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Selection for *Phytophthora* field resistance in the $F_2$ generation of organic outdoor tomatoes

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Received: 9 August 2010 / Accepted: 31 January 2011 / Published online: 13 February 2011
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Abstract  Tomatoes are the most important vegetable, globally as well as in Germany. Outdoor tomato production is seriously impaired due to increasing infections with evolving late blight (*Phytophthora infestans*) populations. Within organic agriculture, research is being conducted to develop regionally adapted and open pollinated cultivars of outdoor tomatoes with late blight field resistance. In the present experiment, three crosses, including wild, cocktail, and beefsteak tomatoes, were selected for field resistance against late blight in $F_2$ at one location per cross. The comparison of positive and negative selection in $F_3$ revealed the selection of single $F_2$ plants to be efficient in all three crosses. $F_2$ selection has proved to be a robust and efficient tool for breeding programs. The correlated response to selection in other traits, including yield, fruit weight, days to maturity, harvest period, and plant height, depended on the cross. It was evident that selection for desired traits combined with field resistance against late blight is promising, even in wide crosses. The most undesired attribute of wild tomatoes is the formation of shoots on leaves and in inflorescences. No correlation was observed between field resistance and shoot formation, allowing the selection of genotypes with improved field resistance and yield, but without morphological disadvantages.

Keywords  Late blight · Organic breeding · *Phytophthora* · Resistance · Tomato

Introduction  Tomatoes are the most important vegetable on the global scale. In 2008, approximately 130,000,000 t were produced (FAO 2010). Germany imported approximately 660,000 t in 2008. Commercial horticulture produced approximately 63,000 t (FAO 2010) while approximately the same amount was harvested in home gardens (unpublished data). As in most neighboring regions, tomatoes intended for processing (canning, etc.) are imported in large quantities, while the domestic crop is almost exclusively used for the fresh market. In Germany, cheap and resource-efficient outdoor tomato production has almost ceased to exist due to increasing infections with late blight (*Phytophthora infestans* (Mont.) de Bary). Analysis of *Phytophthora* isolates from both host species, the tomato and the potato, collected during 1967–2000 revealed massive changes in population structure, resulting in more aggressive pathogen genotypes (Rullich et al. 2002). Similar changes were observed in France (Lebreton et al. 1998) and England (Day and Shattock 1997). Many
populations collected since the 1980s belonged to ‘new’ populations, which are characterized by the presence of both mating types in France and by new haplotypes in England. Prior to this time, only the A1 mating type was observed outside Mexico. The presence of the A2 mating type outside Mexico created the opportunity for sexual reproduction and the creation of new, more aggressive genotypes (Foolad et al. 2008). A2 outside of Mexico was first reported in Western Europe, but was observed worldwide soon after (Fry et al. 1993). The occurrence of oospores has been reported in many parts of the world, including Europe (Rullich et al. 2002; Drenth et al. 1995), North America (Gavino et al. 2000), Israel (Rubin et al. 2001), and Taiwan (Deahl et al. 2008). Recently, Klarfeld et al. (2009) have shown experimentally that known sources of resistance can easily be overcome by recombinant Phytophthora genotypes derived from oospores.

The experiment presented here is part of the Organic Outdoor Tomato Project, which was started in 2003 as a participatory selection and breeding program in Germany. A screening based on 3,500 accessions identified cultivars to improve amateur gardening (Horneburg 2007). The best parent cultivars were chosen to develop breeding strategies to be carried out in the breeding program in three organic market gardens in Central and Northern Germany. The first open pollinated cultivars resulting from the Organic Outdoor Tomato Project were released in 2010.

The accelerated recombination of P. infestans must be balanced by continuous resistance breeding in the target environments. Knowing in which segregating generation selection can be done under field conditions is of crucial importance for the development of an efficient breeding strategy. In the present experiment, the selection efficiency in F2, the first segregating generation after crossing, was tested. The use of genotypes not adapted to present production techniques was also considered. In wide crosses, we have used ‘wild tomatoes’ as sources for important traits, including earliness, high field resistance against Phytophthora, and fruit quality. However, these positive attributes are often combined with less-desired traits, like strong vegetative growth, rapid succession of short internodes, and abundant shoot formation on leaves and in inflorescences. The correlated response to selection in undesired traits when selecting desired traits was investigated.

**Materials and methods**

Three crosses were used in the present experiment, including wild, cocktail, and beefsteak tomatoes.

Cross 1: Wild tomato ‘Rote Murmel’ × cocktail tomato ‘Zuckertraube’.

Cross 2: Wild tomato ‘Golden Currant’ × beefsteak tomato ‘Paprikaformige’.

Cross 3: Cocktail tomato ‘Celsior’ × beefsteak tomato ‘Paprikaformige’.

The pedigrees of the parent genotypes are unknown. These genotypes were chosen due to their different susceptibilities to late blight infection in earlier field trials (Table 2). Information about resistance to specific late blight races was not available. In 2009, Rote Murmel was resistant against Phytophthora race US-11 in a test with detached leaflets from field-grown plants (Miles et al. 2010). One cross per location was grown and selected in central or northwest Germany. Typical to the research area, plants were grown as staked tomatoes and pruned to one main shoot. Locations are characterized in Table 1 and genotypes in Table 2. The term “wild” tomato is used for genotypes with very small fruits and heavy side shoot formation. “Wild” tomato is not a botanical term, as we do not know the history of the genotypes used in the experiment. Wild tomatoes have a very low yield if grown as staked tomatoes; however, fruits are abundant when the plants are grown with little or no pruning.

In 2004, 10 parent plants, 10 F1 plants, and 30 F2 plants were grown from each cross in two replications. The plants at Schönhagen and Ellingerode were damaged by late frosts shortly after being transplanted to the field plots. Early growth was severely impaired and it was not possible to determine yield level. The five best and five worst F2 plants per cross were selected according to the scores for Phytophthora leaf and fruit infections, general plant health, and fruit set at the end of the season. In 2005, F3 progenies of the 10 selected F2 plants and their parents were grown in a randomized block design with three replications and two plants per plot. Phytophthora infections were scored according to Table 3 at intervals of 5–28 days during periods of infection. The area under the disease progress curve (AUDPC) was calculated according to Kranz (1996):
AUDPC = \sum_{i=1}^{n-1} \left( \frac{x_{i+1} + x_i}{2} \right) (t_{i+1} - t_i)

with \( x_i \) is the score at time \( i \), \( t_i \) is the day of the \( i \)th observation, and \( n \) is the number of scores. High AUDPC values indicate high infection levels.

The tomatoes were sown on 18 March 2004 and 31 March 2005 in the greenhouse. They were transplanted to the fields on 19 May 2004 and 25 May 2005, respectively. The number of inflorescences with at least one flower in bloom was counted 91–101 days after transplanting. Plant height was measured 49–51 days after transplanting. Shoot formation on leaves and shoot formation in inflorescences were scored 75–101 days after transplanting;

\( 1 = \text{no shoots}, \ 9 = \text{maximum shoot formation} \). Planting to maturity indicates the period between the establishment of the field plots and full maturity of the first fruit. The total amount of healthy fruit produced per plant, or yield per plant, was determined in intervals of 14 days. The size of the fruit produced in each cross was calculated as the average fruit weight for the entire season. The highest truss with at least one mature and healthy fruit was counted. Harvest period is the period between the date the first healthy mature fruit was observed and the date the last healthy mature fruit was harvested.

Data were analyzed with Plabstat, Version 3A-pre (Utz 2005). Deviation of \( F_2 \) from the parent mean was tested using the \( t \)-test. Mean values of \( F_3 \)

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**Table 1** Description of the experimental sites

| Experimental sites | Schönhausen | Ellingerode | Rhauderfehn |
|--------------------|-------------|-------------|-------------|
| Management         | Bio-dynamic | Organic     | Organic     |
| Geographical location | 51°19’60” N, 10°1’0” E | 51°19’60” N, 9°49’0” E | 53°16’51” N, 7°53’71” E |
| Altitude           | 300 m       | 140 m       | 2 m         |
| Soil               | Loamy clay  | Sandy loam  | Sand        |
| Fertilizer         | 150–180 dt/ha composted cattle manure | 150–180 dt/ha composted sheep manure | 150–180 dt/ha sheep manure |
| Pre-crop 2004      | Winter wheat | Oats       | Clover grass |
| Pre-crop 2005      | Malva verticillata | Crimson clover | Clover grass |

**Table 2** Attributes and origin of the genotypes used

| Genotypes                  | Rote Murmel | Golden Currant | Celsior | Zuckertraube | Paprikaförmige |
|----------------------------|-------------|----------------|---------|--------------|----------------|
| Fruit type                 | Wild tomato | Wild tomato    | Cocktail| Cocktail     | Beefsteak      |
| Approx. fruit weight (g)   | 2           | 7              | 14      | 23           | 200–250        |
| Growth type                | Indeterminate | Indeterminate | Indeterminate | Indeterminate | Indeterminate |
| Fruit infection\(^a\)    | 61          | 81             | 73      | 155          |
| Leaf infection\(^a\)      | 135         | 130            | 179     | 229          |
| Origin                     | Dreschflegel\(^b\) | Dreschflegel\(^b\) | SamenArchiv\(^c\) | Commercial cultivar released 1994 by Reinsaat KG\(^d\) | Private seed saver B. Rumkowski |

\(^a\) Area under disease progress curve. Mean value 2003 Schönhausen and Rhauderfehn

\(^b\) In der Aue 31, 37213 Witzenhausen, Germany

\(^c\) Waldstraße 40, 90596 Schwanstetten, Germany

\(^d\) 3572 St. Leonhard am Hornerwald 69, Austria

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\( 1 \) = \text{no shoots}, \( 9 \) = \text{maximum shoot formation}.
progenies from positive and negative selection were compared using the t-test.

Results

In 2004, the average late blight fruit infection of F₂ plants from crosses 1 and 3 differed significantly from parent mean values and was closer to the more resistant parent (Tables 4, 5, 6). In all crosses, the genotypes with smaller fruits (Rote Murmel, Golden Currant, and Celsior) were less susceptible to Phytophthora infections. The F₂ plants from the positive and negative selections were clearly distinguishable by the level of fruit and leaf infections. Leaf infections in all three crosses differed significantly from the parent mean values and were closer to the more resistant parent. In crosses 1 and 3, F₁ was less infected than F₂ and both parents. In crosses 2 and 3, plant height decreased from F₁ to F₂. The wild tomatoes in crosses 1 (Rote Murmel) and 2 (Golden Currant) produced a higher number of inflorescences than the other parent (Tables 4, 5). F₂ plants, on average, produced an intermediate number of inflorescences. Plant height of the F₂ populations from crosses 1 and 2 was intermediate between the parent genotypes, but closer to the height of the wild tomato parents (Rote Murmel and Golden Currant). In cross 3 (Table 6), F₂ plants surpassed the height of the less vigorous parents (Celsior and Paprikaformige). For both wild tomato parents, abundant undesired shoot formation in inflorescences and on leaves (Golden Currant only) was observed, while the F₂ populations displayed intermediate levels (Tables 4, 5).

In 2005, F₃ progenies of the F₂ plants selected in 2004 were grown. In all three crosses, the positive selection had a higher field resistance against late blight than the negative selection (Tables 4, 5, 6). Differences were highly significant, the one exception being leaf infection in the cross Celsior × Paprikaformige. As in the previous year, differences in fruit infection were more pronounced than differences in leaf infection.

Selection for Phytophthora field resistance had an effect on morphology, phenology, and agronomic traits. Significant differences between positive and negative selection were observed for plant height in

Table 3 Key for the assessment of damages by late blight (Phytophthora infestans (Mont.) de Bary) on leaves and fruits of tomatoes in field experiments

| Leaf infections                                                                 | Fruit infections including small, immature fruits |
|--------------------------------------------------------------------------------|--------------------------------------------------|
| 1 No infections                                                               | 1 No infections                                   |
| 2 First symptoms as grey-green to brown leaf spotsa                           | 2 Up to 12.5% of the fruits with grey-green to brown spots |
| 3 Symptoms obvious. Yellowing or browning of some leaves or small leaf spots to 50% of plant height | 3 Up to 25% of the fruits with typical dark spots |
| 4 Increased yellowing or browning, or small leaf spots to 75% of plant height | 4 Up to 37.5% of the fruits with typical dark spots |
| 5 Plant severely affected                                                      | 5 Up to 50% of the fruits with typical dark spots |
| 6 Yellowing or browning to 50% of plant height                                 | 6 Up to 62.5% of the fruits with typical dark spots |
| 7 Yellowing or browning to 75% of plant height                                 | 7 Up to 75% of the fruits with typical dark spots |
| 8 Entire plant yellow to brown, all leaves infected                           | 8 More than 75% of the fruits with typical dark spots |
| 9 All leaves dead                                                              | 9 All fruits infected                              |

a At a low level of infection, discerning between late blight and early blight (Alternaria solani (Ell. & Mart.) L. R. Jones & Grout) is not always possible.
### Table 4  Effect of the selection for *Phytophthora* field resistance in cross 1 ‘wild tomato Rote Murmel × cocktail tomato Zuckertraube’ in Schönhagen, 2004 and 2005

| Schönhagen       | Fruit infection\(^a\) | Leaf infection\(^a\) | Number of inflorescences\(^b\) | Plant height (cm)\(^c\) | Shoot formation on leaves\(^d\) | Shoot formation in inflorescences\(^d\) | Planting to maturity (days) | Highest truss | Harvest period (days) | Fruit weight (g) | Yield (g) |
|------------------|------------------------|----------------------|--------------------------------|-------------------------|--------------------------------|----------------------------------------|--------------------------|---------------|---------------------|------------------|----------|
| **2004**         |                        |                      |                                |                         |                                |                                        |                          |               |                     |                  |          |
| Rote Murmel      | 31                     | 131                  | 3.6                            | 200                     | 1.3                            | 4.7                                    |                          |               |                     |                  |          |
| Zuckertraube     | 68                     | 150                  | 0.6                            | 100                     | 1.0                            | 1.7                                    |                          |               |                     |                  |          |
| F\(_1\)          | 35                     | 112                  | 2.9                            | 191                     | 1.3                            | 3.8                                    |                          |               |                     |                  |          |
| F\(_2\)          | 35***                  | 115***               | 2.3                            | 195                     | 1.4                            | 2.4                                    |                          |               |                     |                  |          |
| Positive selection F\(_2\) | 31                | 100                  | 2.8                            | 193                     | 1.0                            | 2.0                                    |                          |               |                     |                  |          |
| Negative selection F\(_2\) | 44                | 121                  | 2.4                            | 192                     | 2.6                            | 1.8                                    |                          |               |                     |                  |          |
| **2005**         |                        |                      |                                |                         |                                |                                        |                          |               |                     |                  |          |
| Rote Murmel      | 57                     | 142                  | 4.2                            | 187                     | 2.8                            | 2.8                                    | 63.0                     | 8.0           | 77.0                | 2.2              | 172      |
| Zuckertraube     | 271                    | 322                  | 3.0                            | 146                     | 1.8                            | 1.8                                    | 71.8                     | 4.0           | 38.8                | 22.5             | 342      |
| Positive selection F\(_3\) | 107                | 170                  | 4.2                            | 193                     | 3.0                            | 3.0                                    | 64.1                     | 7.5           | 72.2                | 11.1             | 681      |
| Negative selection F\(_3\) | 185***               | 276***               | 4.1                            | 177***                  | 3.4                            | 3.4                                    | 67.2*                    | 6.4           | 55.3***             | 8.6***           | 418***   |

\(^a\) Area under disease progress curve

\(^b\) 9 July 2004 and 13 July 2005

\(^c\) 26 August 2004 and 25 August 2005

\(^d\) 3 August 2004 and 8 August 2005

* *, ** Significant deviation from the parent mean (2004) or differences between positive and negative selection (2005) in the t-test at \(p = 0.05\), or \(p = 0.001\), respectively
### Table 5  Effect of the selection for *Phytophthora* field resistance in cross 2 ‘wild tomato Golden Currant × beefsteak tomato Paprikaformige’ in Ellingerode, 2004 and 2005

| Ellingerode | Fruit infection | Leaf infection | Number of inflorescences | Plant height (cm) | Shoot formation on leaves | Shoot formation in inflorescences | Planting to maturity (days) | Highest truss (days) | Harvest period (days) | Fruit weight (g) | Yield (g) |
|-------------|----------------|----------------|--------------------------|-------------------|--------------------------|-----------------------------------|-------------------------------|-------------------|---------------------|----------------|----------|
| 2004        |                |                |                          |                   |                          |                                   |                               |                   |                     |                |          |
| Golden Currant | 47             | 94             | 4.5                      | 185               | 7.0                     | 8.5                               |                               |                   |                     |                |          |
| Paprikaformige | 56             | 205            | 2.7                      | 116               | 1.0                     | 1.0                               |                               |                   |                     |                |          |
| F1          | 50             | 111            | 4.3                      | 186               | 2.2                     | 3.7                               |                               |                   |                     |                |          |
| F2          | 55             | 111**          | 3.4                      | 163               | 3.3                     | 4.5                               |                               |                   |                     |                |          |
| Positive selection F2 | 44          | 90             | 4.6                      | 178               | 4.2                     | 5.8                               |                               |                   |                     |                |          |
| Negative selection F2 | 74          | 181            | 2.8                      | 174               | 1.8                     | 5.0                               |                               |                   |                     |                |          |
| 2005        |                |                |                          |                   |                          |                                   |                               |                   |                     |                |          |
| Golden Currant | 45             | 108            | 6.7                      | 194               | 9.0                     | 8.3                               | 70.0                         | 7.3               | 64.0                | 6.7           | 473      |
| Paprikaformige | 174            | 176            | 3.5                      | 145               | 1.0                     | 1.0                               | 86.3                         | 1.7               | 23.5                | 248.4         | 1877     |
| Positive selection F3 | 76          | 109            | 4.8                      | 190               | 4.3                     | 5                                 | 77.0                         | 6.0               | 56.5                | 31.7          | 1072     |
| Negative selection F3 | 130***       | 162***         | 4.7                      | 194               | 3.9                     | 4                                 | 82.1*                        | 5.3               | 44.6**              | 59.8***       | 1316     |

* *, **, *** Significant deviation from the parent mean (2004) or differences between positive and negative selection (2005) in the t-test at $p = 0.05$, $p = 0.01$, or $p = 0.001$, respectively

a Area under disease progress curve
b 9 July 2004 and 14 July 2005
c 24 August 2004 and 25 August 2005
d 12 August 2004 and 3 September 2005
Table 6  Effect of the selection for *Phytophthora* field resistance in cross 3 ‘cocktail tomato Celsior × beefsteak tomato Paprikaförmige’ in Rhauderfehn, 2004 and 2005

| Rhauderfehn         | Fruit infection<sup>a</sup> | Leaf infection<sup>a</sup> | Number of inflorescences<sup>b</sup> | Plant height (cm)<sup>c</sup> | Shoot formation on leaves<sup>d</sup> | Shoot formation in inflorescences<sup>d</sup> | Planting to maturity (days) | Highest truss | Harvest period (days) | Fruit weight (g) | Yield (g) |
|---------------------|-----------------------------|----------------------------|-------------------------------------|----------------------------|-------------------------------------|-------------------------------------|---------------------------------|---------------|---------------------|-----------------|----------|
| 2004                |                             |                            |                                     |                            |                                     |                                     |                                 |               |                     |                 |          |
| Celsior             | 363                         | 395                        | 3.0                                 | 131                        | 1.2                                 | 1.7                                 | 80.9                            |               |                     |                 |          |
| Paprikaförmige      | 402                         | 438                        | 3.9                                 | 162                        | 1.0                                 | 1.5                                 | 79.8                            |               |                     |                 |          |
| F<sub>1</sub>       | 368                         | 389                        | 3.5                                 | 200                        | 2.1                                 | 2.2                                 | 76.9                            |               |                     |                 |          |
| F<sub>2</sub>       | 386<sup>**</sup>            | 403<sup>*</sup>            | 3.3                                 | 186                        | 1.1                                 | 2.1                                 | 80.0                            |               |                     |                 |          |
| Positive selection F<sub>2</sub> | 346                | 394                        | 3.6                                 | 191                        | 1.0                                 | 1.1                                 | 81.7                            |               |                     |                 |          |
| Negative selection F<sub>2</sub> | 439             | 422                        | 2.8                                 | 169                        | 1.2                                 | 2.5                                 | 76.3                            |               |                     |                 |          |
| 2005                |                             |                            |                                     |                            |                                     |                                     |                                 |               |                     |                 |          |
| Celsior             | 315                         | 380                        | 3.8                                 | 106                        | 5.0                                 | 3.5                                 | 85.7                            | 3.3           | 35.8                | 13.8            | 177      |
| Paprikaförmige      | 406                         | 406                        | 4.2                                 | 143                        | 2.0                                 | 2.0                                 | 87.8                            | 0.0           | 0.0                 | 47.5            | 119      |
| Positive selection F<sub>3</sub> | 319                | 345                        | 3.6                                 | 126                        | 4.2                                 | 3.2                                 | 95.5                            | 1.9           | 21.5                | 40.6            | 124      |
| Negative selection F<sub>3</sub> | 350<sup>**</sup>        | 358                        | 4.2                                 | 141<sup>***</sup>          | 4.0                                 | 2.5                                 | 86.2                            | 1.7           | 9.3<sup>*</sup>      | 47.5            | 119      |

<sup>a</sup> Significant deviation from the parent mean (2004) or differences between positive and negative selection (2005) in the *t*-test at *p* = 0.05, *p* = 0.01, or *p* = 0.001, respectively
<sup>b</sup> Area under disease progress curve
<sup>c</sup> 8 July 2004 and 13 July 2005
<sup>d</sup> 25 August 2004 and 24 August 2005
<sup>e</sup> 4 August 2004 and 21 August 2005
<sup>f</sup> Fruit weight approx. 200–250 g
crosses 1 and 3 (Tables 4, 6). In cross 1, the more resistant parent (Rote Murmel) grew taller than the less resistant parent (Zuckertraube) and the positively selected F2 grew taller than the negatively selected F2. In cross 3, the opposite was observed (and again, the plant height of the positive selection more closely resembled that of the more resistant parent). Earliness was improved in the positive selection of crosses 1 and 2 by 3.1 and 5.1 days, respectively (Tables 4, 5). The parents of cross 3 did not differ much in earliness (Table 6). The harvest period of the positively selected offspring of all crosses was prolonged by at least 11.9 days. Fruit weight of the F3 populations did not reach the mean fruit weights of the parents. Positive selection for field resistance led to lower fruit weight in crosses 2 and 3; the opposite occurred

|   | Fruit infection | Leaf infection | Number of inflorescences | Plant height | Shoot formation on leaves | Shoot formation in inflorescences | Planting to maturity | Highest truss | Harvest period | Fruit weight |
|---|----------------|---------------|--------------------------|--------------|--------------------------|----------------------------------|----------------------|--------------|---------------|-------------|
| Leaf infection | 0.92*** | 0.91*** | 0.71** | -0.64** | -0.54 | -0.57 | -0.34 | -0.78*** | -0.49 | -0.50 | -0.53 | 0.66** | -0.04 | -0.09 | -0.29 | 0.48 | 0.67** | -0.06 |
| Number of inflorescences | -0.10 | 0.10 | 0.27 | 0.42 | -0.18 | -0.02 | 0.61 | 0.13 | -0.45 | -0.30 | 0.60 | -0.40 | -0.10 | 0.10 | 0.27 | 0.42 | -0.18 | -0.02 | 0.61 | 0.13 | -0.45 | -0.30 | 0.60 | -0.40 |
| Shoot formation on leaves | -0.49 | -0.30 | 0.74** | -0.69** | 0.23 | -0.61 | -0.49 | 0.48 | -0.69** | 0.73** | -0.04 | -0.09 | -0.29 | 0.48 | 0.67** | -0.06 |
| Shoot formation in inflorescences | 0.72** | 0.64** | -0.78*** | -0.27 | 0.20 | 0.20 | 0.68** | 0.46 | -0.82*** | 0.29 | -0.21 | -0.69** | -0.22 | -0.02 | -0.08 | -0.29 | 0.32 | 0.12 | -0.72** | -0.69** | 0.72** | 0.80*** | 0.24 | 0.42 | 0.64** | -0.85*** |
| Planting to maturity | -0.57 | -0.34 | 0.94*** | -0.26 | 0.42 | 0.64** | -0.85*** | -0.63 | -0.14 | 0.87*** | 0.17 | 0.56 | 0.24 | 0.25 | 0.76** | 0.55 | 0.04 | 0.04 | -0.79*** | 0.79*** | -0.72** | -0.53 | 0.82*** | 0.27 | 0.17 | 0.60 | -0.95*** | 0.87*** |
| Harvest period | -0.63 | -0.14 | 0.87*** | 0.17 | 0.56 | 0.24 | 0.25 | -0.95*** | 0.87*** | -0.58 | -0.63** | 0.57 | 0.26 | -0.52 | -0.11 | 0.27 | -0.16 | -0.41 | -0.11 | -0.22 | -0.87*** | -0.87*** | -0.32 | -0.02 | -0.25 | -0.16 | -0.41 | -0.11 | -0.22 | -0.87*** | -0.87*** | -0.32 | -0.02 | -0.25 |
| Fruit weight | 0.61 | 0.27 | -0.84*** | 0.27 | -0.39 | -0.69** | 0.90*** | -0.84*** | -0.86*** | 0.71** | 0.17 | -0.59 | 0.13 | -0.43 | -0.34 | -0.34 | -0.58 | -0.69** | -0.09 | -0.59 | -0.77*** | 0.40 | 0.68** | 0.80*** | 0.27 | 0.12 | -0.10 | 0.18 | -0.15 | -0.35 | 0.20 | 0.11 | -0.17 | 0.39 | -0.40 | -0.21 | 0.85*** | 0.27 | 0.46 | 0.24 | -0.18 | 0.79*** | 0.47 | -0.26 |

***, *** Significant differences between positive and negative selection in the t-test at \( p = 0.05 \) or \( p = 0.01 \), respectively

a Combined score for shoot formation on leaves and in inflorescences
in cross 1. Significant differences in yield were observed in cross 1, where positive selection improved yield by more than 50%.

Correlations of the observed traits between the F3 progenies are presented in Table 7. In all crosses, fruit and leaf infection were positively correlated. No correlation was observed between level of infection and shoot formation in inflorescences and on leaves. In cross 3, leaf infection and plant height were correlated and a correlation was also observed between shoot formation in inflorescences and on leaves. Days to maturity and harvest period were negatively correlated in crosses 1 and 2, while in cross 3 the parent genotypes differed in days to maturity by only 2.1 days. In most cases, fruit and/or leaf infection were negatively correlated with harvest period. Surprisingly, a positive correlation between harvest period and yield was observed only in cross 1. Yield and fruit weight were positively correlated only in cross 1. A positive correlation between number of inflorescences and highest truss existed in all crosses, although highest truss and yield were only positively correlated in cross 3. A positive correlation between fruit infection and fruit weight occurred only in cross 3. A positive correlation between plant height and infection level was only significant for leaf infections in cross 3.

Discussion

Despite difficult experimental conditions, the selection for Phytophthora field resistance in F2 was successful in all three crosses investigated. Frost damage at two sites had resulted in uneven, delayed, and atypical development of the tomato plants. Therefore, this technique proved to be a robust and efficient tool for breeding programs.

A key for the assessment of damages by late blight was developed by the Organic Outdoor Tomato Project for the rapid scoring of large numbers of field-grown tomato plants. The assessment keys for late blight of potatoes were insufficient for this purpose. Keys scoring the percentage leaf area covered (e.g., James 1971) are only suitable for limited numbers of plants or laboratory assays. While it is possible to score the percentage of necrotic tissue in potatoes (e.g., Cruickshank et al. 1982), tomato late blight can develop in different ways. Symptoms either spread from older to younger leaves (resulting in die-off of entire leaves), or they spread more evenly over large parts of the plant (causing multiple of leaf spots).

We scored leaf infections in addition to fruit infections for two reasons. First, the level of leaf infection will influence the vitality and photosynthetic activity of the plant (and as a consequence, yield). Second, in some environments, the low level of late blight infections does not allow observers to score fruit infections. In the present experiment, selection for field resistance against fruit infections (significant in all three crosses) has been more efficient than selection for field resistance against leaf infections (significant in two crosses). Nevertheless, if the scoring of fruit infections is not possible, selection for leaf resistance will also result in a selection gain due to the high correlation of fruit and leaf infections. At early stages of leaf infections, it is not always possible to distinguish between leaf spots caused by late blight or early blight (Alternaria solani). This situation provides an explanation for reduced selection efficiency for leaf infections, and may explain the phenomenon observed in cross 1 Rote Murmel × Zuckertraube (Table 4): F1 and F2 suffered less from leaf infections than the resistant parent, Rote Murmel.

In screenings, a high level of field resistance is easier to find in genotypes with smaller fruits. However, a significant correlation between fruit weight and fruit infection was only observed in one of the three crosses.

Other traits, including yield, fruit weight, days to maturity, harvest period, and plant height, were not considered in selection. The indirect effects of a selection for resistance on these traits depended on the cross. However, it was evident that selection for suitable traits combined with field resistance is promising, even in wide crosses. Harvest period was mainly influenced by earliness and field resistance. Both traits were successfully incorporated from wild tomatoes. Harvest period and yield were not always correlated. Both traits are particularly important for the fresh tomato supply. As shown earlier (Horneburg and Becker 2008), selection at suitable locations with frequent exchange of breeding lines might be the best strategy to combine a long harvest period with high yield.

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The least desirable attribute of wild tomatoes is the formation of shoots on leaves and in inflorescences, as their removal is necessary and labor intensive. The fact that no correlation between field resistance and shoot formation was observed allows for the selection of genotypes with both improved field resistance and yield, but without morphological disadvantages.

A major advantage of selection in field trials versus selection under more controlled conditions in the laboratory or greenhouse is their relatedness to the target environment in practical horticulture. Field resistance is directly assessed, including interactions with pedoclimatic conditions, cultivation practices, pests, and other diseases. In most indoor experiments with artificial infection, only one or a few Phytophthora strains have been applied on tomatoes (Michalska and Pazio 2005; Drenth et al. 1995) and potatoes (Zimnoch-Guowska et al. 2003; Vleeshouwers et al. 1999; Swiezynski et al. 1997). According to the recent review by Foolad et al. (2008), no particular method for the artificial infection of tomato fruits has been published to date. Serious drawbacks in field tests occur when climatic conditions are unfavorable for Phytophthora infections. For example, during the unusually hot and dry summer of 2003, late blight did not appear in approximately 50% of our field experiments.

Selection for field resistance between adult plants in the field limits the population size, because training tomatoes is laborious. However, the advantage is that all other important agronomic and morphological traits can be selected simultaneously. F2 selection depends on scoring individual plants, and the experimental error is expected to be high. F3 selection would reduce experimental error, because progenies can be assessed. However, this approach would delay selection for one year. The present study has demonstrated that F2 selection is a successful approach. These results will help to improve the efficiency of outdoor tomato breeding for Phytophthora resistance.

Acknowledgments Many thanks to the German Federal Organic Farming Scheme, our colleagues on farm, and the seed savers who generously donated heritage cultivars.

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