: 23–30.

Madsen, K.H. (1994): *Weed Management and Impact on Ecology of Growing Glyphosate Tolerant Sugarbeets (Beta vulgaris L.).* PhD Thesis, Royal Veterinary and Agricultural University, Copenhagen.

Manasse, R. (1992): Ecological risks of transgenic plants: effects of spatial dispersion on gene flow. *Ecol. Appl.* **2**: 431–438.

Manasse, R. & Kareiva, P. (1991): Quantifying the spread of recombinant genes and organisms. In: Ginzburg, L. (ed.): *Assessing Ecological Risks of Biotechnology,* pp. 215–231, Butterworth-Heinemann, Boston.

Miller, H.I., Huttner, S.L. & Beachy, R. (1993): Risk assessment experiments for 'genetically modified' plants. *BiolTechnology* **11**: 1323–1324.

Miller, H.I., Beachy, R. & Huttner, S.L. (1994): Risk assessment redux [Correspondence]. *Biol Technology* **12**: 216–217.

Okubo, A. & Levin, S.A. (1989): A theoretical framework for data analysis of wind dispersal of seeds and pollen. *Ecology* **70**: 329–338.

Price, T. & Langen, T. (1992): Evolution of correlated characters. *Trends Ecol. Evol.* **7**: 307–310.

Raybould, A.F. & Gray, A.J. (1993): Genetically modified crops and hybridization with wild relatives: a UK perspective. *J. Appl. Biol.* **30**: 199–213.

Reboud, X. (1992): *Les risques associés aux manipulations génétiques: le cas de la résistance aux herbicides.* PhD Thesis, Institut National Agronomique Paris-Grignon, France.

Reboud, X. & Till-Bottraud (1991): The cost of herbicide resistance measured by a competition experiment. *Theor. Appl. Genet.* **82**: 690–696.

Regal, P.J. (1994): Studying and managing the risk of cross-fertilization between transgenic crops and wild relatives. *Mol. Ecol.* **3**: 5–13.

Reheul, D. (1987a): Ruimtelijke isolatie in de plantenveredeling. 1. Ruimtelijke isolatie bij windbestuivers. *Landbouwtijdschrift* **40**: 5–14.

Reheul, D. (1987b): Ruimtelijke isolatie in de plantenveredeling. 2. Ruimtelijke isolatie bij insektenbestuivers. *Landbouwtijdschrift* **40**: 15–23.

Rieseberg, L.H. & Brunsfeld, S.J. (1992): Molecular evidence and plant introgression. In: Soltis, P.S., Soltis, D.E. & Doyle, J.J. (eds.): *Molecular Systematics of Plants*, pp. 151–176, Chapman & Hall, New York.

St Pierre, M.D. & Bayer, R.J. (1991): The impact of domestication on the genetic variability in the orange carrot, cultivated *Daucus carota* ssp. *sativus* and the genetic homogeneity of various cultivars. *Theor. Appl. Gen.* **82**: 249–253.

Santoni, S. & Berville, A. (1992): Evidence for gene exchanges between sugar beet (*Beta vulgaris* L.) and wild beets: consequences for transgenic sugar beets. *Plant Mol. Biol.* **20**: 578–580.

Schneider, F. (1944): Züchtung der Betarüben. In: Roemer, T. & Rudorf, W. (eds.): *Handbuch der Pflanzenzüchtung 4. Rüben, Kartoffeln, Ol und Gespinstpflanzen, Tabak*, pp. 1–96. Paul Parey, Berlin.

Small, E. (1978): A numerical taxonomic analysis of the *Daucus carota* complex. *Can. J. Bot.* **56**: 248–276.

Small, E. (1984): Hybridization in the domesticated weed–wild complex. In: Grant, W.F. (ed.): *Plant Biosystematics*, pp. 195–210, Academic Press, Toronto.

Tomiuk, J. & Loeschke, V. (1993): Conditions for the establishment and persistence of populations of transgenic organisms. In: Wöhrmann, K. & Tomiuk, J. (eds.): *Transgenic Organisms, Risk Assessment of Deliberate Release*, pp. 117–133, Birkhäuser Verlag, Basel.

Van den Bosch, F., Zadoks, J.C. & Metz, J.A.J. (1988): Focus expansion in plant disease. I: The constant rate of focus expansion. *Phytopathology* **78**: 54–58.

Van Raamsdonk, L.W.D. (1986): The grey area between black and white: the choice between botanical code and cultivated code. *Acta Hort.* **182**: 153–158.

Van Raamsdonk, L.W.D. (1993): Wild and cultivated plants: the parallelism between evolution and domestication. *Evol. Trends Pl.* **7**: 73–84.

Van Raamsdonk, L.W.D. (1995): The effect of domestication on plant evolution. *Acta Bot. Neerl.* **44**: 421–438.

Van Raamsdonk, L.W.D. & Van der Maesen, L.J.G. (1996): Crop–weed complexes: the complex relationship between crop plants and their wild relatives. *Acta Bot. Neerl.* **45**: 135–155.

Wellensiek, S.J. (1943): *Grondslagen der algemeene plantenveredeling*, Tjeenk Willink en Zoon, Haarlem.

Wijnheimer, E.H.M., Brandenburg, W.A. & Ter Borg, S.J. (1989): Interactions between wild and cultivated carrots (*Daucus carota* L.) in the Netherlands. *Euphytica* **40**: 147–154.

Wilson, H.D. (1990): Gene flow in squash species. Domesticated *Cucurbita* species may not represent closed genetic systems. *Bioscience* **40**: 449–455.

Williamson, M. (1993): Risks from the release of GMOs: ecological and evolutionary considerations. *Evol. Trends Plant Environm. Update* **1**: 5–9.