ASSESSING COMPENSATION FOR INSECT DAMAGE IN MIXED PLANTINGS OF RESISTANT AND SUSCEPTIBLE POTATOES

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Abstract

Plant mixtures have been proposed for pesticidal transgenic potatoes as a means to reduce selection intensity favoring resistant insect genotypes. Colorado potato beetle, *Leptinotarsa decemlineata* (Say), defoliation was simulated in mixed plantings of susceptible and resistant potato "mimics" to evaluate yield compensation. Various mixtures of susceptible and resistant potato were planted at two densities and two locations in eastern North Carolina. Resistant plants were undamaged throughout the season whereas susceptible plants were completely defoliated by hand either during early or late bloom. The ability of non-defoliated plants to compensate for neighboring defoliated plants was investigated through single-plant and small-plot field experiments for 2 years.

Yield compensation for defoliated plants by neighboring non-defoliated plants was not evident in our studies. Yield of two potato plants, positioned on either side of a defoliated plant, was not different from yield of two potato plants positioned on either side of a non-defoliated potato plant. Compensation in mixtures of resistant and susceptible potato was not evident using several non-linear regression analyses. A negative linear relationship existed between yield and an increasing percent of susceptible plants in the mixture for all planting densities, at each location, every year.

Compendio

Las mezclas de plantas han sido propuestas para papas transgénicas a los pesticidas como una manera de reducir la intensidad de selección que favorece a los genotipos resistentes de insectos. Se simuló la defoliación causada por el escarabajo de la papa de Colorado, *Leptinotarsa decemlineata* (Say), en siembras mixtas que "imitan" plantas de papas susceptibles y resistentes, para evaluar la compensación en rendimientos. Se sembraron varias mezclas de papas susceptibles y resistentes a dos densidades y en dos localidades del este de Carolina del Norte. Las plantas resistentes permanecieron sin daños durante toda la temporada mientras que las

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susceptibles fueron totalmente defoliadas a mano al comienzo o al final de la floración. Se investigó por dos años la capacidad de las plantas no defoliadas, para compensar la defoliación de las plantas vecinas mediante experimentos con plantas individuales y en pequeñas parcelas en el campo.

La compensación en rendimiento-para las plantas defoliadas-por las plantas vecinas no defoliadas, no se hizo evidente en los estudios. El rendimiento de dos plantas de papa, localizadas a ambos lados de una planta defoliada, no se diferenció del rendimiento de dos plantas de papa localizadas a ambos lados de una planta de papa no defoliada. La compensación en mezclas de papas resistentes y susceptibles no se hizo evidente al utilizar varios análisis de regresión no linear. Se presentó una relación linear negativa entre el rendimiento y un creciente porcentaje de plantas susceptibles de la mezcla, para todas las densidades de siembra, en cada localidad y año.

Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), has become increasingly difficult to manage on potato, *Solanum tuberosum* L., in the eastern US because of pesticide resistance (9, 12, 17, 33). Field populations of potato beetles have become resistant to all major classes of synthetic insecticides (10). In addition, potato beetle resistance to *Bacillus thuringiensis* (Bt)-based products has been demonstrated in the laboratory (35). Recently, the toxic constituents of Bt have been genetically engineered into potato plants. These transgenic plants are presently designed to express toxin all season long, and consequently can be expected to exert selection pressure for beetles to adapt if these plants are widely grown commercially (22). The first transgenic potatoes to be available commercially will have high levels of Bt-toxin.

A number of resistance management strategies have been proposed to mitigate pest adaptation to resistant plants including Bt endotoxin expressing transgenic plants (13, 14, 21, 27). Planting mixtures of susceptible and resistant potato within a field may be the most promising strategy for managing the Colorado potato beetle (15). Mixtures would provide a refuge from toxicity that would permit survival of susceptible potato beetles that can mate with resistant individuals and slow the evolution of resistance. However, under certain conditions mixtures could fail as a resistance management strategy for the potato beetle. Using a computer simulation model, Mallet and Porter (20) reported that if larval movement from susceptible plants to resistant plants is significant, the population could adapt more rapidly to a toxin. Ferro (7) pointed out that if the initial larval density exceeds the carrying capacity of the susceptible plants in the mixture, those plants would be defoliated and would not be available as a refuge for emerging adults. These adults would feed on the remaining resistant plants and would be subjected to high selection pressure to adapt to the toxin. However, if larval movement is limited and the potato beetle population is maintained below the carrying capacity of the susceptible plants in the mixture, the mixture strategy is likely to be effective in delaying resistance. Empirical evidence is needed to quantify beetle movement and defoliation in mixtures of resistant and susceptible potato under a range of potato beetle densities to identify the optimum conditions for deploying this strategy.

Because transgenic potato with the Bt-toxin will be the first insect resistant potato to be marketed specifically for Colorado potato beetle control, primary focus has been to use mixtures to manage the potential for Bt resistance. However, mixtures should also be considered in managing resistance to future modalities of plant resistance such as those found in the glandular trichomes of the wild species, *S. berthaultii* Hawkes.

Before adopting a mixture strategy, growers will want to know how yield from a mixture compares with yield from a monoculture of resistant plants. We had two questions: Will yield of a mixed planting be affected by the percentage of susceptible plants in the mixture that are defoliated, and do neighboring resistant plants have the ability to compensate for defoliation of susceptible plants in the mixture? Studies have indicated that potato has the ability to compensate for hail damage (1) and decreases in planting density (5, 28). Other research has shown that potatoes can tolerate varying levels of defoliation by insects, depending on the growth stage of the crop. In general, potatoes are most tolerant of defoliation very early or late in the season (8, 16). Hare (16) and Zehnder and Evanylo (36) demonstrated that potatoes could even withstand 100% defoliation by Colorado potato beetles within a few weeks before harvest without significantly reducing yield. Most researchers have shown that potato is most susceptible to yield loss when defoliation occurs during the bloom stage (4, 30, 34); nevertheless, potato was shown to tolerate a certain level of defoliation during bloom in each of these studies. Despite extensive literature covering yield compensation in potato, there has been no research identifying whether or not undamaged potatoes can compensate for neighboring, completely defoliated potatoes. Perhaps, the compensatory response might reduce yield losses in a mixture of resistant and susceptible potatoes.

The objective of this study was to quantify the ability of potato plantings in North Carolina to compensate for damage to susceptible plants in a mixture of susceptible and resistant plants. In our experiments, we assumed that all susceptible plants in the mixture would be completely defoliated. Although this represents a "worst case" scenario, in terms of plant damage, it provided maximum opportunity for yield compensation by plants adjacent to the defoliated susceptible plants. The magnitude of compensation was investigated at two different planting densities and times of defoliation within the bloom stage. Our experiments focused on simulating defoliation during bloom because this is the period when defoliation occurs naturally in North Carolina potato fields. To determine the level at which potatoes could compensate for damage of susceptible plants in a mixture of resistant and susceptible potato, we conducted a series of experiments including: a comparison of effects of hand and beetle defoliation, measurement of single plant compensatory response, and measurement of compensatory response of small plots.

Materials and Methods

Defoliation Comparison Experiment—Potato seed pieces, Atlantic, were planted on March 17, 1992 at the Tidewater Res. Stn. near Plymouth in Washington Co., NC where the soil type is a Portsmouth loam. Seed pieces were treated with captan at a rate of 45 g per 45 kg of potatoes and with thiabendazole at a rate of 1.2 l per 379 l of water. Potatoes were planted at 0.23 m spacing in 9.14 m rows that were spaced at 0.97 m. Each plot was one row with a guard row on either side. Plots within rows were separated by 1.5 m of row planted to the red-skinned potato cultivar, Norland, to facilitate separation of plots at harvest.

The experiment had 3 treatments (hand, insect, and no defoliation) replicated 4 times in a randomized complete block design. On May 28, approximately 75 to 100 third and fourth instar potato beetles per plant were transferred to plots designated to be insect defoliated. Plants in these plots were severely defoliated (near 100%) by June 8 (during late- to post-bloom period) and at this time plants in the hand-defoliated plots were completely stripped of foliage (100% defoliated). Each leaflet was individually stripped so that most apical or axillary buds were undamaged. Esfenvalerate was applied at a rate of 0.056 kg (AI)/ha on May 28 to prevent insect damage in the control and hand-defoliated plots while oxamyl was applied at a rate of 1.1 kg (AI)/ha to all plots on June 10 and 21 to prevent further defoliation. The test was harvested on July 6, 111 d after planting, using a one-row digger. Tuber quality was graded in the field as US no. 1 or 2.

Before planting, plots were fertilized (17: 17: 17) (N: P: K) at a rate of 818 kg/ha and during bloom (34: 0: 0) (N: P: K) at a rate of 163 kg/ha. Weeds were managed using one application of metribuzin at a rate of 0.84 kg (AI)/ha and foliar diseases were prevented using two applications of chlorothalonil at a rate of 0.58 kg (AI)/ha.

1992 Single-Plant Compensation Experiment—Atlantic seed pieces treated with captan were planted on March 17 at the Washington Co. site. Potatoes were planted every 0.23 m in rows spaced at 0.97 m. Because some seed pieces rotted, the distance between plants averaged (±SE) 0.273 ± 0.009 m (range = 0.203 - 0.362 m). Forty plots, each consisting of 3 consecutive plants within a row, were chosen randomly such that half had the middle plant defoliated by hand during late- to post-bloom on June 8 and half remained

non-defoliated. Buffer plants flanked each plot. Insecticides, fungicides, herbicides and fertilizer were used in accordance with the Defoliation Comparison Experiment. Cultural practices also coincided with the Defoliation Comparison Experiment. All plots were harvested and graded as US no. 1 or no. 2 on July 3, 108 d after planting. Data on weight of no. 1 and 2 grade potatoes were recorded separately for each plant.

1993 Single-Plant Compensation Experiment—Atlantic seed pieces were planted in a commercial potato field on March 23 near Alliance in Pamlico Co., NC where the soil type is a Yonges loamy fine sand/Ardent loam. Potatoes were planted at 0.23 m spacing in rows spaced at 0.97 m. Distance between plants in each plot averaged (±SE) 0.273 ± 0.005 m (range = 0.203 - 0.368 m). The design had 3 treatments (defoliation during early bloom, late bloom or no defoliation) arranged in a randomized complete block design. One-hundred-sixty plots, each consisting of five consecutive plants within a row, were randomly chosen such that 80 remained non-defoliated, 40 had the middle plant defoliated by hand during early bloom on May 21 and 40 had the middle plant defoliated during late- to post-bloom on June 4. The middle 3 plants were harvested and the tubers graded as US no. 1 or no. 2 on July 1, 100 d after planting. Data on weight of no. 1 and 2 potatoes were recorded separately for each plant.

The field was fertilized before planting at a rate appropriate for the location (73: 23: 23) (N: P: K) and foliar applications of fertilizer (9: 4.5: 9) (N: P: K) were made on May 11, 21 and 30 at rates of 3.9, 6.7 and 6.7 kg/ha, respectively. Weeds were managed with one application of metribuzin on April 5 at a rate of 0.74 kg (AI)/ha and disease was prevented with applications of metalaxyl + chlorothalonil on May 21 at a rate of 1.7 kg (AI)/ha and mancozeb at a rate of 1.1 kg (AI)/ha on May 30 and June 7.

1992 Small-Plot Mixture Experiment—Pamlico Co. Atlantic seed pieces were planted in a commercial potato field on March 5 near Alliance, NC where the soil type is a Yonges loamy fine sand. Each plant in a treatment was designated as resistant or susceptible based on a binomial expansion that identified the pattern of susceptible and resistant plants in each mixture treatment that was expected to be encountered most frequently in a randomized planting of seed pieces (Table 1). All three plant combinations of resistant (R) and susceptible (S) were grouped into four major categories: all susceptible (S-S-S), two susceptible and one resistant (S-S-R, S-R-S, or R-S-S), one susceptible and two resistant (S-R-R, R-S-R, R-R-S), and all resistant (R-R-R). The expected frequency of a 3-plant combination in which one of the plants is susceptible (S-R-R, R-S-R, or R-R-S) in a mixture where 80% of the plants are resistant is: (0.2 x 0.8 x 0.8) x 3= 0.384 or 38.4%. If a row was 9.14 m long and planted at 0.30 m spacing, there would be 30 plants and 28 possible 3-plant combinations to consider in calculating actual frequencies. Actual frequencies of R and S plants in each mixture were

TABLE 1.—*Expected and actual frequencies of various mixtures of susceptible and resistant potato based on groups of three plants. Resistant plants are represented as "R" and susceptible plants are represented as "S". Expected frequencies were determined using a binomial expansion.*

		Mixture (% resistant plants)								
		Expected frequency			Actual frequency					
					Pamlico Co.			Washington Co.[2]		
Combination	Density[1]	90%	80%	50%	90%	80%	50%	91/92%	79/80%	50/52%
S-S-S	High Low	0.1 0.1	0.8 0.8	12.5 12.5	0.0 0.0	0.0 0.0	13.2 14.3	0.0 0.0	0.0 0.0	15.2 16.7
S-S-R, S-R-S, or R-S-S	High Low	2.7 2.7	9.6 9.6	37.5 37.5	2.6 3.6	10.5 14.3	34.2 35.7	3.0 0.0	12.1 12.5	36.4 33.3
S-R-R, R-S-R, or R-R-S	High Low	24.3 24.3	38.4 38.4	37.5 37.5	26.3 25.0	39.5 35.7	39.5 35.7	21.2 25.0	36.4 37.5	36.4 33.3
R-R-R	High Low	72.9 72.9	51.2 51.2	12.5 12.5	71.1 71.4	50.0 50.0	13.2 14.3	75.8 75.0	51.5 50.0	12.1 16.7

[1] High planting density at 0.23 m and 0.27 m spacing in Pamlico Co. and Washington Co., respectively; and low planting density at 0.30 and 0.37 m spacing for Pamlico Co. and Washington Co., respectively.

[2] The first percentage in each heading refers to resistant plants at high density and the second at low density.

calculated so that they were as similar as possible to the expected frequencies. When a pattern was identified for each mixture treatment, it was used for replicates of that treatment.

After plants emerged, they were designated as either resistant or susceptible, marking the latter with wooden stakes approximately 0.61 m long which were driven into the ground on either side. Resistant potato plants were mimicked by treating normal plants with *Bacillus thuringiensis* subsp. *tenebrionis* (Foil®, Ecogen Inc., Langhorne, PA) at a rate of 9.4 l formulation per ha with a CO_2-powered backpack sprayer equipped with a 3 nozzle (D-3/ 25 core hollow cone) single row boom calibrated to deliver the spray at 318 l/ha at 275.7 kPa. Susceptible plants were covered with paper grocery bags, which were supported by the wooden stakes, during the application to shield them from the Bt spray. Plants were treated twice weekly beginning on May 11 to control first generation larvae.

The indigenous potato beetle population during the pre-bloom period was not of sufficient size to uniformly defoliate susceptible plants in all treatments before bloom. Therefore, each susceptible plant in the experiment was defoliated by hand during late-bloom on May 18 and a second time on June 24 to simulate first generation larval and adult feeding, respectively. Each compound leaf was individually stripped such that most apical and axillary buds were undamaged. A late season, foliar application of oxamyl was applied at a rate of 1.1 kg (AI)/ha to the entire test on June 11 to control first generation adults. Defoliation estimates were recorded prior to each hand-defoliation using a pre-transformed rating scale, modified by Little and Hills (19).

The experiment was a 2 (0.23 or 0.30 m plant spacing) x 5 (mixtures of resistant and susceptible plants such that 0, 50, 80, 90, 100% were resistant plants) factorial arranged in a randomized complete block design replicated 8 times. Each single row plot was 9.14 m long, with a guard row on either side. Rows were spaced at 0.97 m with a 1.52 m alley between plots within a row. Red-skinned potatoes, Norland, were planted in the center of each alley to distinguish plots at harvest. Plants were harvested on June 29, 116 d after planting, using a one-row digger. Tuber quality was graded in the field as US no. 1 or 2. The test site was fertilized before planting at a rate of (81: 25: 81) (N: P: K). Herbicides and fungicides were used in accordance with the commercial growers typical pest management practices.

Washington Co.—Atlantic seed pieces treated with captan at the rate mentioned previously were planted on March 17 adjacent to the Single-Plant Compensation Experiment. Plot size, experimental design and method of defoliation were identical to that of the Pamlico Co. location. Due to a marginal plant stand, spacing was modified to 0.27 and 0.37 m for the high and low densities, respectively. Because these densities were lower than those used in Pamlico Co., percentages of resistant plants in each mixture were slightly different (Table 1). Hand-defoliations on May 29 and June 30 were preceded by defoliation estimates using the procedure mentioned previously. Oxamyl was applied at a rate of 1.1 kg (AI)/ha to the entire study on June 10 and 21 to prevent defoliation. The test was harvested on July 6, 111 d after planting. All data analyses followed methods described previously for the experiment at the Pamlico Co. location. Fungicides, herbicides and fertilizer were applied as described for the Defoliation Comparison Experiment.

1993 Small-Plot Mixture Experiment—Atlantic seed pieces were treated with captan and planted on March 23 adjacent to the Single-Plant Compensation Experiment. Plants in the mixture treatments were designated as resistant or susceptible again using the binomial expansion described in 1992. Rather than infesting the test with potato beetles, plants were defoliated by hand when they were in late-bloom on June 4 using the same method as in 1992. Endosulfan was applied at a rate of 1.12 kg (AI)/ha on May 6 and oxamyl was applied at a rate of 0.56 kg (AI)/ha on May 21 for potato beetle control.

The experiment was a 2 (0.23 and 0.30 m planting density) x 8 (mixtures of resistant and susceptible plants such that 0, 30, 50, 59, 69, 82, 89 and 100% were resistant at high density and 0, 30, 50, 60, 70, 80, 90 and 100% were resistant at low density) factorial arranged in a randomized complete block design replicated 6 times. Because plant stand was marginal in sections of the field, two treatments (30 and 59% resistant plants in the mixture) at high planting density, and one entire replicate of the remaining high density x mixture treatments were eliminated. Plots were each 6.10 m long, single rows with a guard row on either side spaced at 0.97 m. A 1.52 m alley separated plots and were planted with several red-skinned potatoes, Norland, to distinguish between plots at harvest. Plants were defoliated on May 19 during the end of bloom and were harvested on July 1, 100 d after planting. Insecticides, fungicides and herbicides were used in accordance with the 1993 Single-Plant Compensation Experiment.

Statistical Analyses—Data in all studies were subjected to an analysis of variance to detect differences among treatments and their interactions using the procedure PROC GLM of SAS ($P \leq 0.05$) (29). Treatment means from the Defoliation Comparison Experiment were compared using a protected LSD ($P \leq 0.05$). Means from the Single-Plant Compensation Experiments were separated using the LSMEANS procedure of SAS ($P \leq 0.05$) because of unequal sample size (29). In all Small-Plot Mixture Experiments, non-linear regression analyses including a quadratic (PROC GLM) and a Weibull function (PROC NLIN) were used to identify the relationship between yield and percent susceptible plants in the mixture ($P \leq 0.05$) (29).

Results and Discussion

Defoliation Comparison Experiment—Simulating insect defoliation has been a controversial method of assessing yield loss (26). Insects tend to irregularly defoliate leaves through time within specific areas of the canopy, whereas mechanical defoliation often involves discriminate foliage removal and typically occurs one time. Studies have shown that insect defoliation may be less, the same, or more damaging than mechanical defoliation in various crops. Hare (16) estimated that Colorado potato beetle defoliation was only 65-70% as debilitating as mechanical defoliation when his results with naturally occurring defoliation were compared with those from an artificial insect defoliation experiment by Sparks and Woodbury (32). Poston *et al.* (25) reported that there was no difference in soybean, *Glycine max* (Merrill), net photosynthesis when plants were artificially defoliated with a cork borer, paper punch, or removing a portion of leaflet along the midrib compared with equivalent defoliation by either the green cloverworm, *Plathypena scabra* (F.) or the painted lady, *Vanessa cardui* (L.). However, when a portion of the leaflet was removed across the midrib, net photosynthesis increased compared with the same level of insect defoliation. Capinera and Roltsch (3) have shown that defoliation of wheat, *Triticum sp.* by grasshoppers, *Melanoplus sanguinipes* (F.), lowered the rate of seedling regrowth more than an equal amount of manual clipping.

TABLE 2.—*Yield comparison of potatoes that were defoliated during late to post-bloom either by hand or by Colorado potato beetle larvae in Washington Co., North Carolina, in 1992. Yield was extrapolated to T/ha from kg/9.14 m.*

Defoliation Treatment	Yield (T/ha)[1] US no. 1	Yield (T/ha)[1] Total
No (0% defoliated)	22.316 ± 0.486 a	27.808 ± 0.532 a
Insect (near 100% defoliated)	13.242 ± 1.845 b	18.622 ± 2.171 b
Hand (100% defoliated)	12.166 ± 0.500 b	18.044 ± 0.993 b

[1] Means (±SE) followed by the same letter within a column are not significantly different ($P > 0.05$) using a protected-LSD (29).

Artificially defoliating potatoes to simulate either hail or insect damage is a common technique used to investigate the relationship between defoliation and yield (4, 30, 34). Results from our study indicated that there was no difference in hand versus insect defoliation treatments on either total or US no. 1 grade yield (Table 2). Hand-defoliating potatoes enabled one to discretely investigate yield/defoliation relationships while avoiding the large labor requirements involved in manipulating beetle densities to obtain uniform damage across all replications at the same time.

1992 Single-Plant Compensation Experiment—The question we posed was, when a potato plant is defoliated, does compensation occur in non-defoliated neighboring plants? Compensation might occur through reduced competition between plants for nutrients and space above or below ground. If flanking plants compensated for damage to the single neighbor, the two plants flanking a completely defoliated plant would be expected to yield significantly more than plants flanking a non-defoliated plant. There were no significant differences in either total yield (F=1.67; df= 1,38; P=0.2043) or in US no. 1 yield (F= 0.02; df= 1,38; P=0.9029) from two non-defoliated plants flanking a defoliated neighbor or two non-defoliated plants flanking a non-defoliated neighbor (Fig. 1a). Non-defoliated middle plants had significantly greater total yield (F= 21.63; df= 1,38; P=0.0001) and US no. 1 yield (F= 31.48; df= 1,38; P=0.0001) than defoliated middle plants (Fig. 1b). In this experiment, the average total yield lost by the defoliated plant was 41.1%, indicating that each of its neighbors would have to yield 20.55% more than other non-defoliated plants to achieve full compensation. Yet, total yield from each of the plants flanking the defoliated plant was only

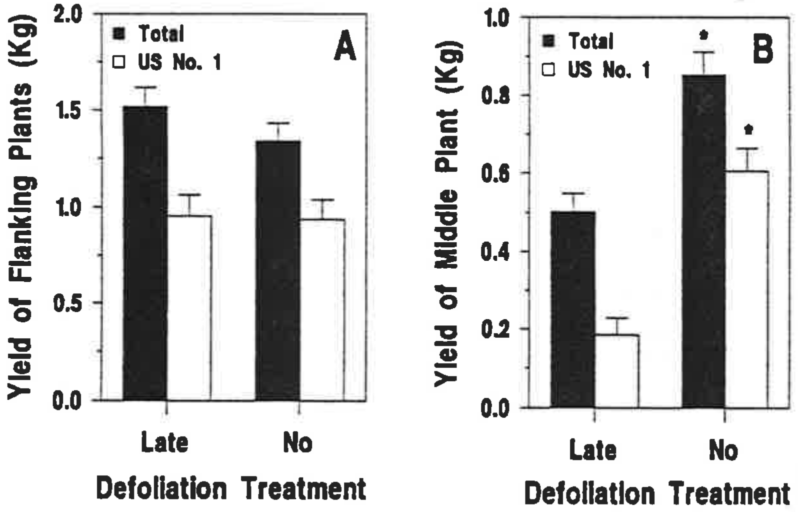

FIG. 1.–(a) Yield (±SE) of two potato plants that either flanked the same defoliated or non-defoliated neighbor. (b) Yield (±SE) of the middle plant that was either defoliated during late bloom or not defoliated. Defoliated middle plants were completely stripped by hand during late to post bloom on June 7 in 1992 in Washington Co., NC. Asterisks signify a significant difference between treatments for each dependent variable ($P \leq 0.05$; df=1) (F test).

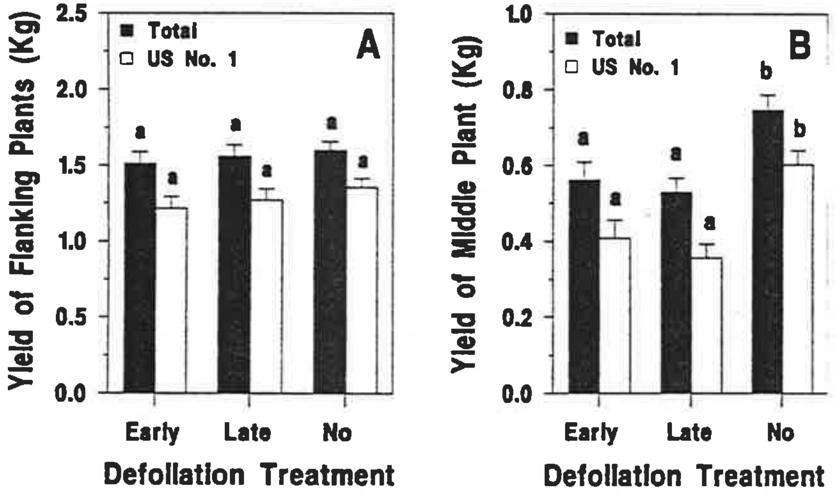

FIG. 2.-(a) Yield (±SE) of two potato plants that either flanked the same defoliated or non-defoliated neighbor. (b) Yield (±SE) of the middle plant that was either defoliated during early bloom, late bloom or not defoliated. Defoliated middle plants were completely stripped by hand either during early bloom on May 21 or late bloom on June 3 in 1993 in Pamlico Co., NC. Means followed by a different letter are significantly different for each dependent variable ($P \leq 0.05$; LSMEANS).

5.8% greater in yield compared with plants flanking non-defoliated plants. If two neighboring, susceptible plants became completely defoliated, the non-defoliated plants flanking them would have had to each yield 41.1% more than other non-defoliated plants in the field.

Plant spacing in non-defoliated plots did not influence either total (F= 1.75; df= 1,18; P=0.2029) or US no. 1 yield (F= 1.40; df= 1,18; P=0.2516). These results suggested that marketable yield would not increase with an increase in plant density within the range of plant spacings tested (0.213 - 0.362 m) under North Carolina growing conditions.

1993 Single-Plant Compensation Experiment—No differences were apparent in either total yield (F= 0.36; df= 2,105; P=0.6990) or US no. 1 yield (F= 1.01; df= 2,105; P=0.3694) between plants flanking a defoliated neighbor and those with a non-defoliated neighbor (Fig. 2a). Mean total yield and US no. 1 yield from non-defoliated middle plants were significantly greater than total yield (F= 8.62; df= 2,112; P= 0.0003) and US no. 1 yield (F= 11.24; df= 2,112; P=0.0001) from middle plants that were defoliated (Fig. 2b). Plants that were defoliated during early bloom had 14 d longer to recover from complete defoliation compared with those plants defoliated during late bloom. Yet, there were no differences in either total yield (F= 0.21; df= 1,38; P=0.6515) or US no. 1 yield (F= 0.56; df= 1,38; P=0.4574) between plants that were either defoliated during early bloom (May 21) or late bloom (June 3) (Fig. 2b). In Virginia, Zehnder and Evanylo (36) reported a greater difference in yield loss between Superior potatoes that were defoliated by potato beetles (81-100% foliage removed) during early and late bloom (9.2% in 1986 and 37.1% in 1987). This greater difference in yield loss in Virginia may reflect a cultivar difference (Superior is an earlier maturing variety than Atlantic), a shorter period between defoliation and harvest, or an environmental difference. In North Carolina, defoliation by first generation larvae rarely occurs before or after the bloom stage. Our data suggested that attempts to delay Colorado potato beetle colonization through rotating fields, causing a temporal shift in defoliation, would not prevent yield loss. However, rotation can reduce the size of the population colonizing the field, and thereby reduce the overall defoliation.

Gradual increases in plant spacing between 0.203 and 0.362 m within non-defoliated plots did not affect either total yield (F= 2.70; df= 1,31; P=0.1102) or US no. 1 yield (F= 2.32; df= 1,31; P=0.1383). These results were similar to those in 1992 indicating that an increase in plant density within the densities evaluated would not increase marketable yield.

1992 Small-Plot Mixture Experiment—Cool temperatures over the course of the experiment at both locations favored plant growth but slowed potato beetle larval development and resulted in low levels of defoliation during bloom. Because we wanted to simulate the worse case scenario, 100% defoliation of all susceptible plants in the mixture, plants designated as susceptible were hand-defoliated at appropriate times. Prior to the first hand-defoliation during late bloom in plots designated to have 100% susceptible plants, there was a mean (±SE) percent defoliation of 4.3 ± 1.3 and 28.1 ± 2.3 in Washington and Pamlico Co., respectively. In Washington Co., following defoliation on May 29, the plants produced new foliage so that the mean defoliation of plants in 100% susceptible plots was 3.9 ± 1.3 on June 30, 6 d before harvest. A similar vegetative recovery was not seen in Pamlico Co. where plants in the 100% susceptible plots had a mean percent defoliation of 94.6 ± 1.2 a week before harvest. Defoliation in the 100% resistant plots was 0% for the entire season in Washington Co. and rose slightly in Pamlico Co. from 0.4% to 8.1% between June 8 and harvest.

Mean total yields (± SE) in plots with 100% resistant plants were similar in Washington Co. (23.828 ± 0.915 T/ha) and Pamlico Co. (23.653 ± 1.058 T/ha). Total yields were significantly greater at the higher density compared with the lower density in Washington Co. (22.145 ± 0.796 versus 19.297 ± 0.766 T/ha) and Pamlico Co. (21.347 ± 0.778 versus 18.581 ± 0.726 T/ha) (Table 3). However, US no. 1 grade yield did not significantly differ between the high and low planting densities for either Washington Co. (11.690 ± 0.752 versus 11.055 ± 0.773 T/ha) or Pamlico Co. (5.763 ± 0.511 versus 6.277 ± 0.520 T/ha). This indicated that significantly more US no. 2 grade potatoes were produced and suggested reduced tuber bulking at the higher planting density. Past studies have shown an increase in total yield and number of small tubers when seed pieces were spaced close together (2, 18, 24). In Maine, Houghland & Akeley (18) found no differences in US no. 1 yield (5.08 cm minimum size) of varieties Kennebec, Katahdin, or Merrimack when planted at 0.23 m or 0.30 m. When planting large acreages, this relatively small difference in density translates to a significant savings in seed for growers.

If compensation by healthy plants for completely defoliated adjacent plants would occur in small plots, the relationship between yield and an increase in susceptible plants in the mixture would be non-linear. Also, the greatest opportunity to see compensation would be at the 50% susceptible plants in the mixture treatment because this treatment has the most undamaged plants that could increase in yield in the absence of one or more neighbors. Compensation would be evident at the 50% susceptible plants in the mixture treatment if yields tended to be greater than the predicted yield at the midpoint between the 0% and 100% susceptible plants in the mixture treatments (*i.e.* yield expected if there is no compensation). Plant mixtures significantly influenced total yield and had a similar effect at both locations (Table 3). The relationship between total yield (extrapolated to T/ha from kg/9.14 m) and percent susceptible plants in the mixture was negatively linear at the high planting density (Washington Co.: y=25.913-0.105x; df=1, 38; R^2 = 0.58; F=52.67; P=0.0001; and Pamlico Co.: y=25.662-0.120x; df=1, 38; R^2 = 0.79; F=146.99; P=0.0001) and low planting density (Washington Co.: y=23.285-0.111x; df=1, 38; R^2 = 0.70; F=90.11; P=0.0001; and Pamlico Co.: y=22.384-0.106x; df=1, 38; R^2 = 0.71; F=92.57; P=0.0001). Standard errors of the regression line slopes were ±0.014 and ±0.010 at the high planting density and ±0.012 and ±0.011 at the low planting density for Washington and Pamlico Co., respectively. Neither the Weibull function nor the lack-of-fit to linear variables accounted for variation in the data. A negative linear relationship between yield and percent susceptible plants in the mixture indicated that if all susceptible plants in a mixture strategy were completely defoliated during late bloom in North Carolina, yield loss would result. Additionally, plot yields from the 50% susceptible plants in the mixture treatment did not tend to be greater than the predicted yield at the midpoint between the 0% and 100% susceptible plants in the mixture treatments. Results from this small-plot experiment confirmed findings of the 1992 and 1993 Single-Plant Compensation Experiments, indicating the lack of compensation by healthy neighboring plants for complete foliage loss of an adjacent plant.

TABLE 3.—*Significance of planting density, mixture of susceptible and resistant plants and their interactions on total and US no. 1 potato yield in Pamlico and Washington Co., North Carolina, in 1992. Mixture main effect and density x mixture interaction sums of squares were partitioned to identify their specific relationship with yield (29).*

		Washington Co.				Pamlico Co.			
		Total Yield		US no. 1 Yield		Total Yield		US no. 1 Yield	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Replication	7	0.38	0.9126	0.68	0.6888	1.80	0.1037	0.60	0.7533
Planting Density	1	16.45	0.0001	1.15	0.2874	30.91	0.0001	1.60	0.2103
Mixture (linear)	1	123.17	0.0001	185.00	0.0001	267.92	0.0001	180.94	0.0001
(quadratic)	1	0.23	0.6315	0.23	0.6329	1.55	0.2184	0.06	0.8004
(cubic)	1	1.16	0.2846	2.61	0.1112	0.06	0.8034	0.22	0.6372
(quartic)	1	0.38	0.5402	0.01	0.9387	0.11	0.7426	0.31	0.5787
Planting Density x									
Mixture (linear)	1	0.10	0.7530	0.25	0.6214	1.07	0.3050	0.09	0.7673
(quadratic)	1	0.10	0.7577	0.01	0.9110	0.27	0.6050	0.49	0.4852
(cubic)	1	1.31	0.2567	2.93	0.0916	5.73	0.0197	0.78	0.3804
(quartic)	1	0.59	0.4462	0.01	0.9376	4.12	0.0465	0.69	0.4092

The only factor significantly affecting US no. 1 yield was mixture (Table 3). Data indicated that compensation did not occur at either the high (Fig. 3a) or low planting density (Fig. 3b) as reflected by the inability of the Weibull function to fit the data and the absence of non-linearity.

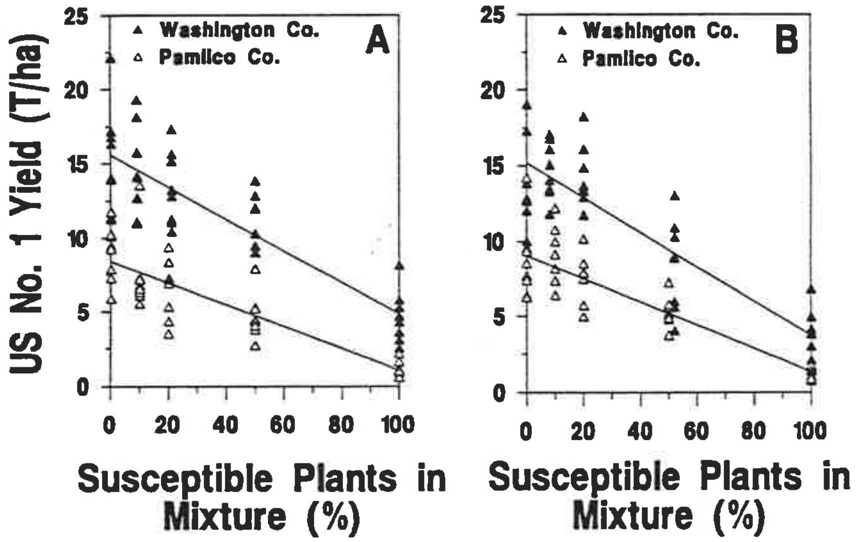

FIG. 3.-(a) Relationship between US no. 1 yield and percentage of susceptible plants (entirely defoliated during late bloom) when grown in mixtures of "resistant/susceptible" plants spaced at 0.23 m in Washington Co. ($y = 15.546-0.107x$; df = 1,38; $R^2 = 0.68$; $F = 81.61$; $P = 0.0001$) or Pamlico Co. ($y = 8.427-0.074x$; df = 1,38; $R^2 = 0.74$; $F = 89.58$; $P = 0.0001$) in 1992, or (b) plants spaced at 0.30 m in Washington Co. ($y = 15.203-0.115x$; df = 1,38; $R^2 = 0.75$; $F = 112.49$; $P = 0.0001$) in 1992. Standard errors of the regression slopes were ±0.012 and ±0.008 at high planting density and ±0.011 and ±0.007 at low planting density for Washington and Pamlico Co., respectively. Yield represented as T/ha was extrapolated from kg/9.14 m.

Initial data analyses of some of the 1992 Small-Plot Mixture Experiments using a mean comparison procedure indicated no difference in yield among mixture treatments that had 100%, 90% and 80% of the plants resistant. The results suggested that an optimal mixture strategy of 80% resistant: 20% susceptible could be deployed without losing marketable yield. However, there was not enough power in this statistical procedure to detect a difference among these treatments even if one existed (*i.e.*, reject the null hypothesis that there are no significant differences among treatments). Therefore, a better experimental design to identify an optimal ratio of resistant: susceptible potatoes to be used in a mixture would include fewer treatments, within the range of 10-30% susceptible plants, and a high number of replicates. If the expected variance in yield for the region is known, appropriate replication for a given probability of obtaining a significant result can be determined using a formula presented by Sokal and Rohlf (31). In our studies, we would have needed 304 replications to detect a 5% difference in yield between two treatment means (*e.g.* 0% and 10% susceptible plants in the mixture) with 90% probability at a 95% significance level given a coefficient of variation of 19% (the average for 1992). North Carolina potato breeders experience coefficients of variation that range between 15 and 23% using the same plot design described in our experiments with only 4 replications (W. Collins personal communication).

TABLE 4.—*Significance of planting density, mixture of susceptible and resistant plants and their interactions on total and US no. 1 yield in Pamlico Co., North Carolina, in 1993. Mixture main effect and density x mixture interaction sums of squares were partitioned to identify their specific relationship with yield (29).*

		Total Yield		US no. 1 Yield	
Source	df	*F*	*P*	*F*	*P*
Rep	5	2.99	0.0173	3.44	0.0082
Planting Density	1	0.92	0.3407	1.15	0.2874
Mixture (linear)	1	41.63	0.0001	46.94	0.0001
(quadratic)	1	0.36	0.5534	0.55	0.4624
(cubic)	1	0.02	0.8989	0.02	0.8818
Planting Density x					
Mixture (linear)	1	0.08	0.7767	0.05	0.8309
(quadratic)	1	0.02	0.8815	0.05	0.8280
(cubic)	1	0.00	0.9624	0.08	0.7825

1993 Small-Plot Mixture Experiment—Mean yield (±SE) in the 100% resistant plots averaged 27.221 ± 1.522 T/ha which was higher than yield from the previous year in the same county. An increase in planting density tended to increase total yield (high density = 25.239 ± 0.972 T/ha; low density = 23.910 ± 0.823 T/ha), but the difference between the two densities was not significant (Table 4). Planting density had no effect on US no. 1 yield (high density = 20.709 ± 0.983 T/ha; low density = 20.012 ± 0.822 T/ha) (Table 4).

Plant mixture significantly affected total yield (Table 4) at both high planting density and low planting density. In both cases, a negative linear relationship existed between total yield and percent susceptible plants in the mixture (high density: y=28.314-0.095x; df=1, 26; R^2 = 0.43; F=19.93; P=0.0001; and low density: y=28.014-0.103x; df=1, 46; R^2 = 0.31; F=21.08; P=0.0001), with no indication of non-linearity. Standard errors of the regression line slopes were ±0.021 and ±0.022 at the high and low planting densities, respectively. These results supported findings in 1992 indicating that yield compensation by potato was not evident in mixtures of resistant and susceptible plants in which all susceptible plants were completely defoliated during bloom.

US no. 1 yield was also significantly affected by planting mixture (Table 4). A negative linear relationship between yield of US no. 1 tubers and percent susceptible plants in the mixture was evident at high density (Fig.

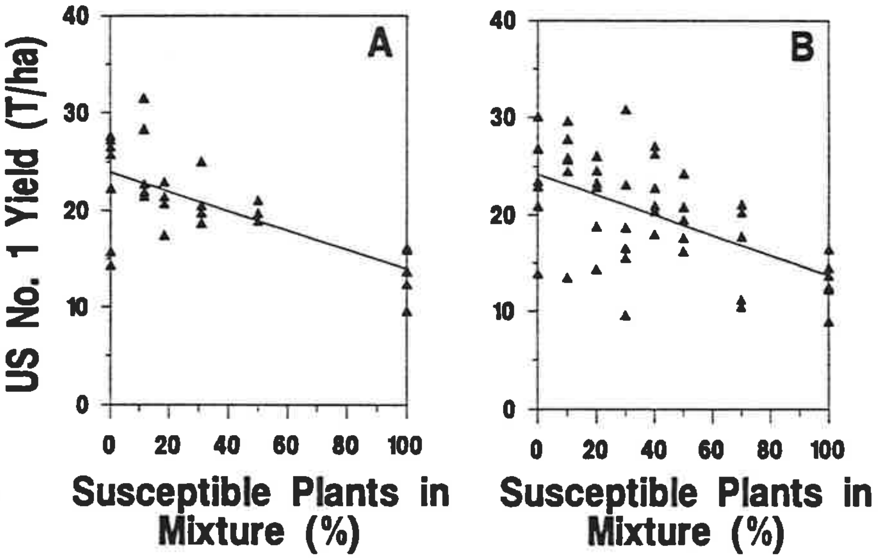

FIG. 4. - (a) Relationship between US no. 1 yield and percentage of susceptible plants (completely defoliated during late bloom) when grown in mixtures of "resistant/susceptible" plants spaced at either 0.23 m ($y = 23.93 - 0.100x$; df = 1,26; $R^2 = 0.47$; $F = 22.64$; $P = 0.0001$) or (b) 0.30 m ($y = 24.196 - 0.105x$; df = 1,46; $R^2 = 0.33$; $F = 22.39$; $F = 0.0001$) in Pamlico Co. in 1993. Standard errors of the regression slopes were ±0.021 at high planting density and ±0.022 at low planting density. Yield represented as T/ha was extrapolated from kg/6.14 m.

4a) and low density (Fig. 4b). There was no evidence of compensation by undamaged plants for damaged plants in the mixture.

Replicated studies in North Carolina have shown a negative linear relationship between potato yield and an increase in percent Colorado potato beetle defoliation during bloom (23). This relationship was consistent for both an early- (Superior) and a later-maturing cultivar (Atlantic). When potatoes enter the bloom stage in May, the soil environment becomes increasingly hot and dry which tends to reduce tuberization and tuber enlargement (6). Sub-optimal potato growing conditions in May and June may be responsible for the absence of significant yield compensation by potato in North Carolina. Although compensation by healthy potato for adjacent damaged plants was not evident in North Carolina, future studies should be conducted in other regions where defoliation occurs when potatoes are at an earlier growth stage.

Deployment Implications—Our results indicated that yield declined in a linear fashion as the percentage of defoliated susceptible plants in the mixture increases. Therefore, to the extent that susceptible plants in the mixture are heavily defoliated, the use of a mixture strategy would result in reduced yields. The value of these reduced yields would represent a cost to the grower of using this resistance management strategy.

In all experiments, there were no significant differences in the yield of US no. 1 grade potatoes when plants were spaced at varying densities between 0.23 and 0.30 m. Plant spacing on commercial farms of the commonly grown potato cultivars in North Carolina can vary from 0.23 to 0.30 m, typically depending on initial cost of seed pieces. Because transgenic seed pieces will be more costly, a low density (0.30 m spacing) may be the most cost-effective.

In this study we created the worse case scenario, 100% defoliation of all susceptible plants during bloom. This situation is unlikely to occur in the field unless beetle populations are extremely high because populations are aggregated within the field, and they typically colonize potato fields from the edges which often results in only a portion of the field being invaded (11). These infestation patterns would preclude defoliation of many susceptible plants and mitigate the yield reductions observed in this study.

Because potatoes may refoliate, the timing of defoliation may be important. In our study, plants refoliated in Washington Co. in 1992 and 1993, and in Pamlico Co. in 1993, such that the plant canopy was equal to or greater than the canopy at the time plants were defoliated. Additionally, the fully refoliated plants coincided with the emergence of first generation adults. Timing of defoliation and the frequency at which the plant is defoliated differs geographically and year to year. In certain northeastern growing regions there are two generations of potato beetle that complete development on potato, whereas in North Carolina only one generation is typically completed on potato before the crop is harvested. Therefore, the ability of susceptible potato plants to refoliate may be more significant in providing a refuge for susceptible beetles in certain areas in the northeast than in North Carolina. Compensation patterns also might be affected by the timing of defoliation.

Whether or not refoliation of susceptible plants makes a difference in selection pressure to the beetle population, a mixture strategy would be most effective if used with other management tactics such as crop rotation, biological control and selective use of insecticides (7). To minimize potential defoliation of susceptible plants, a mixture strategy should be used in fields where the predicted colonizing beetle population would be low (15). Subsequent populations in that field would be expected to be even lower, minimizing the chances that susceptible plants would be defoliated.

Conclusions

Absence of significant differences in yield when potatoes were hand or insect defoliated indicated that hand defoliation was an appropriate mimic of Colorado potato beetle defoliation. Hand-defoliating potatoes enabled discrete yield/defoliation relationships to be investigated without the huge amount of labor required to manipulate beetle populations to obtain uniform defoliation across all replications during the same phenological period.

Because potato beetles rarely cause economically damaging levels of defoliation before bloom in North Carolina, our study focused on the relationship between yield and defoliation during bloom. Healthy Atlantic potatoes lacked the ability to significantly compensate for neighboring plants that were defoliated during early or late bloom in North Carolina. If significant defoliation levels occurred early in the season, compensation may be more likely which is often the case in northern growing regions (8, 16). In our Single-Plant Compensation Experiments, there were no significant differences in either total or US no. 1 yield from two plants flanking a defoliated neighbor or two plants flanking a non-defoliated neighbor. If flanking plants compensated for damage to the single neighboring susceptible plant, the two plants flanking a completely defoliated plant would be expected to yield significantly more than plants flanking a non-defoliated plant. In our small-plot experiments we consistently observed a negative linear relationship between yield (total and US no. 1) and the percentage of defoliated susceptible plants in the mixture. If a compensatory response by potato occurred, this relationship would have been non-linear.

A higher planting density (1 plant per 0.23 m) significantly increased total yields but not yields of US no. 1 grade tubers compared with a lower planting density (1 plant per 0.30 m). Because transgenic seed pieces will be more expensive than currently grown cultivars, a 0.30 m planting density may be the most cost-effective.

Both the Single-Plant Compensation and Small-Plot Mixture Experiments indicated that a mixture strategy could result in a loss in yield if the susceptible plants were defoliated during bloom. The associated benefit that must be weighed against potential yield loss would presumably be a delay in the onset of resistance. Future work with mixtures should emphasize studies that observe movement and oviposition preferences of potato beetle in the field to see whether non-toxic plants actually provide a refuge and whether interplant movement will negate the usefulness of refuges provided by the susceptible plants (15, 20). This information will be needed before using this resistance management strategy.

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